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Prepared by the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

FAO JECFA Monographs 19 bis

Safety evaluation of certain contaminants in food

Food and Agriculture Organization of the United Nations

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World Health Organization, Geneva, 2018

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CONTENTS

Preface v

Safety evaluations of specific contaminants in food 1

Aflatoxins (addendum) 3
4,15-Diacetoxyscirpenol 281
Fumonisins (addendum) 415
Glycidyl esters 575
3-MCPD esters and 3-MCPD 665
Sterigmatocystin 773
Co-exposure of fumonisins with aflatoxins 879

Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives 961

Annex 2

Abbreviations used in the monographs 973

Annex 3

Participants in the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives 981

PREFACE

The monographs contained in this volume were prepared at the eighty-third meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 8–17 November 2016. These monographs summarize the data on selected food additives, including flavouring agents, reviewed by the Committee.

The eighty-third report of JECFA has been published by WHO as WHO Technical Report No. 1002. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scentific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Foods and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and dietary exposure and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by WHO and FAO experts. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by J. Odrowaz, Toronto, Canada.

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Any comments or new information on the biological or toxicological properties of or dietary exposure to the compounds evaluated in this publication should be addressed to: WHO Joint Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (jecfa@who.int).

SAFETY EVALUATIONS OF SPECIFIC CONTAMINANTS IN FOOD

Aflatoxins (addendum)

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1. Explanation 7
2. Biological data 9
2.1 Biochemical aspects 9
2.1.1 Absorption, distribution and excretion 9
2.1.2 Biotransformation 10
(a) Phase II metabolism of AFB_1 mainly occurs with the oxidized metabolites of AFB_1 13
(b) Metabolic polymorphisms and risk of liver cancer from aflatoxin 15
2.1.3 Effects on enzymes and other biochemical parameters 16
(a) Hepatitis B 16
2.1.4 Physiologically based pharmacokinetic modelling 17
2.1.5 Transfer from feed to food 19
2.2 Toxicological studies 20
2.2.1 Acute toxicity 20
2.2.2 Short-term studies of toxicity 20
2.2.3 Long-term studies of toxicity and carcinogenicity 21
2.2.4 Genotoxicity 22
2.2.5 Reproductive and developmental toxicity 23

2.2.6 Special studies 24
(a) Immunotoxicology 24
2.3 Observations in domestic animals/veterinary toxicology 24
2.4 Observations in humans 24
2.4.1 Biomarkers of exposure 24
2.4.2 Biomarkers of effects 27
(a) Effects of pharmacological intervention on biomarkers of aflatoxin exposure 29
2.4.3 Clinical observations 32
(a) Short-term effects 32
(b) Effects on child growth and development 34
(c) Immunotoxicology 41
2.4.4 Epidemiology of primary liver cancer 42
(a) Etiology of primary liver cancer 43
(b) Vaccination programmes against HBV and liver cancer epidemiology 43
(c) Epidemiology studies on aflatoxin and liver cancer 45
(d) HBV, HCV and aflatoxin in the etiology of liver cancer 53
(e) Genetic susceptibility 54
3. Analytical methods 58
3.1 Introduction 58
3.2 Sample preparation 58
3.3 Quantitative methods 59
3.3.1 TLC 59
3.3.2 HPLC 61
3.3.3 HPLC-MS or HPLC-MS/MS 62
3.3.4 Capillary electrophoresis 62
3.4 Semiquantitative methods 62
3.4.1 ELISA 63
3.4.2 Lateral flow tests 63
3.4.3 Direct fluorescence 64
3.4.4 Fluorescence polarization immunoassay (FPIA) 64
3.4.5 Biosensors 64
3.5 Indirect methods 65
3.5.1 Spectroscopy 65
3.6 Emerging technologies 65
3.6.1 Hyperspectral imaging 65
3.6.2 Electronic nose 66
3.6.3 Aptamer-based biosensors 66
3.6.4 Molecularly imprinted polymers (MIPs) 67
3.7 Special considerations 67
4. Sampling protocols 68
5. Effects of processing 70
5.1 Physical methods 70
5.1.1 Sorting 71
5.1.2 Wet milling and dry milling 72
5.1.3 Heat treatments 72
5.2 Fermentation 73
5.3 Extrusion 73

6. Prevention and control 74
6.1 Introduction 74
6.2 Preharvest control 75
6.2.1 Management practices 75
(a) Soil amendments and type 75
(b) Fertilizer use 76
(c) Crop rotation 76
(d) Tillage 76
(e) Seed or variety selection 76
(f) Planting and harvesting dates 76
(g) Planting density 77
(h) Weed, fungal and insect control 77
(i) Irrigation management 77
6.2.2 Biological control 77
(a) Bacteria 78
(b) Yeasts 78
(c) Non-toxigenic *Aspergillus flavus* 79
(d) Special considerations on biocontrol 81
6.2.3 Host resistance 82
(a) Plant breeding 82
(b) Molecular breeding 84
6.3 Postharvest control 86
6.3.1 Moisture control at storage 86
6.3.2 Storage conditions 87
6.4 Decontamination 88
6.5 Enteroabsorbents 89
6.6 Predictive modelling 90
6.6.1 Field application of models 90
(a) Peanuts 90
(b) Maize 91
6.6.2 Storage application of models 93
(a) Maize 93
(b) Pistachios 93
(c) Rice 93
(d) Chilli powder 94
6.6.3 Neural networks 94
6.6.4 Special considerations on modelling 94
7. Levels and patterns of contamination of food commodities 95
7.1 Surveillance data 95
7.1.1 GEMS/Food contaminants database 95
7.1.2 Open literature search: FAO scoping review 96
7.1.3 Open literature search: aflatoxin in human breast milk 98
7.2 Distribution curves 98
7.3 Data on annual variation in contaminant levels 98
8. Food consumption and dietary exposure assessment 99
8.1 Concentrations in food used in the dietary exposure estimates 99
8.2 Food consumption data used in the dietary exposure estimates 100
8.3 Assessments of dietary exposure 115

8.3.1 National estimates of dietary exposure from the scientific literature 115
(a) African Region 115
(b) Argentina 115
(c) Brazil 115
(d) People's Republic of China 127
(e) Egypt 129
(f) France 130
(g) Greece 130
(h) Islamic Republic of Iran 131
(i) Ireland 131
(j) Japan 131
(k) Kenya 132
(l) Lebanon 132
(m) Malaysia 133
(n) Mexico 135
(o) Morocco 135
(p) The Netherlands 135
(q) New Zealand 135
(r) Pakistan 136
(s) Portugal 136
(t) Republic of Korea 136
(u) Serbia 137
(v) Spain 137
(w) Sri Lanka 139
(x) Tunisia 140
(y) Turkey 140
(z) United Republic of Tanzania 141
(aa) Summary 141
8.3.2 National estimates of dietary exposure derived by the Committee 142
8.3.3 International estimates of dietary exposure 143
8.4 Potential effect of limits and their enforcement on chronic dietary exposure 154
8.5 Dietary exposures for infants 159
8.5.1 Estimated dietary exposure for breastfed infants 159
8.5.2 Estimated dietary exposure for fully formula-fed infants 160
9. Dose–response analysis and estimation of toxic/carcinogenic risk 161
9.1 Identification of key data for dose–response analysis 161
9.1.1 Pivotal data from human clinical/epidemiological studies 161
9.1.2 Biomarker studies 162
9.2 General modelling considerations 162
9.2.1 Selection of data 162
9.2.2 Measure of exposure 163
9.2.3 Measure of response 163
9.2.4 Selection of mathematical model 163
9.3 Potency estimates 164
9.3.1 Potency estimates in humans based on epidemiological data 164
(a) Potency estimates not accounting for HBV infection 164

(b) Potency estimates accounting for HBV infection 164
9.3.2 Potency estimates in humans based on biomarkers 169
9.3.3 Potency estimates in test species 169
9.3.4 Combining cancer potency estimates with dietary exposure estimates across GEMS/Food cluster diets and prevalence of HBsAg status to estimate the increases in liver cancer incidences worldwide associated with aflatoxin contamination 171
10. Comments 174
10.1 Biochemical aspects 174
10.2 Toxicological studies 175
10.3 Observations in domestic animals/veterinary toxicology 176
10.4 Observations in humans 177
10.5 Analytical methods 180
10.6 Sampling protocols 182
10.7 Effects of processing 182
10.8 Prevention and control 183
10.9 Levels and patterns of contamination in food commodities 186
10.10 Food consumption and dietary exposure assessment 187
10.11 Impact assessment of implementation of Codex maximum levels in ready-to-eat peanuts 189
10.12 Dose–response analysis 191
11. Evaluation 194
11.1 Recommendations 196
12. References 197
Appendix 1 242

1. Explanation

Aspergillus flavus is a fungus that was first recognized to cause aflatoxicosis in domestic animals and is the most important aflatoxin-producing species in food on a global basis. It produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) and affects many commodities, but most human exposure comes from contaminated corn (also referred to as maize), peanuts (also referred to as groundnuts) and rice. Another important producer of aflatoxin, *A. parasiticus*, produces AFB_1, AFB_2, aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) and is primarily associated with peanuts in the Americas, but can also occur on corn, figs and pistachios (Horn, 2003). Of these four aflatoxins, AFB_1 is most frequently present in contaminated samples; AFB_2, AFG_1 and AFG_2 are generally not reported in the absence of AFB_1. Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1; in areas of high aflatoxin exposure, humans are exposed to AFM_1 more or less exclusively through milk and milk products, including breast milk (Magoha et al., 2014) (Fig. 1).

Fig. 1
Chemical structures of key aflatoxins

Most of the available toxicological data relate to AFB_1. However, information regarding the relative potency of aflatoxin congeners is available from bacterial mutagenicity and hepatocarcinogenic effects in the rainbow trout and rats, in the order of AFB_1 > (AFG_1, AFM_1) >> (AFB_2, AFG_2) (Sinnhuber et al., 1974; Wong & Hsieh, 1976). The apparent order of mutagenic and carcinogenic activity is in accord with the presence (AFB_1, AFM_1 and AFG_1) and absence (AFB_2 and AFG_2) of a chemically reactive double bond that can be converted metabolically to a DNA-reactive epoxide (Guengerich et al., 1998). Based on these biosynthetic, structural and toxicological properties, this evaluation focused primarily on the toxicity of AFB_1 and the exposure to AFB_1 and total aflatoxins (AFT).

Aflatoxins were previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its thirty-first, forty-sixth, forty-ninth, fifty-sixth and sixty-eighth meetings (Annex 1, references *77*, *122*, *131*, *152* and *187*). At the thirty-first meeting, the Committee considered aflatoxins to be a potential human carcinogen and urged that dietary exposure to aflatoxins be reduced to the lowest practicable levels, so as to reduce the potential risk as far as possible. At its forty-sixth meeting (1997, no monograph prepared), the

Committee considered estimates of the carcinogenic potency of aflatoxins and the potential risk associated with their intake and recommended a detailed assessment. This detailed assessment was undertaken at the forty-ninth meeting (1999), when the Committee provided potency estimates for human liver cancer resulting from AFB_1 exposure, taking hepatitis B virus surface antigen (HBsAg) status into account. The effects of applying hypothetical maximum levels of AFT (10 and 20 µg/kg) for contamination in maize and groundnuts were also analysed, and the Committee concluded that reducing the maximum level from 20 to 10 µg/kg is unlikely to result in detectable differences in population cancer risks. For populations with a high prevalence of hepatitis B surface antigen-positive (HBsAg+) individuals and high mean intake of aflatoxins, population health would benefit from reductions in aflatoxin intake. At its fifty-sixth meeting (2002), the Committee evaluated the impact of different maximum levels for AFM_1 in milk, and at the sixty-eighth meeting (2007), the Committee evaluated the impact of different hypothetical maximum levels for tree nuts and dried figs.

The Committee updated the aflatoxin risk assessment at the current meeting at the request of the Codex Committee on Contaminants in Food (CCCF). The toxicological review made use of the literature in three International Agency for Research on Cancer (IARC) publications (IARC, 2002, 2010, 2012), a review by Eaton et al. (2010), a review of the global burden for aflatoxin-induced liver cancer (Liu & Wu, 2010) and a recent IARC publication on aflatoxin and child health (Wild, Miller & Groopman, 2015). Key references from these publications and the monograph from the forty-ninth meeting of JECFA were collected, and searches of the more recent literature (1999 to present) were conducted in PubMed, SciFinder (ACS-PubMed) and/or Web of Science (ISI). In addition, where needed and if possible, important raw data were solicited from the authors of key publications or unpublished reports. The literature search on the occurrence of and dietary exposure to aflatoxins was conducted using three databases (Scopus, PubMed and Ovid) for the period from January 2007 to end of 2015.

2. Biological data

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

The toxicokinetics of aflatoxin in domestic and laboratory animals has been exhaustively reviewed by Hsieh & Wong (1994) with updates in IARC (2002, 2010). There are considerable data comparing AFM_1 concentrations in human urine and breast milk with dietary exposure to aflatoxin; however, there is limited

information relating absorption and distribution of aflatoxin with the presence of other aflatoxin metabolites in body fluids (Annex 1, reference *152*). There is considerable interindividual variation in the rate of activation and elimination of aflatoxins; at low doses, this is likely to be relevant to the pharmacokinetics of aflatoxins in humans. Thus a detailed understanding of the pharmacokinetics in humans from ecological studies is not likely feasible unless conducted on a very large scale.

There are also few studies in non-human primates. A study of eight rhesus monkeys injected with 0.3 mg/kg body weight (bw) radiolabelled aflatoxin indicated a half-life ($t_{½}$) of 36.5 minutes, longer than in the mouse and rat strains tested, and the volume of distribution was much higher than in either the mouse or rat strains tested. Disposition studies of injected ^{14}C-labelled AFB_1 showed that after 100 hours, monkeys excreted similar amounts of radioactivity into urine (38% of total dose) and faeces (35% of total dose), while the rodents excreted approximately 2 times more radioactivity through the faeces than the urine. Monkey liver retained approximately 14% of total radioactivity (Wong & Hsieh, 1980).

Jubert et al. (2009) investigated the kinetics of oral low-dose ^{14}C-labelled AFB_1 in 4 volunteers (30 ng labelled aflatoxin per 32–65 kg bw). After dosing, absorption of AFB_1 equivalents into the systemic circulation was also rapid, with peak plasma concentrations reached within approximately 1 hour with first order kinetics. Pharmacokinetic modelling of absorption and disposition showed that 95% of the total urinary AFB_1-associated radioactivity was excreted within the first 24 hours. Similar to the disposition in monkeys (cited above), only approximately 33% of the total ^{14}C-labelled AFB_1 dose was excreted through the urine over 72 hours. The faeces were not analysed.

AFB_1 was shown to cross the human placenta in ex vivo perfusion experiments; the amount of AFB_1 increased in fetal circulation and decreased in maternal circulation over time, but the concentrations did not equilibrate between fetal and maternal circulation during perfusions (Partanen et al., 2009). The kinetics for AFB_1 diffusion differed from those of antipyrine, the reference compound tested, which is known to diffuse passively through the placenta. Reductive metabolism to aflatoxicol, in the absence of evidence for oxidative metabolism, was observed either during the perfusions or in incubations with placental cytosolic and microsomal fractions in vitro.

2.1.2 Biotransformation

The apparent mutagenic and carcinogenic activity of aflatoxin congeners is in accord with the presence (AFB_1 and AFG_1) and absence (AFB_2 and AFG_2) of an activated double bond that can be converted via cytochrome P450 (CYP) activity to a DNA-reactive epoxide. Based on these structural and toxicological properties, the preponderance of research on biotransformation has been conducted using AFB_1.

The metabolism of AFB_1 in humans and laboratory animals has been well characterized (Fig. 2). There are important differences between humans and various laboratory animals in aflatoxin metabolism that markedly affect the toxicology (IARC, 2002, 2010; Eaton et al., 2010). Understanding the biochemical basis for these species differences provides perspective on the varied responses to aflatoxin. A critical aspect of AFB_1 metabolism is a suite of reactions by microsomal CYP mixed function monooxygenases. This involves multiple CYP isozymes that show considerable variation in kinetic characteristics and product specificity between species (Guengerich et al., 1998). The predominant AFB_1 metabolites from CYP-catalysed reactions in mammals are AFM_1, aflatoxin Q_1 (AFQ_1), aflatoxin P_1 (AFP_1) and AFB_1-8,9-epoxides. Oxidations of AFB_1 to AFQ_1, AFM_1 and AFP_1 produce metabolites that are less toxic and carcinogenic than AFB_1-*exo*-8,9-epoxide (IARC, 2002; Eaton et al., 2010).

The CYP isoforms, CYP1A2, 2B6, 3A4, 3A5 and 3A7, and glutathione *S*-transferase M1 (GSTM1) mediate aflatoxin metabolism in humans (see Fig. 2, showing the primary enzymatic pathways). AFB_1 metabolism in vivo depends on the affinity for each reactant (K_m) and maximum velocity for conversion (V_{max}) and on their expression level in different tissues. These parameters are used to calculate k_{cat} (V_{max} × total enzyme) and the intrinsic clearance (k_{cat}/K_m), also referred to as the specificity constant, which approximates the second order rate constant between enzyme and substrate at substrate concentrations far below the K_m (Guengerich et al., 1998).

Hepatic microsomal metabolism of AFB_1 has been studied in animal models. A study in male F344 rat liver microsomes showed the presence of CYP1A1, 1A2, 1B1/2, 2C11 and 3A1/2 (Stresser, Bailey & Williams, 1994). Formation of AFB_1 epoxide predominated over formation of AFM_1 and AFQ_1 (~340:40:30 pmol/min per mg microsomal protein, respectively). Similarly, rainbow trout liver contains CYP1A1, 1A2, 2M1 and 2K1, of which CYP2K1 was reported to have high activity for formation of AFB_1-*exo*-8,9-epoxide (Bailey, Williams & Hendricks, 1996). During phase I metabolism in human liver microsomes, CYP3A4 catalyses the formation of the 8,9-epoxide(s) of AFB_1 and AFQ_1, which have approximately 1% of the hepatocarcinogenic activity of AFB_1 in trout liver (Hendricks et al., 1980), at a rate constant ratio of approximately 0.29:1, respectively (Kamdem et al., 2006). CYP3A5 oxidizes AFB_1 mainly to the *exo*-8,9-epoxide, with a rate constant 3-fold lower than CYP3A4 (Kamdem et al., 2006). CYP1A2 action produces equimolar amounts of AFB_1-*exo*-8,9-epoxide and its *endo*-8,9 isomer (Ueng et al., 1998). AFB_1-*endo*-8,9-epoxide is approximately 1000-fold less reactive with DNA than the corresponding *exo*-8,9 isomer (Guengerich & Johnson, 1999). CYP1A2 also produced AFM_1, which has approximately 10% of the hepatocarcinogenic activity of AFB_1 in trout (Bailey et al., 1998), with a rate constant 4.6-fold greater than that for AFB_1 epoxide (Kamdem et al., 2006).

Fig. 2
Metabolism and disposition of AFB_1

CYP: cytochrome P450; GSH: glutathione; GST: glutathione *S*-transferase; NADPH: nicotinamide adenine dinucleotide phosphate; UGT: uridine 5′-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase)

The DNA binding and carcinogenicity of AFB_1 result from its conversion to the AFB_1-*exo*-8,9-epoxide, primarily by CYP3A isoforms in human liver, with kinetic properties reflected by those determined using human CYPs expressed in baculosomes (Kamdem et al., 2006). The *exo*-epoxide stereoisomer is highly reactive with the guanine bases of DNA. It also binds to proteins, resulting in cell injury (Eaton et al., 2010). While the *endo*-epoxide isomer is much less reactive with DNA, it does bind to proteins (Table 1 shows the enzyme kinetic constants for hepatic CYP isoform–mediated biotransformations of AFB_1). Moreover, because CYP3A4 is often expressed at much higher quantities than CYP3A5 (and 1A2) in most adult livers, it is thought to be a major contributor towards AFB_1-*exo*-8,9-epoxide formation in vivo (IARC, 2010). However, hepatic CYP3A5 expression differs markedly between individuals and displays several identified variants, which suggests that CYP3A5 can be an important contributor to AFB_1 bioactivation (Kamdem et al., 2006). Human, marmoset (*Callithrix jacchus*) and

Table 1
Kinetic constants for baculovirus-expressed human hepatic CYP isoform–mediated transformations of AFB_1 in vitro

Kinetic parameter	CYP1A2	CYP3A4	CYP3A5
AFB_1 epoxide			
V_{max} (pmol per pmol P450 per min)	0.39 (*endo*)	1.64 (*exo*)	1.2 (*exo*)
K_m (µmol/L)	55	130	302
V_{max} / K_m (µL per pmol P450 per min)	0.007	0.012	0.004
AFQ_1			
V_{max} (pmol per pmol P450 per min)	–	13.3	–
K_m (µmol/L)	–	324	–
V_{max} / K_m (µL per pmol P450 per min)	–	0.041	–
AFM_1			
V_{max} (pmol per pmol P450 per min)	0.95	–	–
K_m (µmol/L)	29	–	–
V_{max} / K_m (µL per pmol P450 per min)	0.032	–	–

AF: aflatoxin; CYP: cytochrome P450; min: minute; K_m: affinity; V_{max}: maximum velocity for conversion
Source: Kamden et al. (2006)

macaque (*Macaca nemestrina*) hepatic microsomes have similar rates of oxidation of AFB_1 to the 8,9-epoxide, although the rate of glutathione *S*-transferase (GST)–catalysed conjugation by macaques was at least 50-fold higher, given that it was undetectable in marmosets and humans (Bammler, Slone & Eaton, 2000). No information about the biotransformation of AFB_2, AFG_1 or AFG_2 was identified beyond that previously reviewed by JECFA in 1999 and by IARC (1993).

(a) Phase II metabolism of AFB_1 mainly occurs with the oxidized metabolites of AFB_1

Considerable interspecies variation exists in the expression of the various phase II enzymes in AFB_1 detoxification, in particular with the GSTs. The amount of AFB_1-*exo*-8,9-epoxide that will bind to DNA is diminished by the fraction of the epoxide that is enzymatically conjugated with glutathione via GSTs. The selectivity of GST isozymes towards AFB_1-8,9-epoxide is a critical determinant of the susceptibility of mammals to aflatoxin-induced liver cancer.

Studies of GST–aflatoxin metabolism have been conducted in mice, rats, hamsters, quail, duck and monkeys, among other species (Roebuck & Wogan, 1977; Degen & Neumann, 1981; O'Brien et al., 1983; Lotlikar et al., 1984). Mouse liver cytosolic fractions have AFB_1-8,9-epoxide conjugating activities 50- to 100-fold greater than rat (Monroe & Eaton, 1988). This is the primary reason why mice are more resistant to the hepatocarcinogenic effects of AFB_1 than rats (Wogan & Newberne, 1967). A 50- to 100-fold lower level of AFB_1–DNA adduct formation after AFB_1 exposure is seen in mice compared to rats (Monroe & Eaton,

1987). A study of nine different mouse strains showed that each had similar GST activities towards the epoxide (Borroz, Ramsdell & Eaton, 1991). Mice have at least three GST subunits, two of which are inducible by chemoprotective agents (IARC, 2002; Eaton et al., 2010). The resistance of mice to aflatoxin-induced liver cancer is explained by the constitutive hepatic expression of an α-class GST, mGSTA3–3, a detoxifying enzyme with a high affinity for AFB_1 (Buetler & Eaton, 1992). Studies conducted in liver cytosolic preparations in vitro showed that mouse GSTs conjugate the *exo*-epoxide of AFB_1 with higher efficiency than either the rat or human preparations tested (~400:25:1, respectively; Raney et al., 1992). Furthermore, mouse liver GSTs conjugate AFB_1 *exo*-epoxide almost exclusively, whereas the analogous rat and human liver cytosolic preparations preferentially conjugate the *endo*-epoxide (Raney et al., 1992). In the non-human primate, *M. fascicularis* (long-tailed macaques), the GST-conjugating ability of the epoxide was partially due to a μ-class GST with 96% amino acid homology to human GSTM2. The long-tailed macaque enzyme mfaGSTM2–2 was predominantly active towards the *endo*-epoxide, whereas the mfaGSHA-GST was almost exclusively active towards the *exo*-epoxide. However, this was 2 orders of magnitude lower than that of the rodent α-class GSTs (Wang et al., 2000). GST-mediated conjugation of AFB_1-8,9-epoxide does occur in humans, which was particularly evident in an intervention study using induction of phase II metabolism by oltipraz (Wang JS et al., 1999).

The glucuronides of AFP_1 and 4,9-dihydroxyaflatoxin B_1 are biliary metabolites in rats treated with AFB_1 (Eaton et al., 1988). Originally described in rhesus monkeys (*M. mulatta*), this phenolic derivative of AFB_1 is found in urine in humans (Wild & Turner, 2002). Similarly, evidence for excretion of "free" and glucuronidated AFM_1 and AFQ_1 in urine and faeces was observed in humans (Mykkänen et al., 2005). It is not clear which hepatic enzyme(s) is responsible for AFP_1 production in humans or rodents (Eaton et al., 2010). The major conjugates in trout injected with radiolabelled AFB_1 were aflatoxicol–glucuronide and AFM_1–glucuronide; sulfate conjugates were not found (Loveland, Nixon & Bailey, 1984). The sulfate conjugates of AFM_1 and AFP_1 were formed in rhesus monkeys (Dalezios, Hsieh & Wogan, 1973).

The hydrolysis of the AFB_1-*exo*-8,9-epoxide to the corresponding dihydrodiol occurs spontaneously in aqueous media ($t_{½}$ ~1 second). However, Johnston et al. (1997) found that recombinant rat epoxide hydrolase only modestly augmented the rate of AFB_1-*exo*-8,9-epoxide hydrolysis in vitro (10–20%) and human epoxide hydrolase did not; this does not support a role for epoxide hydrolase in the detoxification of AFB_1. The researchers found that hydrolysis of the AFB_1-*endo*-8,9-epoxide occurs in aqueous media at a rate of approximately 12% of that of the *exo* isomer, and the presence of epoxide hydrolase does not enhance the rate (Johnston et al., 1997). The dihydrodiol produced from

either *exo*- or *endo*-epoxide undergoes a base-catalysed rearrangement to the corresponding dialdehyde, which binds covalently to lysine amino groups in serum albumin (Montesano, Hainaut & Wild, 1997; Guengerich et al., 1998). The AFB_1–albumin lysine adduct (AFB_1–lys) is the most widely used biomarker of aflatoxin exposure.

The conversion of AFB_1 to aflatoxicol in hepatic postmitochondrial and microsomal fractions by the reduction of the 1-keto group by a nicotinamide adenine dinucleotide phosphate (reduced) (NADPH)-dependent reductase occurs in mice, rats, hamsters, rabbits, chickens, trout, rhesus monkeys and humans (Salhab & Edwards, 1977). Aflatoxicol can be further metabolized by undergoing a 9-hydroxylation to form aflatoxicol-M_1 (Loveland et al., 1988). The formation of aflatoxicol does not appear to be an important detoxification pathway for AFB_1, as it can be converted back to AFB_1 by a microsomal dehydrogenase, thereby increasing the physiological half-life of AFB_1 (Salhab & Edwards, 1977).

Aflatoxin toxicity (e.g. mutagenicity and cytotoxicity) in humans and animal species and strains is a result of the proportion of aflatoxin metabolized to the 8,9-*exo*- versus *endo*-epoxide, detoxification through oxidative phase I metabolism to AFQ_1 and AFM_1, and the contributions of phase II pathways forming nontoxic conjugates (glucuronide, GST and sulfate conjugates; Eaton & Gallagher, 1994; Eaton et al., 2010). Because both activation and detoxification pathways occur concurrently (and can be interactive), the ratio of activation to inactivation, rather than the absolute rate of overall biotransformation, determines species and individual susceptibility to AFB_1 toxicity, including hepatocarcinogenesis, and is the basis of variability.

(b) Metabolic polymorphisms and risk of liver cancer from aflatoxin

DNA and protein adducts of aflatoxin have been detected in many studies of human liver tissues and body fluids (IARC, 1993). Such aflatoxin-specific biomarkers have enabled studies of individual exposures and genetic polymorphisms. An analysis of a variety of genetic polymorphisms as probable modifiers of risk from aflatoxin has been undertaken in regions with high incidence of hepatocellular carcinoma. These case–control studies have tested the hypothesis that differences in either detoxification or activation pathways affect internal exposure to the aflatoxin epoxide and liver cancer risk (IARC, 2010). So far, these studies are limited due to the relatively small numbers of participants in the high-risk genotype. The most consistent finding both for aflatoxin adduct concentrations and increased risk for hepatocellular carcinoma is the GSTM1-null genotype at elevated aflatoxin exposures.

In a study of 357 Gambian adults, aflatoxin–albumin adduct levels were examined in relation to genetic polymorphisms in the GSTM1, GSTT1, GSTP1 and

epoxide hydrolase genes and the CYP3A4 phenotype. Of these, only the GSTM1-null genotype was associated with a modest increase in aflatoxin–albumin adduct levels. This effect was seen in individuals not infected with hepatitis B virus (HBV; Wild et al., 2000). Similarly, there was no association between aflatoxin–albumin adducts and GSTM1 genotype in 234 adults from Qidong County, China (Kensler et al., 1998). A study of 216 cases and 408 controls in the Gambia found the GSTM1-null genotype and the heterozygote XRCC1–399 AG DNA repair genotype were significantly associated with hepatocellular carcinoma. Associations between liver cancer and a number of other polymorphisms were also detected but their significance was not clear (Kirk et al., 2005a).

The influence of polymorphisms in the DNA repair enzyme, XRCC1, on the levels of AFB_1–DNA adducts in samples of placental DNA was studied in 120 women. The presence of at least one allele of polymorphism, 399Gln, was associated with a 2- to 3-fold higher risk of having detectable AFB_1–DNA adducts at intermediate but not high adduct levels compared with individuals homozygous for the 399Arg allele. This outcome was attributed to saturation of repair processes (Lunn et al., 1999).

A number of studies have been conducted on the impact of high aflatoxin exposure on genetic mutations, mainly in populations in Africa and China. In general, the studies are too small to assess the effect on population health (IARC, 2002, 2010). Studies of the types of human genetic alterations associated with exposure to aflatoxin have been even less informative.

Population-level studies of adducts, exposure and cancer outcome are affected by dietary components known or suspected to affect the metabolism of aflatoxin (Groopman, Kensler & Wild, 2008; Gross-Steinmeyer & Eaton, 2012). Aside from small population size, and the magnitude of aflatoxin exposure, the variability in an individual's diet would thus further limit the power of studies linking consumption and biomarkers of exposure with cancer rates.

2.1.3 Effects on enzymes and other biochemical parameters

(a) Hepatitis B

As discussed in the previous JECFA evaluation in 1999 (Annex 1, reference *131*), exposure to aflatoxin in seropositive HBV patients increases the relative risk of liver cancer and appears to be synergistic in most, but not all, studies (IARC, 2002, 2010; Kew, 2003). The mechanisms involved are still being explored, but some animal studies have provided evidence. In HBV-transgenic mouse lineages, acute viral hepatitis induced CYP1A2, CYP2A5 and π-class GST (Chemin et al., 1996, 1999). Π-class GST is ineffective in conjugating aflatoxin epoxide (Stewart, Serabjit-Singh & Massey, 1996). Upregulation of CYPs is also seen in animals with liver injury associated with bacterial and parasitic infections (Kirby et al., 1994).

Human liver specimens with evidence of HBV infection had significantly lower total GST activity than noninfected livers (Zhou et al., 1997). GST conjugation of the aflatoxin epoxide is critical to reducing the carcinogenicity in exposed individuals.

2.1.4 Physiologically based pharmacokinetic modelling

No comprehensive effort to use physiologically based pharmacokinetic modelling to incorporate all the elements of AFB_1 metabolism and disposition, including DNA or protein adduct formation, has been reported; however, some elements that could be useful in future model design have been reported separately. A controlled dosing human study of ^{14}C-labelled AFB_1 pharmacokinetics conducted in four volunteers who consumed an oral bolus of 30 ng (equivalent to a dose range of 0.46–0.94 ng/kg bw) used accelerator mass spectrometric detection to produce time–concentration profiles of total radioactivity in plasma and urine (Jubert et al., 2009). Absorption was rapid ($t_{½abs}$ = 0.14 hour), with a mean time to reach the maximum concentration (T_{max}) of 1.16 hours and urinary excretion more than 95% complete by 24 hours. Cumulative recovery of administered radioactivity in the urine was approximately 33% by 72 hours in the three of four study participants evaluated (faeces were not analysed).

The activation of AFB_1 by CYP isoforms has been extensively investigated and some degree of consensus regarding human metabolic pathways is available: CYP1A2 and CYP3A4/5/7 are the primary isoforms catalysing oxidation of AFB_1, with CYP1A2 producing AFM_1- and AFB_1-*exo*/*endo*-8,9-epoxides, and CYP3A isoforms producing AFQ_1 and the *exo*-8,9-epoxide. While CYP1A2 has the highest affinity for AFB_1 (lowest K_m), CYP3A4 is most abundant in adult human liver. Human liver microsome studies (n = 13 individuals) identified CYP3A4 levels as the most important indicator of AFB_1-*exo*-8,9-epoxide and AFQ_1 formation, in a ratio of 1:7.2 (Kamdem et al., 2006). Expressed individual enzyme studies indicate that only CYP1A2 is associated with AFM_1 formation (Kamdem et al., 2006). Mykkänen et al. (2005) showed that AFQ_1 was extensively excreted into faeces as a glucuronide and into urine as the unconjugated metabolite in similar quantities by study participants with a high consumption of AFB_1. Moreover, AFM_1 excretion in urine and faeces was at least 60-fold lower than the corresponding AFQ_1 levels.

Studies using fetal human liver preparations showed minimal difference in DNA or protein adduct formation compared with adults, despite the absence of detectable CYP1A2 in fetal liver (Doi, Patterson & Gallagher, 2002). Another factor to include in modelling of AFB_1 metabolism and carcinogenic effects is the relative trout liver tumorigenic potencies of AFM_1 and AFQ_1, which are approximately 1/10 and 1/100 that of AFB_1 (Hendricks et al., 1980; Bailey et

al., 1998). The formation of AFB_1 adducts with serum albumin through lysine residues occurs through the AFB_1 dialdehyde produced from either *endo*- or *exo*-8,9-epoxides via the dihydrodiol (Guengerich et al., 1998). The formation of these adducts has been validated as a biomarker of exposure to AFB_1 for use in human intervention studies (Scholl et al., 2006). Controlled dosing studies in F344 rats showed that 24 hours after a single gavage dose of either 50, 250 or 1000 µg/kg bw, 1.1–2.0% of the administered dose had been converted to AFB–lys adducts; that repeated dosing with AFB_1 (5–25 µg/kg bw per day) linearly increased adduct levels over 5 weeks; and that the elimination half-life for adducts was 2.3 days (Qian et al., 2013). The ranges of values for total binding to albumin and AFB_1–albumin (AFB_1–alb) half-life were similar to those reported by Wild et al. (1986), 0.98–2.2% of administered dose and 2.3 days, respectively. The turnover of the AFB_1–alb adduct is markedly faster than the half-life for human albumin of approximately 20 days (Scholl et al., 2006). Cupid et al. (2004) administered ^{14}C-labelled AFB_1 to male F344 rats (0.000 16–12.3 µg/kg bw) and human volunteers (~0.015 µg/kg bw) undergoing colon cancer surgery, and subsequently measured the formation of DNA–albumin adducts using accelerator mass spectrometry. A linear relationship was observed between log AFB_1–alb adducts and log AFB_1 doses administered to the rats. The corresponding values obtained from 7 humans fit closely with the rat data, indicating similar activation and binding: the mean value of bound AFB_1 was 38 ± 20 pg/mg albumin per µg AFB_1 administered per kg bw. The corresponding value for rats was 42 ± 7.1 pg/mg albumin per µg AFB_1 administered per kg bw (range: 6.8–42), values approximately 10-fold higher than previous estimates. DNA binding was measured in rat liver, kidney, colon, lung and spleen and in human colon tissue. DNA binding levels were approximately 100-fold higher in liver than in the other rat tissues. However, no consistent indication of DNA adduct formation was obtained from analysis of human colon tissue samples (signal/noise >2 in 2/10 samples). However, ^{14}C-labelled AFB_1–DNA binding was consistently measured at the equivalent dose in rats (45 ± 11 adducts per 10^{12} nucleotides), indicating that humans form fewer DNA adducts than rats per unit dose of AFB_1. Ratios of AFB_1 binding to liver DNA (in pg AFB_1 per mg DNA) versus serum albumin (pg AFB_1 per mg albumin) in rats were determined to be 2.8 ± 0.48 to 5.0 ± 1.7, which are similar to ratios of 3.2–3.7 produced by doses of ^{3}H-labelled AFB_1 (10–200 µg/kg bw) as determined by Wild et al. (1986). Wild et al. (1996) showed that after 14 days of repeated dosing with AFB_1 (20 µg/kg bw), the formation of serum AFB_1–alb adducts was similar in F344, Sprague Dawley and Wistar rat strains (~1.2 pg per mg albumin per µg/kg bw AFB_1). While AFB_1–adduct formation in guinea-pigs was similar in rats, hamsters and mice produced far lower adduct levels (Wild et al., 1996). In addition, Wild et al. (1996) estimated the corresponding human AFB_1–alb adduct/dose relationship

to be similar to the rat values (57–75%) using maximum exposure estimates from high AFB_1–consuming populations in China and the Gambia, when adjusted using allometry for interspecies differences in body surface areas.

Collectively, the quantitative enzymology relationships involving bioactivation and deactivation, DNA adduct formation, urinary and serum biomarker formation and other disposition pathways provide important elements needed for modelling AFB_1 exposures by physiologically based pharmacokinetic approaches and incorporating with the resultant tumorigenic risks into more complete biologically based dose–response models.

2.1.5 Transfer from feed to food

Although the presence of aflatoxin in primary human food products is the main concern, the carry-over of aflatoxin or its metabolites from animal feed to animal-derived human food products also raises concerns. In this regard, the presence in milk or its products of the hydroxylated AFB_1 metabolite, AFM_1, is the major concern. For this reason, the fifty-sixth JECFA meeting evaluated AFM_1 as a separate toxin (Annex 1, reference *152*). Prior to this evaluation, the forty-ninth JECFA (Annex 1, reference *131*) noted that AFM_1 is found associated with the casein in milk; is not affected by pasteurization; is not destroyed by domestic cooking; and is concentrated during cheese making. These findings were confirmed at the fifty-sixth JECFA meeting, which concluded that most studies show the stability of AFM_1 during various heat treatments and the manufacture of cultured dairy products such as kefir and yoghurt. Studies on cheese indicated concentration increases by a factor of as much as 3.3 in soft cheese and 5.8 in hard cheese.

The fifty-sixth JECFA meeting summarized available studies on the carry-over of aflatoxin into milk and found the process to be highly variable in individual cows and to depend on factors such as the presence of mastitis and the extent of milk production, with high milk-producing cows showing the greatest carry-over. Whereas high producers may exhibit carry-overs of up to 6.2%, lower producers have carry-over rates that can be an order of magnitude lower. Attempts have been recently made to fit a linear relationship between milk yield and carry-over to provide guidance on maximum levels in feed in order to comply with regulated AFM_1 levels (Masoero et al., 2007).

Risk management for human exposure to AFM_1 in milk may involve either control of AFB_1 presence in feed or the monitoring of AFM_1 in milk. Given the complexity of feed composition and the heterogeneity of AFB_1 contamination, sampling of feed does not obviate the need to monitor contamination levels in milk (Trevisani et al., 2014).

At the fifty-sixth meeting, the Committee also examined carry-over of AFB_1 or its metabolites into animal-derived foods other than milk. In brief, the

ratio of feed contamination to food contamination was 75 for dairy milk, 1200 for chicken liver, 2200 for laying hens' eggs and 14 000 for beef liver (Park & Pohland, 1986). A literature search in PubMed on aflatoxin in meat or eggs or residues yielded few papers relevant to occurrence or carry-over, indicating limited interest in carry-over to human food other than in milk and dairy products. Subsequent to the fifty-sixth JECFA assessment, studies in various poultry species found quail liver with a ratio of feed contamination to food contamination of 383, which may be a particular concern (Bintvihok et al., 2002). Given these low aflatoxin carry-over rates, AFM_1 in milk remains the main concern for aflatoxin contamination in animal-derived foods.

The occurrence and exposure assessment of AFM_1 in commercial dairy products and in human breast milk are addressed in sections 7 and 8.

2.2 Toxicological studies

2.2.1 Acute toxicity

Aflatoxin is acutely toxic in all animals tested, with median lethal dose (LD_{50}) values between 0.4 and 18 mg/kg bw (Newberne & Butler, 1966; IARC, 1993, 2002; McKean et al., 2006) and a report of 150 mg/kg bw in Swiss mice (Eaton et al., 2010). Adult rabbits, trout and cats are more sensitive than adult rats, monkeys, catfish and mice (Eaton et al., 2010). Based on limited data, juvenile animals may be more susceptible than adults.

In domestic animals, aflatoxin is most toxic to poultry. Some rodent strains appear to be quite tolerant.

The acute toxicity was 7.8 mg/kg bw in macaque *M. fascicularis* (Shank et al., 1971a) and 3.7 mg/kg bw in vervet monkeys (*Cercopithecus aethiops*; van der Watt & Purchase, 1970). A preliminary LD_{50} of 2.2 mg/kg bw was estimated for the long-tailed macaque (*M. irus*; Rao & Gerhing, 1971).

2.2.2 Short-term studies of toxicity

The use of short-term aflatoxin exposures to induce liver pathology in livestock and experimental animals was an important element involved in the original separation and identification of toxic constituents from dietary constituents contaminated with fungus (Allcroft, 1969). The ability of even short-term exposures to AFB_1 in sufficient doses to induce hepatocarcinogenesis in rats and trout quickly led to a primary focus on chronic exposures in subsequent studies.

The short-term studies of aflatoxin toxicity were previously described in JECFA (Annex 1, reference *131*) and IARC (2002), and no further studies of relevance were identified for use in the current evaluation of growth and development, immunological end-points or hepatotoxicity.

2.2.3 Long-term studies of toxicity and carcinogenicity

The carcinogenicity of AFB_1 is well established in many species, including humans, through the potent initiation of liver tumorigenesis, especially in rats. The most useful AFB_1 dose–liver tumorigenesis response dataset comes from Wogan, Paglialunga & Newberne (1974). Dietary concentrations of AFB_1 from 1 to 100 parts per billion (ppb) induced liver carcinoma incidences from 0 to 100% in male F344 rats and showed evidence for metastatic lesions in the lung; however, limitations of the experimental design complicate the analysis of dose–response. These limitations included the following: (1) the actual AFB_1 doses administered were based on diet consumption, since measurements were not reported. The European Food Safety Authority (EFSA) assumed a consumption of 4% of body weight to estimate daily doses ingested (0.04–0.4 µg/kg bw per day), a default value lower than the 5% currently recommended for chronic studies (EFSA, 2007, 2012; JECFA, 2017); and (2) variable termination (e.g. 74–109 weeks for controls and 54–88 weeks for the 100 ppb group). Each group was reportedly killed when "clinical deterioration of animals was observed", at which time all rats in the group were necropsied. In addition, the authors reported variable "time of appearance of earliest tumour" within each group (e.g. 54 weeks for the 100 ppb group). This deviation from the current rodent lifetime study duration of 104 weeks (2 years) requires a time-dependent dose adjustment based on the total estimated exposure time for the degree of tumorigenesis. Furthermore, not reporting the specific times for early removal of individual moribund animals introduces significant uncertainty into the exposure window. EFSA (2007) adjusted the AFB_1 dose to reflect the total dose consumed if the study had continued for 2 years (i.e. if a dose of 1 µg/kg bw was administered for 1 year, a dose of 0.5 µg/kg bw for a 2-year duration would have produced the same tumour incidence). However, Peto et al. (1984) recommended a dose-correction factor reflecting the squared dependence of dose on time (e.g. a 1-year exposure would be corrected to a 2-year exposure by multiplying the dose by the factor $(½)^2$ or 0.25). The complete time-to-tumour information from the Wogan, Paglialunga & Newberne (1974) study was not reported for individual rats so the reported earliest time of tumour appearance was used in each group to estimate duration-independent doses. EFSA (2007) used benchmark dose analysis to determine the lowest benchmark dose for a 10% response (BMD_{10}) of 0.41 and a lower 95% confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) of 0.17 µg/kg bw per day (confidence limit for benchmark concentration 3.4 ppb in diet) (Table 2).

The rainbow trout is often referred to as the species most sensitive to the hepatocarcinogenic effects of aflatoxin (Eaton et al., 2010), with a 4-week exposure to a dietary concentration of 80 ppb in feed producing a 68% incidence of hepatic tumours after 1 year (Dashwood et al., 1989) versus a historical control rate of about 0.1% (Williams, 2012). Large-scale studies designed to test the ED_{001}

Table 2
Dose–response for AFB_1-induced hepatocarcinogenesis in male F344 rats

Dietary AFB_1				
(ppb)	(µg/kg bw per day)[a]	Time of earliest tumour in weeks	Time-adjusted dose in µg/kg bw per day[b]	Liver carcinoma incidence[c]
0	0	104	0	0/18 (0)
1	0.05	104	0.05	2/22 (9.1%)
5	0.25	93	0.20	1/22 (4.5%)
15	0.75	96	0.64	4/21 (19%)
50	2.5	82	1.55	20/25 (80%)
100	5	54	1.35	28/28 (100%)

AFB_1: aflatoxin B_1; bw: body weight; ppb: parts per billion
[a] Dose estimated using an average feed consumption of 5% of body weight in long-term studies.
[b] Estimated dose using a squared time-dependence of dose-tumour response based on the respective time to earliest tumour (Peto et al., 1984).
[c] Results expressed as number of animals with liver carcinoma / number of animals tested and, in parentheses, as a percentage.
Source: Wogan, Paglialunga & Newberne (1974)

response (effective dose for 0.1% increase in tumour incidence) to AFB_1 were conducted using dietary concentrations of 0.05–110 ppb over a 4-week exposure period with 1-year termination. As reported in 2011, these tumour data have not yet been published; however, analysis of plotted data for a partial dataset produced an estimated 10% increased incidence in liver tumours from a dose of approximately 4 ppb AFB_1 in the diet. Nonetheless, the preliminary data presented to date show no indication of deviation from a log-linear dose–tumorigenesis response curve in trout. This low-dose log-linear relationship also exists between AFB_1 dose and formation of DNA adducts in trout (Bailey et al., 1998) and rat liver (Choy, 1993; Cupid et al., 2004; Pottenger et al., 2014). This low-dose log-linearity of tumour responses is presumably a consequence of the similar lack of constitutive hepatic GST activity towards AFB_1 epoxide in these species (Monroe & Eaton, 1987; Valsta, Hendricks & Bailey, 1988). Continuous dietary exposure studies were conducted previously in the trout model, with 20-month exposure to 0.8 and 4 ppb AFB_1 producing 10% and 14% increases in liver tumour incidence, respectively (Sinnhuber et al., 1968). It is noteworthy that 20-month exposure of trout to 0.8 ppb AFB_1 produces similarly increased incidences of liver tumours as the 24-month exposure of male F344 rat to 1 ppb AFB_1 (see Table 2). This conclusion is supported by the observation of similar dependence of tumour incidences versus steady state levels of AFB_1–DNA adducts in both rats and trout (Bechtel, 1989).

2.2.4 Genotoxicity

The critical elements in genotoxicity of AFB_1 have been extensively reviewed, including the metabolic activation to a reactive epoxide; covalent binding to

guanine bases in DNA; induction of mutagenesis in bacterial and mammalian cells; chromosomal aberrations, sister chromatid exchange and micronucleus formation in mammalian systems; and carcinogenesis in many species (Pottenger et al., 2014). AFB_1 is mutagenic (Chen et al., 2010; Woo et al., 2011) and carcinogenic (Vesselinovitch et al., 1972) in neonatal mice (postnatal days 4–10) at doses (3 × 2 mg/kg bw) that are ineffective in adult mice (postnatal days 120–126), for either mutagenesis or tumorigenesis (Vesselinovitch et al., 1972; Chen et al., 2010). The enhanced susceptibility of neonatal mice was attributable to higher levels of liver DNA adduct formation that resulted from lower levels of hepatic GST and a higher frequency of hepatocyte proliferation that could serve to more efficiently "fix" AFB_1–N^7–guanine (gua) adducts into GC → TA transversions in the liver (Shupe & Shell, 2004; Chen et al., 2010; Woo et al., 2012) that lead to hepatocarcinogenesis later in life (Vesselinovitch et al., 1972).

Animal model studies have shown that AFB_1 can induce time- and dose-dependent mutations in the *HPRT* locus of circulating T-lymphocytes in rats (Casciano et al., 1996; Morris et al., 1999). Subsequently, the frequency of *HPRT* mutations was measured in circulating T-lymphocytes from 42 men and 48 women in Qidong, China, based on the high dietary exposure to AFB_1 in this region (Wang SS et al., 1999), indicating that AFB_1 could serve as a more accessible marker for similar genotoxic events in target tissues for use in human trials. High- and low-exposure groups were delineated by the mean serum AFB_1–alb adduct level (above or below 0.66 pmol/mg albumin), and a significant association was observed between *HPRT* mutant frequencies in the two aflatoxin exposure groups (odds ratio [OR] = 19.3). There was no influence of HBsAg status on the relationship between *HPRT* mutation frequency and AFB_1–alb adduct levels.

2.2.5 Reproductive and developmental toxicity

Some evidence for AFB_1-induced changes in fetal development of neurobehavioural and reproductive functions has come from studies in rat models evaluating dose ranges relevant to high-exposure areas in developing countries. AFB_1 dosing (~7–67 µg/kg bw per day through the diet) during gestation in rats led to significant changes in maternal liver weight and either negligible or small and transient effects on histochemical markers for hippocampal neurogenesis (Tanaka et al., 2015). Intramuscular injections of AFB_1 in rat dams on gestation days 12–19 (10–100 µg/kg bw per day) produced many significant changes in behavioural and fertility parameters in male (Supriya & Reddy, 2015) and female (Supriya et al., 2016) pups (sperm production and reproductive performance in males or irregular estrus cycling and suppressed fertility in females); however, these results are difficult to interpret when the statistical limitations (litter was

not used as the statistical unit) and evidence for maternal toxicity (reductions in live pups, birth weights) are considered.

No multigeneration or reproductive toxicology studies were available for this review.

2.2.6 Special studies

(a) Immunotoxicology

Aflatoxin is immunotoxic in domestic and laboratory animals with oral and inhalation exposures (IARC, 1993, 2002; Annex 1, reference *131*). Cell-mediated immunity (lymphocytes, phagocytes, mast cells and basophils) is more affected than humeral immunity (antibodies and complement; Bondy & Pestka, 2000). Cytokine upregulation occurs in pigs exposed to AFB_1 at approximately 1 mg/kg (Meissonnier et al., 2008). Interleukin-1 (IL-1) levels increased in Fischer 344 rats given AFB_1 intraperitoneally at 1 mg/kg bw (Cukrová, Kurita & Akao, 1992). Repeated exposure of male Fischer rats to AFB_1 at 5–75 µg/kg bw by gavage for 1 week showed dose-dependent decreases in the percentage of splenic $CD8^+$ T cells and $CD3^-$ $CD8a^+$ natural killer (NK) cells. Inhibition of IL-4 and interferon-gamma (IFN-γ) by $CD4^+$ T cells, of IL-4 and IFN-γ expression by $CD8a^+$ cells, and of tumour necrosis factor alpha (TNF-α) expression by NK cells was also reported. However, 5 weeks of repeated dosing with AFB_1 produced increases in many of the same parameters. The differential effects of short-term exposure to AFB_1 on measures of cell-based immunity versus longer-term responses related to possible inflammatory responses were interpreted as a reflection of multiple sites for dysregulation of cytokine expression (Qian et al., 2014).

2.3 Observations in domestic animals/veterinary toxicology

AFB_1 causes a variety of adverse effects in different animal species, especially chickens. The effects of aflatoxin on food-producing animals were reviewed by Eaton et al. (2010) and an IARC working group (Pitt et al., 2012).

2.4 Observations in humans

2.4.1 Biomarkers of exposure

Several linear biomarkers of aflatoxin exposure are used to identify human dietary exposure and to detect associations with disease. Because of the large population studies in Africa and China over the past three decades, there is a good understanding of the probative value of the aflatoxin biomarkers in regular use (IARC, 1992, 2002, 2010; Turner et al., 2012; Annex 1, reference *131*).

The *exo*-epoxide of aflatoxin forms a stable covalent aflatoxin–nucleic acid adduct with the N^7 moiety of guanine (IARC, 1993; Groopman & Kensler,

1999). Depurination releases 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy) AFB_1 (AFB_1–N^7-gua). This DNA adduct has been observed along with AFM_1, the oxidative metabolite, in the urine of aflatoxin-dosed animals and people with dietary exposure to aflatoxin. In two separate studies in chronically exposed people, the urinary concentrations of AFB_1–N^7-gua and of AFM_1 were highly correlated with exposure. AFM_1 and urinary AFB_1–N^7-gua excretion kinetics reflect exposure within the preceding 24–48 hours (Zhu et al., 1987; Groopman et al., 1992a,b, 1993; Egner et al., 2006).

Hydrolysis of both *exo*- and *endo*-epoxides to AFB_1-8,9-dihydrodiol is the precursor to formation of adducts with protein amino groups, particularly lysine. Aflatoxin–albumin adducts are present in the sera of aflatoxin-dosed animals and of humans naturally exposed to aflatoxin through the diet (Sabbioni et al., 1987, 1990; Wild et al., 1992). The concentration of aflatoxin–albumin adducts in sera was strongly correlated with dietary exposure to aflatoxin and provides perhaps the most commonly used validated exposure biomarker (Groopman & Kensler, 1999; Turner et al., 2012). The strongest data come from liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods (McCoy et al., 2005). Aflatoxin–albumin adducts in sera have been demonstrated to be stable in frozen blood samples (Scholl et al., 2008). There remains a continuing need to validate new laboratory methods for aflatoxin–lysine adduct for analytical quality. There is also a need for high purity, commercially available, aflatoxin–lysine/aflatoxin–albumin standards for use with high-performance liquid chromatography (HPLC), LC-MS/MS and other quantitative methods.

While AFB_1 itself can occur in the urine of exposed humans, there is no significant correlation with dietary exposure (Turner et al., 2012). Although AFP_1 occurs in urine, there was no correlation between dietary exposure and this metabolite (Groopman et al., 1992b). However, in the same population, AFP_1 was the most highly correlated of all urinary AFB_1 metabolites in humans with liver cancer (Ross et al., 1992).

As noted, AFM_1 and urinary AFB_1–N^7-gua excretion demonstrate exposure within the preceding 24–48 hours. Thus, although they have frequently been utilized as biomarkers for associated risk of hepatocellular carcinoma risk, these associations are weak when considering the development of a chronic disease with such short-term biomarkers. The standard for observations of chronic exposure is the serum AFB_1–alb adduct biomarker. This biomarker was highly correlated with both AFM_1 excretion and AFB_1 intake in a human population from Guangxi Province, China (Gan et al., 1988). AFB_1–alb has a longer half-life within the body, which closely mimics that of albumin (20–30 days). This biomarker has been the traditional marker utilized in epidemiology studies to assess possible associations with chronic health end-points, such as hepatocellular carcinoma, growth faltering and immunomodulation.

The influence of environmental factors on the metabolism and excretion of certain aflatoxin biomarkers in different human populations has been investigated. A community-based study in Guangxi and Sichuan provinces of China showed statistically significant associations in AFB_1–alb levels and sex and geographical location following multivariate analysis of risk (Tao et al., 2005). In rat models, males are more susceptible to aflatoxin toxicity (Wogan, Paglialunga & Newberne, 1974), and rates of hepatocellular carcinoma in regions of the world with high aflatoxin exposure are typically higher in men (Kirk, Bah & Montesano, 2006). Therefore, higher levels of the toxic AFB_1–alb metabolite in men could be expected.

Although similar associations with sex and AFB_1–alb levels have been observed in human populations, not all epidemiology studies confirm this phenomenon (Wild et al., 2000). In addition, some human studies have shown differences in AFB_1–alb levels in the presence of chronic HBV infection (Sun et al., 2002; Wu et al., 2007a). HBV and other viral infections of the liver, such as hepatitis C virus (HCV), can cause chronic toxicity to hepatocytes and inflammation of the liver; therefore, these stressors on the hepatocytes could alter the efficiency of aflatoxin metabolism within the liver and introduce regenerative hyperplasia (Wild & Montesano, 2009). Such alterations in the metabolic capacity of aflatoxin and production of albumin could alter the relevant interpretation of AFB_1–alb as a biomarker. The association of higher AFB_1–alb levels in people with HBsAg+ has not been verified in populations from the Gambia (Wild et al., 2000) and China (Tao et al., 2005).

Developments in technology have advanced the detection of aflatoxin biomarkers to include the AFB_1–lys adduct by LC-MS/MS, as opposed to by the traditional enzyme-linked immunosorbent assay (ELISA) methodology for AFB_1–alb. The data from these two methods have strong correlations with each other; however, the ELISA method typically measures the level of AFB_1–alb slightly higher (a factor of 2.6) than the LC-MS/MS method (Scholl et al., 2006; McCoy et al., 2008). The LC-MS/MS method specifically measures the AFB_1–lysine pronase digestion product from AFB_1–alb adducts, whereas the ELISA method is less specific and can have cross-reactivity with other aflatoxin–albumin products. Similarly, higher values for binding of ^{14}C-labelled AFB_1 to serum albumin on a dose-adjusted basis (38 ± 20 pg AFB_1 bound per µg of AFB_1 ingested per kg bw) were observed when compared with AFB_1–alb adducts measured by ELISA in high AFB_1-consuming populations in the Gambia using the highest daily estimated intake (1.5 pg AFB_1 bound per µg of AFB_1 ingested per kg bw; Wild et al., 1996). These differences in the measurement of biomarkers are important when comparing estimated exposures among different populations worldwide.

At the forty-ninth meeting, JECFA indicated that a major issue in the utilization of AFB_1–alb adduct as a biomarker of effect was a lack of direct evidence for its link to DNA adducts within the liver tissue of humans (Annex 1, reference *131*). Since then, Zhang et al. (2006) demonstrated a significant association between levels of AFB_1–DNA adducts in hepatocellular carcinoma tumour tissue from liver dissections from patients in China and serum AFB_1–alb.

2.4.2 Biomarkers of effects

AFB_1 is metabolized by CYPs to the highly reactive AFB_1-*exo*-epoxide that can react at the N^7-position of guanine in DNA, causing its genotoxic effects. Binding of the AFB_1-*exo*-epoxide to DNA is the primary mechanism of aflatoxin hepatocarcinogenicity. Biomarkers of this effect include AFB_1–DNA adducts at guanine residues found in liver tissue, which principally result in GC → TA transversion mutations (Smela et al., 2001). A unique feature of aflatoxin-induced hepatocarcinogenesis in people living in areas where exposure to both aflatoxin and HBV is endemic is formation in tumour tissue of the hallmark mutation in p53 at codon 249 (Hussain et al., 2007). Hsieh et al. (1988) developed an immunological detection method for AFB_1–DNA in vivo that has been used to further study the association of AFB_1 exposure and development of hepatocellular carcinoma. However, the correlation between the aflatoxin exposure biomarker AFB_1–alb and liver DNA adducts was not documented in humans until 2006 (Zhang et al., 2006). The immunological detection of AFB_1–DNA adducts in liver tissue was initially associated with hepatocellular carcinoma risk with an OR of 3.9 in one cohort (Lunn et al., 1997). However, Zhang et al. (2006) were the first to verify the linearity of the AFB–DNA biomarker of effect with the predominantly used AFB_1–alb biomarker. AFB_1–alb levels in the sera of patients with dissected liver tissues were 51.0 ± 36.5, 70.5 ± 648.1 and 84.9 ± 848.2 fmol/mg for the low, moderate and high groups of AFB_1–DNA adducts in tumour tissues, demonstrating a positive, dose-dependent linear association. This shows that the less invasive analysis of serum AFB_1–alb biomarkers can be used as a surrogate biomarker of effect in hepatocellular carcinoma models. This association is important because it is often difficult to obtain the liver tissue samples necessary to identify the AFB–DNA biomarkers of effect.

The AFB_1–DNA adducts often result in a GC → TA transversion at codon 249 of the human p53 tumour suppressor gene. This transversion results in a switch from an arginine to serine residue. A p53 mutation is found in up to 60% of hepatocellular carcinoma cases worldwide (IARC, 2014). Quantitative analysis of plasma TP53 249^{ser} mutated DNA showed a highly significant association with hepatocellular carcinoma when compared with cirrhotic patients and non-liver disease patients (Lleonart et al., 2005), suggesting its capacity as a hepatocellular

carcinoma biomarker for diagnosis. Animal models have not provided evidence for the generality of this mechanism (Smela et al., 2001), particularly the observation that hepatocellular tumours from AFB_1-treated rhesus and cynomolgous monkeys contain low gene mutation frequencies in p53 (Fujimoto et al., 1992).

Based on a weight of evidence approach using molecular epidemiological and in vitro mutagenesis data, the AFB_1-induced mutation of the *TP53* tumour suppressor gene in human hepatocytes, by a single base substitution at codon 249 (AGG to AGT), has been causally associated with human hepatocellular carcinoma (Hussain et al., 2007). While this mutation is present in up to 75% of hepatocellular carcinoma cases in regions of developing countries where aflatoxin exposure is high, it is rare in developed countries where aflatoxin exposure is low and intermediate in countries with moderate aflatoxin exposures (Gouas, Shi & Hainaut, 2009). The mutation is also rarely found in other tumour types. Detection of the mutated p53 protein at higher frequencies in serum from hepatocellular carcinoma patients versus healthy study participants in an area of high AFB_1 exposure (Gambia) suggests that it can be considered a biomarker of effect (Kirk et al., 2005a). Moreover, the association between *TP53* codon 249 mutation frequencies in non-malignant liver tissue from people in geographical regions with high, moderate and low exposure to AFB_1 suggests that this may be an early event in the carcinogenic process (Aguilar et al., 1994). In addition to the role of site-specific mutagenesis of *TP53* by AFB_1 epoxide, there is also some evidence that the predominant mutated p53 protein may also confer growth-selective properties that facilitate the transformation and/or progression steps in hepatocellular carcinoma (Gouas, Shi & Hainaut, 2009).

The mutation at codon 249 has long been assumed to be a fingerprint of aflatoxin-induced hepatocellular carcinoma, based on the high correlation observed in two independent studies, in southern Africa and Qidong, China (Bressac et al., 1991; Hsu et al., 1991; Kew, 2003). The case–control study conducted in the Province of Taiwan, China, showed that an overwhelming majority of the hepatocellular carcinoma tumour tissue had GC → TA transversion mutations at codon 249. However, this mutation showed no significant relationship with AFB_1–DNA adducts. In fact, the OR was 10.0 for codon 249 mutations in positive versus negative HBsAg cases (Lunn et al., 1997). In contrast, the Zhang et al. (2006) study showed a positive association between AFB_1–alb levels and p53 mutations, although these mutations occurred at various codons within the gene and were not specific to codon 249. A similar lack of correlation between 249^{ser} mutations at p53 and AFB_1–alb adducts occurred in samples taken from a liver cancer screening programme in Qidong, China, from 1993 to 1998; however, the sample size for testing for this mutation was only 14 (Szymañska et al., 2009). A study conducted in young Guinean children, in whom hepatocellular carcinoma

had not developed, showed neither sense nor antisense fragments for the p53 249^{ser} mutation in any samples, although 96% of the children had detectable AFB_1–alb in their serum (Turner et al., 2005). These authors concluded that the presence of chronic HBV infection might be required to observe this mutation released into the plasma or that the mutation may only occur in developed hepatocellular carcinoma. In either case, although the p53 249^{ser} mutation appears in high frequency in liver tumours of geographical locations with known aflatoxin exposure, its use as an early biomarker for aflatoxin-induced hepatocellular carcinoma risk has yet to be elucidated.

Meta-analysis of epidemiological studies that collected data on p53 mutations in hepatocellular carcinoma cases was conducted by Stern et al. (2001) to determine the association of aflatoxin exposure with the mutation of p53 249^{ser} specific to hepatocellular carcinoma and any role that HBV infection played in these mutation events. This meta-analysis included 49 publications of studies in populations subsequently classified as residing in areas with high, moderate or low exposure to aflatoxin. Mean proportion of tumours with the 249^{ser} mutation was significantly larger in areas with higher aflatoxin exposure, and the mean proportion of tumours with a 249^{ser} mutation among tumours with any p53 mutation was associated with aflatoxin level. The authors concluded that this meta-analysis provided evidence of a dose–response relationship between AFB_1 exposure and prevalence of the p53 249^{ser} mutation in hepatocellular carcinoma. However, the study did not take into account individual dietary aflatoxin exposures but estimated these as an ecological measure; this may have introduced classification bias, as aflatoxin exposure and biomarker levels often differ significantly among individuals in the same geographical location. It is also important to note that the association observed in this meta-analysis does not infer causality. For instance, areas with high aflatoxin exposure were significantly associated with another hepatocellular carcinoma risk factor, HBV infection. Whether AFB_1 causes these mutations or whether AFB_1 leads to differential promotion of cells that acquire the mutation remains unclear in human populations.

(a) Effects of pharmacological intervention on biomarkers of aflatoxin exposure

Several strategies for reducing AFB_1-associated risks of human hepatocellular carcinoma have been evaluated in randomized clinical intervention studies conducted in at-risk groups in developing countries (summarized in Khlangwiset & Wu, 2010; Kensler et al., 2011; Wild, Miller & Groopman, 2015). Wild, Miller & Groopman (2015) also addressed the non-cancer health impacts, primarily in children. The hypotheses tested include (1) trapping actions of clay and chlorophyllin via complex formation with AFB_1; (2) either decreased metabolic

formation or enhanced metabolic deactivation of reactive AFB_1 metabolites by either oltipraz, broccoli sprouts containing glucoraphanin or green tea phenolics; and (3) pre- or postharvest interventions to reduce aflatoxin exposure. All intervention strategies were based on successful results in experimental animal models, typically the rat. Exposure biomarkers were monitored to determine the effectiveness of the strategies, including urinary levels of AFB_1 mercapturate, AFB_1–N^7-gua DNA adducts and AFM_1 and serum levels of AFB_1–alb protein adducts.

NovaSil clay, a common anti-caking agent, has been used to reduce the bioavailability of dietary aflatoxin in animals and humans. The efficacy of NovaSil in reducing AFB_1 bioavailability was evaluated during a 4-month intervention trial. NovaSil or placebo at 1.5 or 3 g/day was administered daily in capsules to groups each comprising 60 Ghanaian individuals (Wang et al., 2008). The serum levels of AFB_1–alb adducts were significantly decreased in both dose groups, relative to placebo, and a significant decrease in urinary AFM_1 levels was observed in the high-dose group versus the placebo group after 3 months.

Similarly, an intervention with chlorophyllin, a water-soluble sodium copper complex of chlorophyll that forms a molecular complex with AFB_1, was conducted in China (Egner et al., 2001). Groups of 90 individuals each consumed either 100 mg of chlorophyllin or a placebo 3 times daily with meals for 4 months. Levels of AFB_1–N^7-gua adducts were measured in urine after 3 months. The chlorophyllin group showed a significant 55% decrease in adduct levels relative to the placebo group.

In a complementary pharmacokinetic study conducted using ^{14}C-labelled AFB_1 (30 ng/person) and accelerator mass spectrometry in four human volunteers, administration of 150 mg of either chlorophyllin or chlorophyll significantly reduced the absorption rate and maximum concentration (C_{max}) values for AFB_1-associated radioactivity (Jubert et al., 2009). These intervention studies demonstrated the plausibility of using dietary agents that bind to AFB_1 and limit its bioavailability, thus reducing hepatocellular carcinoma risks in heavily exposed populations.

A cancer chemoprevention strategy based on modulation of enzymes involved in either phase I activation of AFB_1 or phase II conjugation and deactivation of reactive AFB_1 epoxide was tested in Qidong, China. Oltipraz was administered orally to groups of 80 study participants at either 125 mg daily or 500 mg weekly versus a placebo arm. Urinary AFM_1 and AFB_1 mercapturate levels were measured after 1 month of exposure (Wang JS et al., 1999). The weekly dosing regimen produced a significant decrease in AFM_1 levels in urine (51%) but no change in urinary AFB_1 mercapturate; however, the daily dosing regimen had no effect on AFM_1 excretion but significantly increased AFB_1 mercapturate excretion (2.6-fold), when each was compared with the placebo group.

Similarly, sulforaphane from broccoli sprouts has been evaluated for its chemopreventive effects in Qidong, China (Kensler et al., 2005), based on increased expression of multiple detoxification enzymes through activation of the oxidant responsive Nrf2-KEAP1 pathway (Dinkova-Kostova et al., 2005). Glucoraphanin, in a broccoli sprout infusion, was administered orally daily for 14 consecutive days to 100 study participants along with an identically sized placebo group receiving an inactive broccoli extract. Urinary levels of AFB_1–N^7-gua adducts were reduced, albeit insignificantly, in the treatment group versus the placebo; however, when urinary levels of broccoli-derived isothiocyanate metabolites were included to quantify interindividual variability in bioavailability, a significant association was observed. Finally, encapsulated green tea phenols, equivalent to 1–2 L of tea per day, were given to study participants (40 per group, including 500 and 1000 mg green tea phenols with a placebo) in Guangxi, China, for 3 months. Biomarkers of AFB_1 exposure, urinary levels of AFM_1 and AFB_1 mercapturate and serum levels of AFB_1–alb adducts were measured after 1 and 3 months (Tang et al., 2008). Significant time- and/or dose- × time-related decreases in serum albumin–adduct levels, reductions in AFM_1 levels and increases in AFB_1 mercapturate levels were observed at both times and in both dose groups compared with placebo.

Collectively, these randomized clinical studies demonstrated that biomarkers reflected diminished bioactivation of AFB_1 and enhanced metabolic deactivation of the reactive epoxide metabolite, and indicated that population-level reduction of hepatocellular carcinoma risks from dietary AFB_1 in target populations is plausible using several practical and well-tolerated pharmacological interventions. Kensler et al. (2011) estimated that such chemopreventive strategies could reduce hepatocellular carcinoma incidences attributable to aflatoxins in affected areas like Qidong, China, by 10%. In comparison, an estimated 50% reduction would be expected with implementation of universal vaccination against HBV. Although effective, these interventions do not address the non-cancer health effects, notably in children.

The intervention trials discussed above and all available biomarker-based intervention studies were reviewed by an IARC working group (Wild, Miller & Groopman, 2015). The credibility of the evidence as well as its completeness and transferability at an individual, community or national level were evaluated. Evidence at the community level of reductions in exposure based on biomonitoring was given most weight. Interventions ready for implementation were considered to have reached a mature stage of development, result in significant intervention effects and address the needs of important stakeholders (Rychetnik et al., 2002). All of the available biomarker-based interventions were ranked as Evidence for Public Health Intervention Category 2, that is, needing further field evaluation (Table 3).

Table 3
Excerpt from IARC Working Group evaluations of interventions for reducing aflatoxin exposure biomarkers

Intervention	Category of evidence[a]
Primary prevention	
Dioctahedral smectite clay	2
Chlorophyllin	2
Broccoli sprout extract	2
Dithiolethiones	2
Green tea polyphenols	2

[a] Categories of evidence for public health interventions: (1) sufficient evidence for implementation, (2) needs more field evaluation, (3) needs formative research, and (4) no evidence or ineffective.
Source: Wild, Miller & Groopman (2015)

2.4.3 Clinical observations

(a) Short-term effects

Many epidemiology studies of aflatoxin exposure with various human health end-points have been conducted using multiple biomarkers and through assessment of aflatoxin contamination of foodstuffs since the previous JECFA report. As soon as analytical methods were available, aflatoxin was detected in groundnut samples from the Gambia, Ghana, India, Nigeria, Tanganyika (now part of the United Republic of Tanzania), Uganda and French West Africa (now Benin, Burkina Faso, Côte d'Ivoire, Mali, part of Guinea, Mauritania, Niger and Senegal; Sargeant et al., 1961). In 1963 in South Africa, 75 out of 501 tested samples contained aflatoxin at 2 ppm or greater. In Senegal, 500 samples examined over 2 years contained 0.1–20 ppm (Barnes, 1970). These data allowed early studies on non-cancer health outcomes. For example, Senegalese children aged less than 1 year each received 70–140 g of groundnut meal per day for 10 months as a treatment for protein energy malnourishment, kwashiorkor (Payet et al., 1966). The meal samples were later found to be contaminated with aflatoxin at 500–1000 µg/kg, providing an aflatoxin intake of 35–140 µg per day. Children in Uganda were shown to be similarly exposed (Lopez & Crawford, 1967). Children in India fed groundnut protein supplements containing aflatoxin were found to have serious liver damage (Amla et al., 1971). This was subsequently recognized as a widespread problem in India, notably during drought years (Krishnamachari et al., 1975).

By the late 1960s, the first of the child deaths resulting from high exposure to aflatoxin was reported in the literature. A case from Uganda was reported of a 15-year-old boy who died from acute liver failure in 1967. He had been consuming cassava-based food containing 1.7 ppm aflatoxin. The author

suggested that similar cases at the time were likely under-diagnosed (Serck-Hanssen, 1970).

In 1969 in Thailand, 81 of the 139 people who ate aflatoxin-contaminated noodles died. The incident selectively affected young children and began with sudden onset of coma, fever, respiratory distress and convulsions leading to death 48–72 hours after onset (Bourgeois et al., 1969). A case series described 40 children, between 1 and 12 years old (average age 6 years), whose deaths were attributed to aflatoxin. Encephalopathy and fatty degeneration of the viscera were confirmed at autopsy. Samples of leftover food eaten by two of the children prior to the onset of encephalopathy and fatty degeneration were found to be heavily contaminated with aflatoxin. Chemical assays were performed on brain, liver, kidney, stomach and intestinal content and stool specimens from 22 of the children. The highest levels detected were 93 μg/g AFB_1 per kg in a liver specimen, 123 μg/g AFB_1 per kg stool, 127 μg/g AFB_1 per kg in stomach and intestinal contents, and 8 μg/mL in bile (Shank et al., 1971b). The symptoms reported in the children were similar to those observed in the macaque that died after administration of a lethal or even a sublethal dose of aflatoxin, where AFB_1 itself (as opposed to only its metabolites) was detected in monkey tissues up to 148 hours after detection of the metabolites (Shank et al., 1971a).

A less well-documented case was reported in Malaysia in 1988. Sixteen Chinese children (average age 7 years) and one adult, of whom 13 died of liver failure, were affected (Chao, Maxwell & Wong, 1991; Lye et al., 1995). Aflatoxins were reported in various tissue samples "at lethal levels" (Chao, Maxwell & Wong, 1970). Some food samples were believed to contain boric acid used during food processing, complicating the interpretation of the aflatoxin data (Cheng, 1992).

A cluster of deaths from liver failure was reported in Kenya in 1981. The deaths tended to occur in families. Samples of maize being consumed were found to contain AFB_1 at 3–12 ppm. Liver tissue at necropsy contained up to 89 ppb. The authors believed that most of the cases were caused by acute aflatoxicosis (Ngindu et al., 1981).

In January to June 2004, an aflatoxicosis outbreak in eastern Kenya resulted in 317 cases and 125 deaths (CDC, 2004). Azziz-Baumgartner et al. (2005) compared 40 cases with 80 age-matched controls. Aflatoxin concentrations in maize in the case homes averaged 354.5 ppb versus 44.1 ppb in the control homes. Median AFB_1–lys adduct concentrations at or above the median 0.25 ng/mg albumin led to aflatoxicosis. Serum aflatoxin adduct concentrations in the case children averaged from 1.2 ng/mg of albumin (ranging up to 19 ng/mg albumin) versus 0.15 ng/mg in the controls (Azziz-Baumgartner et al., 2005). This would correspond to a child consuming approximately 100 μg aflatoxin per day. Of all the samples, 35% tested greater than 100 ppb and 7% greater than 1000

ppb. In addition, the areas with the highest maize contamination corresponded to the areas with the highest rate of hepatotoxicity (Lewis et al., 2005).

A number of other cases of child deaths are reviewed in IARC (1993).

Wild & Gong (2010) reviewed data from three incidents where people died of acute toxicity (Table 4). Based on their estimates, doses of AFB_1 of 20–100 µg/kg bw per day were fatal. An estimate of how this would relate to concentration of AFB–lys in pg per mg serum albumin can be made based on data from the single human volunteer study. Cupid et al. (2004) used an accelerator mass spectrometer to analyse human tissues following exposure to ^{14}C-labelled AFB_1 in consenting patients undergoing surgery. The albumin–adduct level was 0.58 pg/mg albumin per 1 µg AFB_1 dose. Groopman et al. (2014) compiled a large dataset of serum adduct data including from the 2004 incident in Kenya in 2004. From this analysis, fatal doses would correspond to aflatoxin–lysine adduct concentrations of 100–1000 pg/mg serum albumin and estimated AFB_1 consumption of 20–120 µg/kg bw per day.

Many epidemiology studies of aflatoxin exposure with various human health end-points have been conducted since the previous JECFA report through the use of multiple biomarkers and through assessment of aflatoxin contamination of foodstuffs. However, it must be noted that determination of aflatoxin concentration in foods is highly variable due to the heterogeneous nature of contamination, making the use of biomarkers preferable in epidemiology studies.

(b) Effects on child growth and development

Animals chronically exposed to aflatoxins in the diet have lower feed conversion efficiency, lower weight gain and slower development (studies summarized in Khlangwiset, Shephard & Wu, 2011). Since the forty-ninth JECFA meeting (Annex 1, reference *131*), epidemiological studies have found an association between aflatoxin exposure and growth faltering in children, mostly from sub-Saharan Africa. Table 5 shows a summary of the studies.

Prenatal exposure

A prospective longitudinal study conducted in June 2000 examined the role of maternal aflatoxin exposure on child growth (Turner et al., 2007). Following Gambian mothers and children from 4.1 months prior to birth to 52 weeks post birth, the study collected maternal blood (2 collections: on average 4.5 and 0.9 months prior to birth), cord blood and infants' blood at week 16 for AFB_1–alb analysis. Anthropometrics were collected periodically from the children over the 52 weeks of the study. AFB_1–alb was found in 100% of the maternal blood samples, 48.5% of the cord blood samples and 11% of the infants' blood samples. AFB_1–alb levels in the mothers above the median had shorter children (n = 138).

Table 4
Estimates of aflatoxin doses resulting in human fatalities

Exposure location / details	Source of exposure	Estimated lethal dose range of AFB_1 (µg/kg bw per day)
Western India (1974), >180 villages 397 patients, 106 deaths (27% fatality)	Maize containing aflatoxin at 6.25–15.6 mg/kg diet	36.5–91
Machakos County, Kenya (1981) 20 cases, 12 deaths (60% fatality)	Maize from homes with fatalities contained AFB_1 at 3.2–12 mg/kg diet	18.7–70
Eastern Kenya (2004) 317 cases, 125 deaths (39% fatality)	Stored maize contaminated with aflatoxin at 0.35 mg/kg (GM); 5–20 mg/kg diet was associated with fatal cases	29.2–116.7

AFB_1: aflatoxin B_1; bw: body weight; GM: geometric mean
Source: Wild & Gong (2010)

Table 5
Aflatoxin-associated child growth outcome studies

Country	Study sample size (*n*) and age range	Study design	Geometric mean AFB_1–alb concentration (95% CI) in pg/mg albumin	Covariates	Findings and inferences	Reference
Postnatal exposures						
Benin and Togo	479; 9 months to 5 years	Cross-sectional	32.8 (5–1064)	Age, sex, SES, agro-ecological zone, weaning status	Dose–response relationship with HAZ and WAZ; overall adjusted negative correlation with HAZ ($P = 0.001$) and WAZ and WHZ ($P = 0.047$)	Gong et al. (2002)
Benin[a]	181; 16–37 months	Prospective	11.8 (9.2–15.2), 31.1 (25.4–38.0), 45.9 (35.7–59.0), 119.3 (96.2–148.1)	Age, sex, baseline height, weaning status, mother's SES, village	AF–alb by quartile was inversely associated with HAZ ($P < 0.000\ 1$); 8 months height increment over three time points was inversely associated with AF–alb level ($P < 0.000\ 1$); no *z*- scores reported; weight increment not associated with AF	Gong et al. (2004)
Egypt[b]	46; 1 month to 4.5 years	Cross-sectional	Child: 51.6 (30.6–62.8) Mother: 50 (35.6–84.9)	N/A	Positive correlation between mother and child AFB_1–alb ($P < 0.000\ 1$); negative correlation between child AFB_1–alb and HAZ ($P = 0.001$), but not WAZ	Shouman et al. (2012)
Gambia	466; 6–9 years	Cross-sectional	22.3 (5–456)	Sex, month of sample collection, birth weight	No relationship with either HAZ or WAZ scores, WHZ was weakly associated with AF–alb ($P = 0.034$); age range of children in the study may be too high to be sensitive to growth effects of aflatoxin	Turner et al. (2003)

Table 5 (continued)

Country	Study sample size (*n*) and age range	Study design	Geometric mean AFB_1–alb concentration (95% CI) in pg/mg albumin	Covariates	Findings and inferences	Reference
Kenya	180; 6–17 years	Cross-sectional	110.5 (95.4–127.9)	Sex, age, school, liver disease state, infectious status	AF–alb levels did not differ by sex or age; inverse association between AF–alb and *IGF1* ($P = 0.039$) and *IGFBP3* ($P = 0.046$); division into three groups of AF–alb; the highest group was significantly shorter than the lowest group after adjustment ($P < 0.001$)	Castelino et al. (2014)
Bhaktapur, Nepal[c]	85; 15–36 months	Prospective	15 months: 3.85 (0.53–130.08) 24 months: 3.05 (0.82–34.72) 36 months: 4.06 (1.01–149.11)	Age, WAMI index (SES), breastfeeding, mother's education, energy, adjusted iron, zinc and vitamin A intake	AF–alb not associated with growth	Mitchell et al. (2016a)
United Republic of Tanzania	166; 6–14 months	Prospective	Baseline: 4.7 (3.9–5.6) 6 months: 12.9 (9.9–16.7) 12 months: 23.5 (19.9–27.7)	Sex, age, baseline length, village, breastfeeding, mother's education, SES, protein and energy intake	AF–alb not associated with growth	Shirima et al. (2015)
Pre/postnatal exposure and postnatal growth						
Gambia	107; birth–14 months	Prospective	Mother: 40.4 (4.8–260.8) Cord blood: 10.1 (5.0–89.6) 16-week infant: 8.7 (5.0–30.2)	Sex, age, placental weight, maternal weight, gestation length, season	Pregnancy AF associated with rate of HAZ and WAZ decline ($P < 0.001$); effects on WHZ not reported	Turner et al. (2007)
	Mean bw 2.9 kg				Pregnancy AF not associated with bw or length	
Gambia	115; Mothers: 1–16 weeks after conception; Children: 2–8 months	Cross-sectional	Dry season: 34.4 (26.0–45.3) Rainy season: 35.2 (27.8–44.7)	Sex, season of conception	Methylation at 71 CpG sites was correlated with AF exposure; 52 of those in annotated genes, including those involved in immune and inflammatory responses; *FGF12* and *IGF1* CpG sites were also methylated and correlated with aflatoxin exposure in utero	Hernandez-Vargas et al. (2015)

Country	Study sample size (*n*) and age range	Study design	Geometric mean AFB_1–alb concentration (95% CI) in pg/mg albumin	Covariates	Findings and inferences	Reference
Kumasi, Ghana	785; birth	Cross-sectional	10.9 (0.44–268.73)	Sex, no. of children, mother's education, mother's income, malaria exposure, anaemia, helminths, *Strongyloides stercoralis*	Rates of all outcomes except preterm highest in Q4 of AFB_1, but only low birth weight significant, Q4 vs Q1 (adjusted OR = 2.09, 95% CI: 1.19–3.68); no significant association with small-for-gestational age or stillbirth	Shuaib et al. (2010)

AF: aflatoxin; AFB_1: aflatoxin B_1; alb: albumin; bw: body weight; CI: confidence interval; CpG: 5′-C-phosphate-G-3′; FGF12: fibroblast growth factor 12; HAZ: height-for-age *z*-score; IGF: insulin-like growth factor; IGFBP3: insulin-like growth factor–binding protein-3; no.: number; OR: odds ratio; Q: quartile: SES: socioeconomic status; TLC: thin-layer chromatography; WAZ: weight-for-age *z*-score; WHZ: weight-for-height *z*-score

[a] Data were provided by individual villages.

[b] AFB_1 levels were analysed by TLC and reported in parts per million.

[c] Data were provided as AFB_1–lys biomarker levels.

Longitudinal weight-for-age *z*-score (WAZ) and height-for-age *z*-score (HAZ) for the children were considered with a generalized estimating equation against mother, cord blood and infant blood ($n = 107$). Utilizing the interaction term age with maternal AFB_1–alb level, the authors found that higher maternal exposure to aflatoxin resulted in a drop in HAZ and WAZ profile over age. The authors concluded that a drop in maternal AFB_1–alb from 110 to 10 pg/mg albumin would result in a child height increase of 2 cm and weight increase of 800 g at 52 weeks. Cord blood aflatoxin levels were not associated with either longitudinal HAZ or WAZ. Confounding variables of sex, age, placenta weight, gestation time and season were included in this analysis. However, no data were collected or controlled for during the analysis of the nutrition status of the mothers or infants; nor was there any adjustment made for infectious or diarrhoeal diseases.

An additional cross-sectional study conducted in Kumasi, Ghana, recruited women presenting for delivery to two separate hospitals serving the area in November and December 2006. Shuaib et al. (2010) identified 785 pregnant women who had uncomplicated or singleton births and invited them to participate in the study. Each participant provided a blood sample for aflatoxin analysis and answered a questionnaire. Preterm delivery was identified as births that occurred before 37 weeks of gestation; low birth weight as less than 2500 g; and small-for-gestational age as sex-specific birth weight at or below the 10th percentile of an international reference population. Lastly, stillborn deliveries were identified. Following categorization of participants into quartiles of AFB_1–alb levels, multivariate analysis indicated that those women in the highest quartile were statistically more likely to have a baby with low birth weight. However,

the confounding variables used in the multivariate analysis were not described (Shuaib et al., 2010).

Hernandez-Vargas et al. (2015) collected blood samples from pregnant women in the West Kiang region of the Gambia at 1–16 weeks of pregnancy for biochemical analysis (n = 115). Following birth, infants (2–8 months of age) from these participating mothers also provided a blood sample for DNA extraction. The maternal blood samples were tested for AFB_1–alb levels by competitive ELISA and the infant blood was assessed for DNA methylation profiles. An association was found between 71 5′-C-phosphate-G-3′ (CpG) sites in the infant DNA and AFB_1–alb from the mothers; these were defined as aflatoxin-associated DNA methylation sites. Covariates included maternal age and maternal body mass index. Important aflatoxin-associated loci included growth factor genes and immune-related genes, such as fibroblast growth factor 12 (*FGF12*), insulin-like growth factor 1 (*IGF1*), chemokine ligand 28 (*CCL28*), toll-like receptor 2 (*TLR2*) and transforming growth factor-β 1 (*TGFB1*). While this study did not follow the children after birth for growth outcomes, it does provide evidence for a possible mode of action for aflatoxin-associated growth stunting in children. Differential methylation of CpG sites in growth factor genes could reduce expression of the IGF1 protein. Reduced expression of IGF1 protein was associated with high AFB_1–alb exposure and reduced growth in a cohort of children in Kenya (Castelino et al., 2015). However, these data show that the effect on *IGF1* expression could be an epigenetic outcome occurring in utero.

Postnatal exposure

Turner et al. (2003) conducted a cross-sectional study in the Gambia, from May 1998 to February 1999, that included children 6–9 years old (n = 472). During the study, a single blood sample was taken from each of the children and their anthropometrics were measured. Of the children surveyed, 93% had detectable serum AFB_1–alb levels. The geometric mean of AFB_1–alb levels was 22 pg/mg, with a range of 5–456 pg/mg, which correlated with a statistically significant reduction in weight-for-height z-scores (WHZ). However, no association was found between AFB_1–alb levels and the other growth indicators, WAZ and HAZ. While this study indicates a significant correlation with one of the three growth parameters, no adjustments were made for other significant etiological factors that could affect growth within this population. Growth deficits observed from age 6 to 9 years were the result of compromised growth earlier in life. Therefore, the association with AFB_1–alb levels ($t_{½}$ ~29 days) should be determined at the age when growth is most affected. Associations of aflatoxin exposure with the standard z-scores of the World Health Organization (WHO), which recommends

that its growth charts be used in children from birth to 5 years of age, would be more meaningful in younger children.

Another cross-sectional study in sub-Saharan Africa (Benin and Togo) that surveyed younger children (9 months to 5 years) had a more appropriate age range to determine any effects of aflatoxins on child growth. In that study, 475/479 children had detectable AFB_1–alb adducts in their blood, with a geometric mean of 33 pg/mg albumin (Gong et al., 2002, 2003). The most important findings from that study were the association of aflatoxin exposure with weaning status and strong inverse relationships between AFB_1–alb and all three *z*-scores (HAZ, WAZ and HWZ) following adjustment for age, sex, socioeconomic status, agro-ecological zone and weaning status. Further categorical analysis of the data by *z*-score ranges demonstrated a significant dose–response relationship between AFB_1–alb and HAZ and WAZ. Effect of nutritional status or chronic diarrhoeal disease was not included in the analysis.

A prospective study in four rural villages of Benin (selected to have different aflatoxin exposures based on diet) included children aged 16–37 months (n = 200) to determine aflatoxin exposure association with longitudinal growth. Children were followed over an 8-month period, from February 2001 to October 2001, and their AFB_1–alb levels, height and weight determined at baseline and 4 and 8 months. Blood micronutrients, vitamin A and zinc were assessed in the children as markers of nutrient status. However, these were not associated with AFB_1–alb and were thus not included as confounders. Growth velocity was calculated as the difference between two time points or over the whole 8-month period. Confounding variables, age, weaning status and the village and mother's socioeconomic status were entered into multivariate models for statistical analysis. AFB_1–alb was detected in almost all children across all the time points, indicating a chronic exposure in this population. Weaning status was associated with increased AFB_1–alb levels in this population after adjusting for age and socioeconomic status; in each village, those children who were fully weaned had higher aflatoxin levels than those who were only partially weaned. Aflatoxin exposure was significantly associated with consumption of groundnuts. However, this association was lost following adjustment for weaning status. After adjustment for confounding variables, there was a significant correlation between HAZ and AFB_1–alb levels at baseline and the mean over the three time points, but no significant correlation between aflatoxin and WAZ or WHZ (Gong et al., 2004). While this prospective study showed promising results of an association between aflatoxin exposure and impaired growth, and included the measure of two important micronutrients, the authors concluded that to fully distinguish the effects of aflatoxin from confounders in the diet would require a randomized intervention study that assessed the effects of reduced aflatoxin exposure on growth outcomes.

Mothers and their children admitted to Mansoura University Children's Hospital in Egypt were recruited to participate in a cross-sectional study of child growth and aflatoxin exposure (n = 46; Shouman et al., 2012). AFB_1 levels were determined for each child and their mother using thin-layer chromatography (TLC). AFB_1 was detected in 37% of both child and maternal serum samples, and these correlated between mother and child. Although this study did look for confounding variables, there were no significant differences between AFB_1 positivity and age, sex, residence, maternal age, parity, education or occupation. AFB_1 concentrations were significantly lower in children who were breastfed than in those given artificial or cow's milk or who were fully weaned. HAZ in AFB_1-positive children was statistically lower than in those who were AFB_1-negative. WAZ showed no correlation with AFB_1 levels. This study did not consider nutritional status of the children and the population size was small (Shouman et al., 2012).

Shirima et al. (2014) conducted a prospective study in the United Republic of Tanzania to assess the growth of seemingly healthy children (n = 166; 6–14 months of age) over a 12-month period. The relationship between aflatoxin exposure and growth was assessed through multivariate regression models for length-for-age, WAZ, weight-for-length z-score (WLZ) and growth velocity. The models were adjusted for village, weaning status, maternal education, socioeconomic status and protein and energy intakes. Growth velocity was also adjusted for sex, baseline age and baseline length. Nonstatistically significant negative associations were found between mean AFB_1–alb at all sampling times and the length-for-age scores and length velocity at the 12-month follow-up.

Mitchell et al. (2016a) followed children from Bhaktapur, Nepal, from birth to 36 months of age and assessed aflatoxin exposure through serum AFB_1–alb at 15, 24 and 36 months of age. Although this cohort had a high frequency of AFB_1–alb detection, the levels were low compared with those in the African studies and, in a more recent study, no associations were found between exposure and z-scores (Mitchell et al., 2017). This study adjusted for multiple cofactors, such as vitamin A, iron and zinc intake as well as gut function biomarkers, socioeconomic status and plasma vitamin A, iron and zinc. None of the parameters were statistically associated with growth, indicating the need for a larger sample size to determine the etiology of poor growth. The best predictor of z-scores in later life was birth weight, indicating that exposures in utero may be more important than those from the external environment.

A number of mechanisms have been proposed for the growth-faltering effect of aflatoxin (Turner, 2013; Wild, Miller & Groopman, 2015). Immune system dysfunction is known to be caused by aflatoxin in relevant animal models (Bondy & Pestka, 2000; Turner et al., 2003). This can increase risk of infections in children (e.g. diarrhoea), leading to growth impairment from energy losses.

Changes in intestinal integrity resulting from aflatoxin exposure could make children more vulnerable to intestinal pathogens (Smith, Stolzfus & Prendergast, 2012). In addition, dietary aflatoxin has been shown to alter the intestinal flora in Fischer 344 rats (Wang et al., 2016). Disruption of the gut microbiome may also be a relevant factor to consider in future studies. Epidemiology work has indicated that aflatoxin exposure may alter expression of the IGF axis (Castelino et al., 2014; Hernandez-Vargas et al., 2015). The liver is the main site for both aflatoxin toxicity and biosynthesis of IGF. IGF1 protein and IGF-binding protein-3 (IGFBP3) were both inversely correlated with AFB_1–alb level in Kenyan schoolchildren, and the authors concluded that IGF1 levels explained 16% of the impact of aflatoxin on child growth (Castelino et al., 2014). The downregulation of IGF1 and IGFBP3 from aflatoxin exposure was verified in vitro with human hepatocyte line (HHL-16) liver cells. In utero exposure of 115 children was associated with epigenomic DNA methylation of growth factor genes *FGF12* and *IGF1* (Hernandez-Vargas et al., 2015), suggesting that maternal aflatoxin exposure during pregnancy can influence growth and other health outcomes in their offspring.

Growth and development within the first years of life are integral to continued growth and development later in life. Deficits in growth are multifaceted and still not fully understood. In areas of the world where the highest frequency and the most severe growth-faltering occurs, the etiology is multifaceted and it is difficult to determine the mode of action and the interaction of all possible risk factors. Malnutrition, malabsorption, socioeconomic status, diarrhoeal disease and chronic infections are constant occurrences in areas with high aflatoxin exposure. The cofactors all need to be assessed in epidemiological studies of growth faltering in relation to aflatoxin exposure. Indeed, such an assessment would require a randomized intervention study to determine the effect of reducing aflatoxin exposure on growth outcomes and the potential pathways by which aflatoxin can affect growth.

Therefore, although there has been significant work on the effect of aflatoxin on child growth since the forty-ninth JECFA meeting (Annex 1, reference *131*), there remains insufficient evidence to utilize the impaired growth outcome as a health end-point for risk assessment.

(c) Immunotoxicology

A number of studies in humans (e.g. Turner et al., 2003; Jiang et al., 2005) suggest that aflatoxin is immunosuppressive in children in highly exposed populations. A population from Ghana was separated into high AFB_1 exposure and low AFB_1 exposure based on the median serum AFB_1–alb level (0.9068 pmol/mg albumin) and assessed for associations with cytokine production and monocyte phagocytic function (Jiang et al., 2005). Mean percentages of CD3 and CD19 cells showing

the CD69 activation marker were lower in the high AFB_1 group than in the low AFB_1 group; however, there were no significant differences in the percentages of CD3, CD4, CD8, CD14, CD19 and $CD3^-CD56^+$ cells between the two groups. There were also no significant differences in the percentage of $CD8^+$ T cells expressing IFN-γ and IL-4, or TNF-α-expressing NK cells (Jiang et al., 2005).

Immunomodulation was also observed in children with AFB_1–alb levels ranging from 5 to 456 pg/mg albumin, with a negative association between AFB_1–alb detectability and salivary IgA level (Turner et al., 2003).

A recent study in the Gambia examined the DNA methylation status of infants potentially exposed in utero via their exposed mothers (n = 115) measured using serum AFB_1–alb adducts. Hernandez-Vargas et al. (2015) showed differential DNA methylation associated with in utero aflatoxin exposure for some growth and immune function-related genes, including *CCL28*, *TLR2* and *TGFBI*.

2.4.4 Epidemiology of primary liver cancer

The most studied human health effect of aflatoxin exposure is its association with hepatocellular carcinoma. AFB_1 was classified as a known human carcinogen by IARC (1987, 2002). It can act synergistically with HBV infection in the development of hepatocellular carcinoma (Kew, 2003; Annex 1, reference *131*). These associations are described in more detail in the following sections.

Liver cancer is prevalent in areas with predisposing conditions, such as chronic HBV and HCV infections and chronic exposure to aflatoxins. Liver cancer incidence is consistently higher in men than in women, with a sex ratio ranging from 2:1 to 4:1 in some populations (Nordenstedt, White & El-Serag, 2010). Hepatocellular carcinoma is the most common type of liver tumour, representing approximately 80% of liver tumours (IARC, 2014). Hepatocellular carcinoma remains most frequent in China, South-East Asia and sub-Saharan Africa, although this pattern has been changing with shifts in predisposing conditions. Changing incidence rates reflect changes in aflatoxin exposure, emphasis on HBV vaccination programmes in newborns, expanded HCV populations in Europe, North and South America and Oceania, and non-alcoholic fatty liver disease (McGlynn & London, 2011; IARC, 2014). Liver cancer is the fifth most common cancer in men and the ninth most common in women, with more than half of the global incidence and mortality in China. Lack of early detection methods and treatments, as well as the occurrence of the majority of cases in low-to-middle-income countries, makes liver cancer the second most common cause of cancer death worldwide (IARC, 2014).

Hepatocellular carcinoma incidence rates also differ between ethnicities. Within the USA, those of Asian/Pacific Islander descent have age-standardized

incidence rates 3 times higher than whites (11.7 and 3.9, respectively), while people of Hispanic and African descent have age-standardized incidence rates of 7.0–8.0 (Altekruse, McGlynn & Reichman, 2009). However, the largest predictor is geographical location, with predisposing conditions and exposures. Although differences in ethnicity indicate a potential genetic predisposition to development of liver cancer in some instances, migration from areas of high risk to lower risk tends to reduce the levels of incidence to those of the host country; this effect can be observed within the first and second generations.

(a) Etiology of primary liver cancer

The etiology of primary liver cancer is well understood, although the progression and modes of action for some of the risk factors have not yet been elucidated. The major contributing factor to development of hepatocellular carcinoma worldwide is chronic viral infection. Hepatitis B viral infection can be attributed to anywhere between 20% (USA) and 65% (eastern China) of the hepatocellular carcinoma cases worldwide, reflecting its geographical variation (Venook et al., 2010; IARC, 2012; El-Serag & Kanwal, 2014). Hepatitis C viral infection is also a significant risk factor in areas of high incidence, like Egypt and Japan (IARC, 2014; Zhu et al., 2016). Coinfection with both HBV and HCV increases the overall risk (Huang et al., 2011).

People in areas with high incidence of hepatitis infection are often coexposed to dietary aflatoxin through consumption of maize and groundnuts. Aflatoxin is a known hepatocarcinogen, and exposure in areas of South-East Asia and sub-Saharan Africa is often chronic. Evidence from both animal models and epidemiological data suggests a potential synergistic mechanism between HBV and aflatoxin in the development of hepatocellular carcinoma. However, the majority of studies on the attributable risk of these factors have been conducted in populations with high frequency of exposure to both; the nature of a possible interaction of these risk factors at low levels remains unknown.

Other risk factors have been identified, and the associated change in the pattern of epidemiology has been attributed to certain risk factors that occur more frequently in populations in developed countries. Chronic alcohol consumption, diabetes, non-alcoholic fatty liver disease and tobacco smoking have all been associated with hepatocellular carcinoma. HBV infection and co-occurrence of these risk factors increase the lifetime risk of development of hepatocellular carcinoma.

(b) Vaccination programmes against HBV and liver cancer epidemiology

HBV is endemic in sub-Saharan Africa and South-East Asia, where 5–10% of the adult population is chronically infected. In 1983, WHO proposed trials of HBV

immunization with medium-term objectives to lower liver cancer incidence; WHO now recommends that HBV vaccines be incorporated into all routine infant and childhood immunization programmes. Schweitzer et al. (2015) was the first to estimate global HBV prevalence, at the country level, through a systematic review of the literature from 1965 to 2013; they indicated that HBsAg seroprevalence was 3.61% worldwide and that approximately 248 million individuals worldwide were seropositive. These authors concluded that this work highlighted the need for continued control and prevention strategies and collection of reliable epidemiological data based on standardized methodologies.

Global vaccination programmes targeting infants and children have proven effective in reducing the overall carriage of HBV in younger populations. In fact, chronic HBV infection has decreased worldwide from 1990 to 2005 (Ott et al., 2012). The universal vaccination programme drastically reduced the prevalence of HBV in children under the age of 15 years over the past 25 years in the Province of Taiwan, China, which previously had one of the highest rates of chronic HBV infection (Kao, 2015). Prevalence of the HBsAg+ in children has decreased from 9.8% in 1984 to 0.7% in 1999 and to 0.3% in 2009 (Ni et al., 2012); and the infection rate (anti-HBc seropositive rate) has also decreased, from 38% in 1984 to 16% in 1999 and 4.6% in 2009 (Ni et al., 2007). Similar results have been reported in China, the Gambia, Italy, the Republic of Korea and Saudi Arabia (Kao, 2015).

The annual incidence rate of hepatocellular carcinoma in children 6–14 years of age in the Province of Taiwan, China, decreased from 0.7 to 0.36 per 100 000 between 1981 and 1994 following initiation of the HBV immunization programme (Chang et al., 1997). Hepatocellular carcinoma rates in China and Singapore have also decreased following introduction of vaccination programmes. However, a 28-year follow-up of hepatocellular carcinoma incidence in Qidong, China, showed that while HBV vaccination programmes had had an impact, a decrease in liver cancer presaged the impacts of global vaccination. WHO-initiated neonatal HBV vaccination began in September 1983 in Qidong but did not become universal until 2002. Follow-up of this population in 2005–2008 in those aged 20–24 years (of whom 27.8% would have been vaccinated) showed a decrease in overall primary liver cancer rates that would account for 1.38 of the total 14.1 decrease in relative risk of this age group (Sun et al., 2013). Chen et al. (2013) demonstrated a longitudinal reduction in aflatoxin exposure in this particular Qidong population following a change in dietary habits due to a new open policy of food distribution in China in 1985. The change in Chinese interregional trade policy resulted in a drastic decrease in the production and consumption of maize in Qidong and an increase in consumption of rice, which is less likely to be contaminated with aflatoxin. AFB–alb detection in this population in 1989 showed 100% positivity ($N = 75$); that detection rate declined

to 23% in 2009 (N = 100) and further to 7% in 2012 (N = 100). HBsAg positivity has remained constant across this population in age groups born prior to the HBV vaccination programme, but the hepatocellular carcinoma incidence has nevertheless been declining, indicating that aflatoxin exposure was the primary effector in this observed decrease. Chen et al. (2013) calculated that 83% of the reduction in primary liver cancer was attributable to decreasing aflatoxin. As both aflatoxin exposure and chronic HBV infection decreased in areas with endemic hepatocellular carcinoma, a dramatic effect on hepatocellular carcinoma incidence should be observed due to the synergistic interactions.

(c) Epidemiology studies on aflatoxin and liver cancer

Better technology and increased research capacity since the Committee's last review of aflatoxin at its forty-ninth meeting have made the use of biomarker analysis techniques more readily available and the preferred methodology for determining aflatoxin exposure in human populations. Some new work that utilizes the survey method of assessing dietary aflatoxin as the exposure parameter in human health end-point studies has been conducted. Summaries of the studies described below, and their individual adjusted ORs, are shown in Table 6.

Yeh et al. (1989), as previously discussed in the Committee's last review, was one of the first publications to address the roles of HBV and aflatoxin on the development of primary liver cancer. This work prospectively followed 7917 men in Guangxi, China, and showed that 91% of liver cancer mortalities were positive for HBsAg at enrolment. In addition, estimated aflatoxin exposure levels from tested food samples were linearly associated with mortality rates of primary hepatocellular carcinoma, independent of HBV status. As described by the previous Committee, there are some limitations to this study (lack of control for other potential confounding variables such as HCV infection and tobacco use); however, this dataset still remains useful for dose–response modelling and risk assessment due to the large population size, the prospective study design and the availability of direct dietary exposure data.

Liu et al. (2012) calculated aflatoxin-related attributable cancer risk based on data in 17 articles on human exposure and hepatocellular carcinoma incidence. The authors summarized that aflatoxin exposure was significantly associated with hepatocellular carcinoma risk, without the presence of HBsAg positivity, and calculated an overall OR of 4.75 (2.78–8.11) from nine studies where the ORs were adjusted by HBsAg positivity. The population-attributable risk of liver cancer from aflatoxin exposure ranged from 2.1% to 63% in hepatocellular carcinoma cases with HBsAg+ populations.

Ross et al. (1992) conducted a prospective epidemiological study of diet and cancer in the metropolitan area of Shanghai. Male participants were recruited

Table 6
Characteristics of hepatocellular carcinoma and aflatoxin epidemiological studies

Location/period	Sex	Age (years)	No. of cases	No. of controls	Biomarker/range of exposure	Adjusted OR/RR	Covariates	Combined OR/RR with HBV	Source
China 1982–1987	M	25–64	76 liver cancer-related deaths	304 matched	AFB_1 in foods	Linear relationship: Pearson correlation coefficient = 1.00	HBsAg positivity, age, race, area of residence	NA	Yeh et al. (1989)
China 1986–1989	M	45–64	22 cases	140 matched	Urinary aflatoxins (detectable vs non-detectable)	3.8 (1.2–12.2)	Level of education, HBsAg positivity, cigarette smoking, alcohol consumption	60.1 (6.4–561.8)	Ross et al. (1992)
China 1986–1992	M	45–64	50 cases	267 matched	AFB_1–N^7-gua (detectable vs non-detectable)	9.1 (2.9–29.2)	HBsAg positivity, cigarette smoking	59.4 (15.6–212)	Qian et al. (1994)
Taiwan, China 1991–1992	M/F	36–65	20 cases	86 matched	AFB_1–alb (detectable vs non-detectable)	5.5 (1.2–24.5)	HBsAg positivity, anti-HCV, family history of liver cancer	N/A	Chen et al. (1996)
Taiwan, China 1988–1992	M	30–65	32 cases	73 matched	AFB_1–alb (low vs non-detectable)	1.6 (0.6–4.0)	Cigarette smoking, alcohol consumption	N/A – all were HBsAg carriers	Wang et al. (1996)
Taiwan, China 1991–1995	F/M	30–64	52 cases	168 matched	AFB_1–alb (detectable vs non-detectable)	3.8 (1.0–14.5)	HBsAg positivity, sex	70.0 (11.5–425.4)	Wang et al. (1996)
Taiwan, China 1988–1992	M	30–65	43 cases	43 controls	Urinary AFM_1, AFB_1–N^7-gua	12.0 (1.2–117.4)	HBsAg positivity, educational level, ethnicity, alcohol consumption, cigarette smoking	NA	Yu et al. (1997)

Location/period	Sex	Age (years)	No. of cases	No. of controls	Biomarker/range of exposure	Adjusted OR/RR	Covariates	Combined OR/RR with HBV	Source
Taiwan, China 1984–1995	F/M	Not reported	105 cases	37 controls	AFB_1–DNA adducts (liver tissue)	3.9 (1.4–11.5)	NA	67.6 (12.2–373.2)	Lunn et al. (1997)
Gambia 1997–1998	F/M	20–73	53 cases	53 matched	ser 249 TP53 mutation	16.4 (3.0–90.5)	Age, sex, recruitment site, HBsAg positivity	NA	Kirk et al. (2005)
Taiwan, China 1991–1997	F/M	30–64	75 cases	140 matched	AFB_1–alb (detectable vs non-detectable)	2.0 (1.1–3.7)	Sex, age, residence	NA	Lunn et al. (2001)
Qidong, China	M	27–64	31 cases	145 HBsAg+ carriers follow-up	AFM_1	3.5 (1.5–8.1)	Age, HCV, family history of liver cancer	NA	Ming et al. (2002)
Qidong, China	F/M	19–87	25 cases	30 controls	ser 249 TP53 mutation	22.1 (3.2–91.7)	Sex, age, recruitment site, HBsAg positivity	NA	Huang et al. (2003)
Gambia	F/M	Not reported	186 cases	348 matched	ser 249 TP53 mutation	20.3 (8.19–50.0)	Study group, season of recruitment, daily groundnut intake	NA	Kirk et al. (2005b)
Gambia	F/M	19–85	89 HCC; 42 cirrhosis	131 non-liver disease	ser 249 TP53 mutation	62 (4.7–820) HCC cases vs controls; 15 (1.6–140) HCC vs cirrhosis	Age, sex, recruitment site, HBsAg positivity, anti-HCV	NA	Lleonart et al. (2005)
Gambia 1997–2001	F/M	<35–>65	97 cases (cirrhosis)	346 matched	ser 249 TP53 mutation	3.8 (1.5–9.6)	Age, sex, recruitment site, HBsAg positivity, anti-HCV	46.0 (8.5–249.1)	Kuniholm et al. (2008)
China 2006–2008	F/M	<35–>65	618 cases	712 controls	AFB_1 exposure years; AFB–DNA (blood leukocytes)	2.11 (1.54–2.90)	Ethnicity, sex, HBsAg positivity, HCV	NA	Long, Ma & Deng (2009)

Table 6 (continued)

Location/period	Sex	Age (years)	No. of cases	No. of controls	Biomarker/range of exposure	Adjusted OR/RR	Covariates	Combined OR/RR with HBV	Source
Taiwan, China 1991–2004	F/M	30–64	230 cases	1 052 matched	AFB_1–alb (detectable vs non-detectable)	0.99 (0.48–2.02)	HBsAg positivity, anti-HCV, habitual smoking, alcohol consumption, BMI, batch of aflatoxin biomarker assay	NA	Wu HC et al. (2009)
					AFB_1–alb (below vs above the mean)	1.54 (1.01–2.36)		10.38 (5.73–18.82)	
China 1989–1998	M	30–59	126 cases	123 matched	AFB_1–alb (detectable vs non-detectable)	0.90 (0.52–1.56)	HBV-positive individuals only	NA	Szymañska et al. (2009)
India 2005–2009	F/M	≤45–>45	266 cases	251 (non–HCC chronic liver disease)	AFB_1-N^7-gua (detectable vs non-detectable)	1.48 (0.65–3.37)	Unadjusted	5.47 (3.07–9.82)	Asim et al. (2011)
China 2004–2010	F/M	<35–>65	1 499 cases	2 045 controls	AFB_1–alb (low, medium, high)	High exposure: 6.52 (5.4–7.88) (≥2.98 fmol/mg alb)	HBsAg positivity, anti-HCV, age, sex, race	NA	Long et al. (2013a)
China 2004–2010	F/M	<35–>65	1 499 cases	2 045 controls	AFB_1–DNA adducts (liver tissue)	High exposure: 6.43 (5.28–7.83) (≥2.01 µmol/mol DNA)	Unadjusted	NA	Long et al. (2013b)
Taiwan, China 1991–2004	F/M	30–64	230 cases	832 controls	AFB1–alb (above vs below mean 59.8 fmol/mg)	1.54 (1.01–2.36)	Batch of aflatoxin biomarker assay, HBsAg positivity, anti-HCV, smoking, alcohol consumption, BMI	NA	Wu et al. (2007a)

AFB_1: aflatoxin B_1; AFM_1: aflatoxin M_1; alb: albumin; BMI: body mass index; F; female; gua: guanine; HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; M: male; NA: not applicable; OR: odds ratio; RR: relative risk; ser 249 TP53: codon 249 on the *TP53* tumour suppressor gene in human hepatocytes

between January 1986 and September 1989. Cancer diagnosis was followed-up via copies of death certificates, data from the Shanghai Cancer Registry and by contacting each study participant every year. Follow-up was completed in March 1990 by which time the researchers had identified 22 cases of primary liver cancer from among the 18 244 people initially recruited. These 22 cases were matched with 140 controls, aged within 1 year of the cases; their urinary and blood samples were collected within 1 month of the matched cases, who lived in the same neighbourhoods. The relative risk and 95% confidence intervals associated with different urinary aflatoxin biomarkers were 4.9 (1.5–16.3) for AFB_1-N^7-gua and 3.0 (1.0–9.3) for AFM_1 (Ross et al., 1992). Positivity of HBsAg was also strongly associated with liver cancer (RR = 7.8; 95% CI: 3.0–20.6). Adjusted relative risk of aflatoxin positivity was 3.8 (95% CI: 1.2–12.2) for liver cancer, and the combined relative risk in study participants with HBsAg+ and detectable aflatoxin was 60.1 (95% CI: 6.4–561.8), indicating a possible synergism of aflatoxin and HBV infection for induction of primary liver cancer.

The urinary biomarkers of aflatoxin exposure reflect only recent dietary exposure, thus making them less supportive of associations with chronic health end-points such as cancer. However, this study was one of the first to demonstrate that such an association, which was long suspected based on animal models, also occurs in humans. A follow-up in 1992, with the same cohort, provided data on 33 additional identified primary liver cancer cases for a total of 55 cases. Similar to the previous study, a combined relative risk of 59.4 (95% CI: 16.6–212.0) was observed in those study participants who were positive for both HBsAg and urinary aflatoxins (Qian et al., 1994).

A majority of the epidemiological work on aflatoxin exposure and hepatocellular carcinoma incidence has been conducted in the Penghu Islets of the Province of Taiwan, China. Chen et al. (1996) enrolled seemingly healthy residents from these Islets to participate in a cohort trial (n = 6487). Participants were screened for hepatocellular carcinoma with follow-up every 3 months. Serum levels of AFB_1–alb were determined in 20 identified cases of hepatocellular carcinoma and 86 matched controls. The adjusted OR for anti-HCV, family history and HBsAg+ in this population with detectable AFB_1–alb was 5.5 (95% CI: 1.2–24.5). Anti-HCV and family history were not statistically significant predictors for development of hepatocellular carcinoma.

A study population of 110 primary liver cancer cases and 42 controls enrolled at the National Taiwan University Hospital from 1984 to 1995 was included in a case–control study to determine the association of aflatoxin exposure with hepatocellular carcinoma through use of both tumour and adjacent non-tumour liver tissues from the same person (Lunn et al., 1997). The adjusted OR for AFB_1–DNA adducts in the liver tissue and incidence of hepatocellular

carcinoma was 3.9 (1.4–11.5). The strength of the association was increased with the lack of association between non-tumorous tissue and AFB_1–DNA adducts.

Another cohort of 12 024 males and 13 594 females enrolled from July 1990 to June 1992 was followed for cancer prognosis over several years (Wang et al., 1996; Lunn et al., 1997; Wu et al., 2007b). These nested case–control studies were conducted within a cancer screening cohort aged 30–64 years who lived within seven townships in the Penghu Islets of the Province of Taiwan, China, between July 1990 and June 1992. During the follow-up period between 1991 and 1997, there were 99 confirmed cases of hepatocellular carcinoma (79 HBsAg-positive, 20 HBsAg-negative); these HBsAg+/hepatocellular carcinoma cases were matched with either one or two HBsAg+ individuals who were not suspected to have hepatocellular carcinoma for analysis of aflatoxin association with hepatocellular carcinoma controlling for HBV status (Lunn et al., 1977). In this follow-up population, those with hepatocellular carcinoma had a higher percentage of detectable AFB_1–alb (62.7% versus 45.7% of controls) and a calculated OR of 2.0 (1.1–3.7). However, following an observed positive association between aflatoxin and GSTT1-null genotype, a multiple logistic regression analysis that accounted for this interaction with AFB_1–alb level and GSTT1 genotype showed that aflatoxin exposure by itself was no longer a statistically significant risk factor for hepatocellular carcinoma (Lunn et al., 1977).

From this cancer screening cohort study of 12 024 males and 13 594 females, 241 hepatocellular carcinoma cases were identified between February 1991 and June 2004 (Wu HC et al., 2009). Of these, 174 cases and 832 controls had available baseline blood samples for aflatoxin analysis. HBsAg positivity and alcohol consumption were associated with hepatocellular carcinoma in these patients. The OR adjusted for aflatoxin biomarker assay, HBsAg, anti-HCV status, habitual smoking, alcohol use and body mass index was 1.54 (95% CI: 1.01–2.36) for those people with AFB_1–alb levels above the mean. The OR of this population was slightly higher (1.65) when HBsAg-negative individuals were included in the calculations (Wu HC et al., 2009a). The work conducted with this population is one of a few studies that determined associations between aflatoxin and hepatocellular carcinoma risk using available baseline serum samples (and thus AFB_1–alb levels) from the enrolment period and that examined longitudinal associations with development of hepatocellular carcinoma. While there are positive associations with AFB_1–alb levels and hepatocellular carcinoma within this cohort, the OR values are much lower than those calculated for the population in Qidong, China (Wu et al., 2007a; see below).

This population was also assessed for environmental exposure to polycyclic aromatic hydrocarbons (PAHs), using serum benzo[a]pyrene tetrol–albumin levels, for any association with aflatoxin and hepatocellular carcinoma status. Of these, 174 cases and 776 matched controls had baseline blood samples

available for AFB_1–alb analysis. The authors evaluated the dose–response relationship between aflatoxin, PAH and hepatocellular carcinoma risk through adjustment for HBsAg, smoking and alcohol consumption (Wu et al., 2007a). Participants were divided into one of four classes of doses of AFB_1–alb and PAH–alb for statistical analysis: above the mean for both PAH–alb and AFB_1–alb; above the mean for PAH–alb and below the mean for AFB_1–alb; below the mean for PAH–alb and above the mean for AFB_1–alb; or below the mean for both PAH–alb and AFB_1–alb. The OR for the combined effect of both of these environmental contaminants and HBV infection was 8.2 (95% CI: 3.6–19.0); this was statistically significantly different from those who had PAH–alb and AFB_1–alb levels below the mean and were negative for HBsAg.

Ming et al. (2002) analysed sera from 145 follow-up (up to 13.25 years) male chronic hepatitis cases identified and enrolled in a cohort with the Qidong Liver Cancer Institute since 1988. The relative risk for this population was 3.5 (95% CI: 1.5–8.1) in men with AFM_1 levels greater than 3.6 ng/L urine. Use of such a short-term biomarker ($t_{½}$ 24–48 hours) gave a glimpse of aflatoxin exposure within an individual, but the variability in individual intakes and excretions made it difficult to reach solid conclusions of association of AFM_1 levels with chronic health end-points such as hepatocellular carcinoma.

A case–control study was conducted in China of patients who were diagnosed with hepatocellular carcinoma from 2004 to 2008. The patients, who all received the same curative resection treatment, were followed for recurrence until August 2013. Liver tumour resection tissue was analysed for AFB_1–DNA adducts, and a mean of 2.87 ± 1.60 µmol/mol DNA was observed in the cancerous tissue. Patients were classified into low and high AFB_1–DNA adduct level groups; high AFB_1 exposure was associated with decreasing 1-year, 3-year and 5-year survival rates (Liu et al., 2014). This study provides evidence for the association between aflatoxin exposure and onset of hepatocellular carcinoma, and also for poor prognosis.

A study in Guangxi, China, assessed genetic markers of hepatocellular carcinoma development and prognosis in 1499 patients diagnosed with hepatocellular carcinoma in two affiliated hospitals from January 2004 to December 2010 and 2045 controls (Long et al., 2013a,b). The researchers published the results of their work with a focus on X-ray repair cross-complementing (XRCC) polymorphisms in hepatocellular carcinoma patien ts versus controls. However, they also collected aflatoxin exposure data based on both serum AFB_1–alb and AFB_1–DNA, and calculated "exposure years" based on an interviewer-administered questionnaire. Mean AFB_1–alb was 2.98 and 2.18 fmol/mg, respectively, in hepatocellular carcinoma cases and controls (Long et al., 2013a). Participants were grouped based on AFB_1–alb levels into low (<2.18 fmol/mg), medium (2.18–2.98 fmol/mg) and high (>2.98 fmol/mg) categories. The ORs for

hepatocellular carcinoma were 2.10 (95% CI: 1.75–2.25) and 6.52 (95% CI: 5.40–7.88) for the medium- and high-exposure groups, respectively. Neither HBV nor HCV status differed statistically between hepatocellular carcinoma cases and controls in this population.

Long et al. (2013b) conducted a similar analysis using, as the aflatoxin biomarker, AFB_1–DNA levels in DNA samples from peripheral blood leukocytes. Once again, the participants were grouped into low-, medium- and high-exposure AFB_1–DNA levels for statistical analysis. Levels in the low-exposure group were 1.00 µmol/mol DNA or less (below the mean of the control group); in the medium-exposure group were 1.01–2.00 µmol/mol DNA; and in the high-exposure group were 2.00 µmol/mol DNA or higher (above the mean in the hepatocellular carcinoma group). Once again, HBV and HCV status were similar in cases and controls in this population. The ORs for AFB_1–DNA and hepatocellular carcinoma risk were similar to those based on the AFB_1–alb levels in the Long et al. (2013a) study, 2.03 for the medium-exposure group and 6.43 (95% CI: 5.28–7.83) for the high-exposure group. Although these studies are not longitudinal, and therefore do not have data on aflatoxin exposure prior to the hepatocellular carcinoma diagnoses, the large population size in both of these studies and the confirmation of the AFB_1–alb biomarker-associated risk with AFB_1–DNA biomarker-associated risk provide complementary evidence of the influence of aflatoxin on hepatocellular carcinoma.

Several studies have included the use of the p53 249^{ser} mutation as a biomarker of aflatoxin exposure to determine any correlations between aflatoxin and hepatocellular carcinoma risk in different populations. While this hotspot mutation has been highly associated with hepatocellular carcinoma in areas with high aflatoxin exposure levels and positive results in some epidemiology studies, the direct relationship between aflatoxin exposure and p53 249^{ser} mutation remains unclear.

Szymañska et al. (2009) analysed tumour tissue samples and plasma DNA for the 249^{ser} mutation in hepatocellular carcinoma patients from Qidong, China. Tumour biopsies were available from 20 cases with matched controls, but plasma was available for 130 participants who were followed from 1989 over a 6-year period. These plasma samples were collected at different time points during the follow-up period, from between 0 and 74 months prior to the individual's cancer diagnosis. The presence of the 249^{ser} mutation in tumour tissues occurred in 61% of the tissues, but did not correlate with histological features of the tumours. There were corresponding plasma and tumour samples from the same individual in 14 participants. Interestingly, the presence of the 249^{ser} mutation in plasma DNA was not predictive of detection within the tumour. While the sample size for this analysis was too small to allow absolute conclusions, this finding should be an indication of cautionary use of these biomarkers in humans.

Szymañska et al. (2009) also calculated AFB_1–alb adduct levels for 123 cases and 126 controls in this group; however, exposure was uniformly low in this group, with most of the positives showing levels between 5 and 10 pg/mg. Using a cut-off of 3 pg/mg to dichotomize the participants into exposed and unexposed, the OR calculated for hepatocellular carcinoma was 0.90 (0.52–1.56).

A study population recruited as part of the Gambia Liver Cancer Study was analysed for hepatocellular carcinoma status and aflatoxin exposure through p53 249^{ser} mutations. Recruitment of study participants occurred from September 1997 to January 2001. Those with suspected liver disease provided a blood sample for analysis. Over this period, 97 people with liver cirrhosis and 397 controls were identified for further assessment. HBV and HCV were strongly correlated with risk of liver cirrhosis with an adjusted OR of 10.3 and 3.3, respectively. Presence of the p53 249^{ser} mutation was also associated with cirrhosis after adjustment for HBV and HCV status, with an OR of 3.8 (1.5–9.6). The joint effect of HBV status and 249^{ser} positivity appeared to be more than multiplicative (OR = 46.0 [8.5–249.1]); however, only two controls were positive for both and the interaction did not attain statistical significance because of this (Kuniholm et al., 2008).

Lleonart et al. (2005) identified 89 cases of hepatocellular carcinoma in addition to 42 cirrhotic cases in the same Gambian cohort. This study demonstrated that 67% of hepatocellular carcinoma cases had detectable plasma 249^{ser} and showed a dose-dependent association of 249^{ser} presence and severity of disease (i.e. controls versus cirrhosis versus hepatocellular carcinoma). Adjusted OR for hepatocellular carcinoma (with the controls as the reference group) was 62 (4.7–820) for persons with greater than 10 000 p53 249^{ser} DNA copies/mL plasma. The OR with cirrhosis as the reference group versus hepatocellular carcinoma was 15 (1.6–140) for greater than 10 000 p53 249^{ser} DNA copies/mL plasma (Lleonart et al., 2005). This study had relatively small numbers of participants compared with some of those that utilized AFB_1–alb as the biomarker of exposure.

(d) HBV, HCV and aflatoxin in the etiology of liver cancer

Some work in animals and epidemiology has found evidence for an interaction of effect between HBV and aflatoxin in the development of hepatocellular carcinoma. Several mechanisms for this interaction have been proposed, including HBV-induced increased cellular proliferation increasing expansion of an existing aflatoxin-induced 249^{ser} mutation; HBV causing an increase in levels of aflatoxin-metabolizing enzymes; HBVx protein interfering with nucleotide excision repair for aflatoxin–DNA adducts; and increase in oxidative stress. The synergistic interaction has been reviewed by Kew (2003). Meta-analysis has indicated that HBV and aflatoxin interact in a multiplicative manner (Liu et al., 2012).

Several of the epidemiology studies described above determined the combined effect of HBV and aflatoxin on hepatocellular carcinoma status. Table 7 summarizes the combined relative risks or ORs for aflatoxin and HBV on hepatocellular carcinoma risk. Many of the epidemiological studies control for HBsAg positivity status and include only HBsAg+ individuals to determine contribution of aflatoxin to hepatocellular carcinoma risk.

A meta-analysis of 17 studies between 1994 and 2009 summarized the multiplicative effect of aflatoxin and HBV exposure in hepatocellular carcinoma risk by calculating a combined OR across the studies with Wu HC et al. (2009) excluded for homogeneity. The combined OR for aflatoxin and HBV on hepatocellular carcinoma risk was 73.0; the individual OR for aflatoxin was 6.37 and for HBV was 11.3, thus making the joint effect based on this meta-analysis almost perfectly multiplicative (Liu et al., 2012). The combined ORs in the studies shown in Table 7 indicate a greater than multiplicative interaction between aflatoxin exposure and HBV status with hepatocellular carcinoma risk. The combined OR across all eight studies is 40.06, while the individual ORs are 3.64 for aflatoxin alone and 8.32 for HBV alone.

In addition to HBV, HCV infection rates have increased worldwide, with populations in certain regions developing chronic infections that are associated with the development of hepatocellular carcinoma. Countries with low rates of hepatocellular carcinoma, such as Japan and the USA, have seen an increase in rates due to an increase in the number of people living with cirrhosis, most often as a result of HCV infection (El-Serag & Kanwal, 2014). Indeed, several studies have shown associations with HCV and hepatocellular carcinoma incidence. Kuniholm et al. (2008) found an OR of 3.3 (1.2–9.5) for study participants with positive anti-HCV for risk of hepatocellular carcinoma compared with those without anti-HCV in their plasma. In a population from Qidong, the OR for HCV was low at 0.94, although this population had a small number of HCV-positive individuals (only 1.6%) among the hepatocellular carcinoma cases, making it unlikely that this lack of association is valid and would be maintained with a larger sample size (Ming et al., 2002).

(e) Genetic susceptibility

The variations in susceptibility of development of hepatocellular carcinoma in different ethnic groups living in similar geographical areas with homogenous environmental exposures imply a genetic predisposition to initiation and progression. Genes related to aflatoxin metabolism and DNA repair are of importance in areas with both chronic HBV infection and aflatoxin exposure.

Epigenetic changes have been measured in liver tumour and adjacent non-cancerous tissue from study participants with AFB_1-associated

Table 7
Findings in studies comparing the risk of HBV alone, dietary aflatoxin alone and the two risk factors together in etiology of hepatocellular carcinoma

	RR or OR (95% CI)		
Reference	**HBV alone**	**AFB_1 alone**	**Combined**
Ross et al. (1992)	4.8 (1.2–19.7)	1.9 (0.5–7.5)	60.1 (6.4–561.8)
Qian et al. (1994)	7.3 (2.2–24.4)	3.4 (1.1–10.0)	59.4 (15.6–212)
Wang et al. (1996)	17.4 (3.6–143.4)	0.3 (0–3.6)	70.0 (11.5–425.4)
Lunn et al. (1997)	17.0 (2.8–103.9)	17.4 (3.4–90.3)	67.6 (12.2–373.2)
Kuniholm et al. (2008)[a]	7.3 (3.9–13.6)	1.8 (0.5–6.7)	46.0 (8.5–249.1)
Wu HC et al. (2009)	7.03 (4.45–11.09)	1.64 (0.89–3.03)	10.38 (5.73–18.82)
Asim et al. (2011)	4.9 (3.27–7.35)	1.48 (0.65–3.37)	5.47 (3.07–9.82)
Qi et al. (2015)[b]	0.90 (0.61–1.34)	1.22 (0.75–1.98)	1.57 (1.03–2.40)

AFB_1: aflatoxin B_1; CI: confidence interval; HBV: hepatitis B; OR: odds ratio; RR: relative risk
[a] Health end-point was liver cirrhosis.
[b] Association was between etiological factors and recurrence of cancer after surgery.
Source: Kew (2003)

hepatocellular carcinoma, including DNA methylation, histone modifications and non-coding RNAs (Chappell et al., 2016). Associations between gene-specific hypermethylation, which can lead to inactivation of tumour suppressors (*RASSF1*, *p16*, *MGMT*) and GSTP1, which is responsible for metabolically inactivating AFB_1 epoxide, and high levels of AFB_1–DNA adducts have been observed in liver tissue from patients in a geographical area with high AFB_1 exposure (Zhang et al., 2002, 2005). In addition, AFB_1-induced changes in post-translational modifications of histone proteins that can affect gene expression and repression and changes in levels of non-coding RNAs have been reviewed (Chappell et al., 2016).

As discussed previously, AFB_1 activation by cytochrome P450s to the AFB_1-*exo*-8,9-epoxide is necessary for genotoxicity. CYP3A4 is considered the most important enzyme in the formation of the *exo*-8,9-epoxide. CYP1A2 may produce some *exo*, but mostly *endo*-8,9-epoxide, which does not have the same genotoxic properties as the *exo* isomer. Two separate studies have indicated that CYP3A5 can also activate AFB_1 to the genotoxic metabolite AFB_1-*exo*-8,9-epoxide (Gillam et al., 1995; Wang et al., 1998). Since CYP3A5 expression can exceed that of CYP3A4 in some humans, its effect on aflatoxin biomarkers was investigated. AFB_1–alb adduct levels from sera from a cohort study of 303 Gambian participants were 23.2% higher in those with corresponding high levels of CYP3A5 expression compared with low expressers after controlling for time of sample collection and geographical residence (Wojnowski et al., 2004). The effect of the CYP3A5 polymorphism was most pronounced in individuals either

with low CYP3A4 activity or with null alleles of GSTM1. Stratification by sex and HBV status did not reveal significant associations with CYP3A5 and AFB_1–alb.

Phase II detoxification pathways are important to aflatoxin (see section 2.1.2). Briefly, GSTM1, GSTT1 and epoxide hydrolase (HYL1) are responsible for converting the carcinogenic AFB_1-*exo*-8,9-epoxide to non-reactive metabolites. Alterations in expression of these genes and function of the enzymes can result in an increase in the half-life and amount of free carcinogen available to create DNA adducts. Wojnowski et al. (2004) found that the observed association of CYP3A5 with AFB_1–alb levels was no longer present following stratification by GSTM1 polymorphisms. The study participants with at least one functional GSTM1 allele showed no statistical difference in AFB_1–alb level by CYP3A5 expression. Stratification by GSTT1 and HYL*2 did not alter the association between CYP3A5 expression and AFB_1–alb levels. This study provides evidence for genetic susceptibility for production of the carcinogenic metabolite of aflatoxin in people with increased expression of CYP3A5 and the absence of GSTM1. However, the authors cautioned that the sample size was limited with respect to conducting multi-gene interaction analyses.

Participants in the Gambia Liver Cancer Study were invited to participate in a genetic study to identify associations with hepatocellular carcinoma, aflatoxin exposure, HBV prevalence and polymorphisms in the suspected aflatoxin-related high-risk genotypes (GSTM1, GSTT1, HLY1*2 and XRCC1). This study analysed the genotype data as dichotomous variables with at least one intact allele versus null for the GST genes; HLY1*2 was considered heterozygote or homozygote for the low activity variant *His* allele, and the XRCC1 polymorphisms were classified by the number of arginine to glycine substitutions at exon10 of codon 339 (AA, AG, GG or AG/GG). Following adjustment for age, sex, recruitment site, recruitment date, ethnicity, socioeconomic status and HBV and HCV status, the GSTM1-null genotype was associated with a 1.86-fold increased risk for hepatocellular carcinoma whereas the XRCC1 AG genotype had a 2.26-fold increase in risk (Kirk et al., 2005b). These associations were increased when adjusted for aflatoxin exposure through the presence of p53 249^{ser} mutations in the circulating cell-free DNA from plasma (OR = 2.45 in GSTM1-null genotype and OR = 3.18 in XRCC1 AG genotype, respectively). This study also showed increasing risk for hepatocellular carcinoma with increasing number of high-risk genotype polymorphisms, with the combination of both GSTM1 and XRCC1 having the highest risk at 9.14.

The authors concluded that the results demonstrated that both XRCC1 and GSTM1 contribute to hepatocellular carcinoma pathogenesis by modulating effects of either endogenous or exogenous carcinogens or through aflatoxin itself. However, the statistical power was limited to test for combinations of genetic

polymorphisms, and the aflatoxin exposure markers used in this study are yet to be validated as biomarkers of effect in humans.

The DNA repair genes comprising the "x-ray repair complementing group" are necessary to repair most double-strand breaks, and polymorphisms of these genes have been associated with DNA repair capacity and cancer risk. The XRCC family of polymorphisms has been implicated in several studies as genetic susceptibility markers for aflatoxin-induced hepatocellular carcinoma. Three studies conducted with liver tissue samples collected from hepatocellular carcinoma patients at two affiliated hospitals associated with Guangxi Medical University and Youjiang Medical College for Nationalities from January 2004 to December 2010 investigated associations of hepatocellular carcinoma prevalence and survival rate with XRCC polymorphisms and AFB_1–DNA levels (Long et al., 2013a,b; Yao, Huang & Long, 2014). Assessment of polymorphisms in XRCC1, XRCC3, XRCC4, XRCC7, XPD and XPC showed a significant increased risk for hepatocellular carcinoma in all of these DNA repair genes (Yao, Huang & Long, 2014). In addition, calculation of the interactive coefficient between AFB_1 exposure (AFB_1–alb) and genotypes of DNA repair genes using logistic regression resulted in a corresponding interactive coefficient of 1.6 between AFB_1–alb and XRCC1, XRCC3, XRCC4 and XRCC7. The authors concluded that AFB_1 exposure acted in a multiplicative manner with the polymorphisms of the DNA repair genes in the development of hepatocellular carcinoma (Yao, Huang & Long, 2014).

Long et al. (2013a) also observed an association between AFB_1–alb levels and hepatocellular carcinoma risk that became more pronounced in people with XRCC4 risk genotypes. This association was again verified with the AFB_1–DNA adduct levels from the liver tissue. Long et al. (2013b) found that hepatocellular carcinoma risk was associated with the number of XRCC4 codon 247 serine alleles, and genotypes with these serine alleles significantly downregulated the XRCC4 expression in tumour tissues compared with the homozygous alanine genotype. The researchers also calculated ORs of 6.12, 7.81 and 14.43 for risk of hepatocellular carcinoma in people with high AFB_1–DNA levels and the XRCC4 genotypes AA, AS and SS, respectively. The authors concluded that the presence of XRCC4 polymorphisms modifies the association of aflatoxin exposure with hepatocellular carcinoma risk. They suggested that these polymorphisms reduce DNA repair capacity, resulting in an inability of hepatocytes to effectively repair aflatoxin-induced DNA damage, leading to higher adduct levels and overall risk for hepatocellular carcinoma. However, these are all cross-sectional studies, from which cause and effect cannot be inferred, making them unsuitable as a basis for risk assessment (Long et al., 2013b).

3. Analytical methods

3.1 Introduction

The toxicity and potency of aflatoxins make them significant health hazards in food and feed. Many countries have stringent regulations for acceptable levels in food and feed. Consequently, analytical methods must be able to accurately, rapidly and precisely measure aflatoxin levels lower than those required by national or international regulations in order to monitor levels in the food chain.

One difficulty that arises in developing methods for detection is the non-homogenous distribution of aflatoxins in agricultural commodities. A sampling error can account for up to 90% variability in aflatoxin levels (Whitaker, 2003). The source of error comes from the skewed distribution of aflatoxin in any commodity. Only a few kernels in an otherwise clean sample of a grain lot may be contaminated. Therefore, sampling and extraction methods (see section 4) become a critical part of aflatoxin analysis. Aflatoxin analytical tools have developed very rapidly and are the subject of many reviews (Cigić & Prosen, 2009; Shephard, 2009, 2016; Turner, Subrahmanyam & Piletsky, 2009; Wacoo et al., 2014; Miller, 2016). The topic of analytical methods is of such significance that special issues of journals highlight the latest developments (Journal of AOAC International Volume 98, 2016). The World Mycotoxin Journal provides an annual update on mycotoxin analysis (Berthiller et al., 2016). The analytical methods can be generally divided into quantitative methods, semiquantitative methods, indirect methods and some emerging technologies.

3.2 Sample preparation

Most analytical methods require that samples be correctly extracted and cleaned prior to analysis. Sample preparation may be the most important and time consuming part of aflatoxin analysis. Aflatoxins can be removed from a commodity by extraction with a polar solvent such as methanol or acetonitrile. The sample can be ground with the solvent and then filtered. Solvent selection must take into consideration the safety of the solvent and the volume of waste generated (Reiter, Zentek & Razzazi, 2009; Shephard, 2009; Turner, Subrahmanyam & Piletsky, 2009).

Additional clean-up is required after extraction (discussed in detail in Shephard, 2009; Turner, Subrahmanyam & Piletsky, 2009). The methods used are (1) liquid–liquid extraction (LLE), which involves extracting the toxin using an aqueous phase and an immiscible organic solvent phase, with aflatoxin ending up in one phase and the majority of other compounds in the other phase; (2) supercritical fluid extraction (SFE), which uses a supercritical fluid such as CO_2 to extract the toxin from the matrix; and (3) solid phase extraction (SPE), which

is also used for cleaning up samples for aflatoxin analysis where the binding matrix of the SPE column can be either porous silica or an antibody and the washing liquid can be an organic solvent or distilled water (Yao, Hruska & Di Mavungu, 2015).

The most commonly used clean-up tool for aflatoxins is the immunoaffinity column (IAC), which is based on monoclonal or polyclonal antibodies. This technique combines the use of liquid chromatography with the specific binding of antibodies or related agents. The method can be, therefore, used in assays for a desired target or to purify and concentrate analytes prior to analysis (Moser & Hage, 2010). IAC can be used with HPLC, capillary electrophoresis or mass spectrometry. This method is effective because of (1) the specificity of the antibody, which allows for clean extracts; (2) the applicability to multiple and complex matrices; (3) the achievement of rapid and precise clean-up; and (4) their limited use of organic solvents. IACs were first developed for a single mycotoxin class, but have been developed and commercialized for multi-mycotoxin analysis and contain antibodies specific to more than one mycotoxin (details provided in Shephard, 2009).

3.3 Quantitative methods

There are several types of chromatographic methods available for aflatoxin analysis and quantification. Most require sample pretreatment. The advantages and disadvantages of each technique are summarized in Table 8, and more detailed information is available in several reviews (Shephard, 2009, 2016; Turner, Subrahmanyam & Piletsky, 2009; Yao, Hruska & Di Mavungu, 2015; Berthiller et al., 2016).

3.3.1 TLC

Ever since the discovery of aflatoxins, TLC has been the technique most used for their separation and quantification. TLC is still used in combination with an ultraviolet (UV) or fluorescence scanner, but with the development of other rapid techniques, it is now significantly less used. Using known standards, scanners can provide quantitative estimation of aflatoxin relative to the quantity of the standard. Silica gel is the most common layer used for TLC, and the samples are developed by a solvent mobile phase. The advantages of this method are that it is easily transportable; it has high-throughput (multiple samples can be screened simultaneously); it is economical; and it can easily identify aflatoxins with adequate sensitivity for most applications of 0.5 µg/kg (Shephard, 2009).

Table 8
Advantages and disadvantages of aflatoxin detection technologies

Method	Pros	Cons	References
TLC	• Reliable quantification method when combined with densitometry • Accuracy and precision comparable to HPLC methods • Official reference methodology for aflatoxins (AOACI 968.22, 970.45, 998.03)	• Outdated equipment • Destructive sample preparation • Largely replaced by HPLC for quantitative analysis of aflatoxins	Rahmani, Jinap & Soleimany (2009); Shephard (2009)
HPLC	• Reliable, sensitive, selective and repeatable quantification methodology • May be automated • Official reference method for aflatoxins (AOACI 999.07, 990.33)	• Expensive equipment requiring dedicated operator and specialist to interpret results • Destructive sample preparation • May require derivatization	Cho et al. (2008); Shephard (2009); Turner, Subrahmanyam & Piletsky (2009)
HPLC-MS or HPLC-MS/MS	• Simultaneous analysis of mycotoxins • Low LOD (LC-MS/MS) • Confirmatory method • No derivatization required	• Very expensive equipment requiring dedicated operator and specialist to interpret results • Sensitivity relies on ionization • Matrix-matched calibration for quantitative analysis • Lacks internal standards	Krska et al. (2008); Pascale (2009); Shephard (2009); Li et al. (2013)
ELISA	• Specific, rapid and relatively easy to use • Inexpensive equipment • Simultaneous analysis of multiple samples • Semiquantitative (screening) or quantitative analysis possible • Limited use of organic solvents	• Possible cross-reactivity with related mycotoxins • Matrix interference • Possible false positives/negatives • Narrow detection range • Confirmatory LC analysis may be required	Pittet (2005); Pascale (2009); Turner, Subrahmanyam & Piletsky (2009)
Direct fluorescence assay	• IAC in combination with liquid fluorometry is comparable to LC for determination of aflatoxins • Official reference method (AOACI 991.31)	• Sample destruction	Pittet (2005)
FPIA	• Rapid, no clean-up required • Very sensitive • Portable	• Limited validation with ELISA or HPLC • Possible cross-reactivity with related mycotoxins • Matrix interference • Limited to detecting one mycotoxin at a time, mycotoxin-specific tracer needed	Pascale (2009); Lattanzio et al. (2011); Lippolis & Maragos (2014)
Capillary electrophoresis	• Useful for separating closely related mycotoxins • Highly sensitive • Capable of multi-constituent analysis when combined with immunoassays	• Limited to lab use due to cumbersome instrumentation	Maragos (2004)
Biosensors	• Rapid, no clean-up required • High selectivity and low LOD • Ease of use, low cost and portability • Self-contained, simple design	• Extraction sample preparation for solid samples • Extract clean-up needed to improve sensitivity • Cross-reactivity with related mycotoxins • Variation in reproducibility and repeatability (improved with use on novel materials)	Pascale (2009); Tothill (2011); Rubert et al. (2012); Malhotra et al. (2014a); Meneely & Elliott (2014)

Method	Pros	Cons	References
NIR spectroscopy	• Rapid, non-destructive • No extraction or clean-up required • User-friendly operation	• Calibration model must be validated • Requires knowledge of statistical methods • Poor sensitivity (high LOD) • Costly equipment	Gordon et al. (1999); Pearson et al. (2001); Dowell et al. (2002); FAO (2004); Berardo et al. (2005); Pearson & Wicklow (2006); Tallada et al. (2011); Hossain & Goto (2014)
Hyperspectral imaging	• Rapid, non-destructive • No extraction or clean-up • User-friendly operation • High spectral and spatial resolution • Potential for in-line detecting applications	• Calibration model must be validated • Knowledge of statistical methods • Poor sensitivity (high LOD) • Low signal level (for fluorescence) • Costly equipment	Yao et al. (2008); Del Fiore et al. (2010); Yao et al. (2010); Hruska et al. (2013)
Electronic nose	• Rapid means of controlling and improving the microbiological quality of food	• Need to improve selectivity and reduce interferences (e.g. to humidity) • Need to compensate for drift effects • Limited feasibility studies and poor validation	Gardner & Bartlett (1994); De Lucca et al. (2012)

AOACI: Association of Official Analytical Chemists International; ELISA: enzyme-linked immunosorbent assay; FPIA: fluorescence polarization immunoassay; HPLC: high-performance liquid chromatography; IAC: immunoaffinity column; LC: liquid chromatography; LOD: limit of detection; MS/MS: tandem mass spectrometry; NIR: near-infrared; TLC: thin-layer chromatography.
Source: Yao, Hruska & Di Mavungu (2015)

3.3.2 HPLC

Because of its greater accuracy and higher sensitivity, this technique has nearly replaced TLC as the analytical tool of choice. Aflatoxins are separated and purified using reversed-phase columns, usually combined with some derivatization system. The compounds can be detected using a variety of detection systems such as fluorescence, UV or diode array detectors. This is one of the advantages of HPLC, coupled with its total automation and the high quality of separation in a short time. The latest development of HPLC (termed ultra-HPLC or UHPLC) involves the use of columns packed with smaller, more uniform particles (typically ≤2 μm). Combined with new pump designs to operate at the high pressures required and detectors capable of handling the sharp chromatographic peaks, UHPLC reduces run times and solvent consumption while increasing efficiency and sensitivity. Aflatoxins are rapidly analysed using UHPLC with fluorescence detection. Aflatoxin B_1, B_2, G_1 and G_2 can be easily resolved in a short run time (4 minutes). A quick analysis can be conducted using an isocratic solvent system of 40% methanol in water or, for more difficult separations, a solvent gradient with acetonitrile in water (both with 0.1% formic acid additive). Fluorescence detection allows for high sensitivity and accurate quantification.

3.3.3 HPLC-MS or HPLC-MS/MS

The application of HPLC-MS or HPLC-MS/MS to mycotoxin analysis is relatively recent (Songsermsakul & Razzazi-Fazelli, 2008) and has been the subject of many reviews (Li et al., 2013). "It was the development of API (atmospheric pressure ionization) techniques such as electrospray ionization and atmospheric pressure chemical ionization that enabled HPLC coupled to MS (HPLC-MS) or to tandem MS (HPLC-MS/MS) to become a versatile analytical tool" (Shephard, 2016). Compared to HPLC, mass spectrometry can identify aflatoxins without the need for derivatization. Single ions can be selectively recorded for enhanced sensitivity. Alternatively, total ion scans can be used to detect and identify aflatoxin analogues and precursors as well as other mycotoxins that are not fluorescent. This method is highly sensitive, but requires several considerations, for example, the composition of extraction mixture and the possibility of negative matrix effects. Additional positive and negative aspects of this technique are summarized in Table 8. For analysis of aflatoxins alone, sample extracts can be cleaned-up as for HPLC. The use of tandem mass spectrometry (MS/MS) results in fragmentation ions, which can provide both quantification and confirmation of the toxin.

In any multi-analyte analysis, such as the simultaneous detection of multiple mycotoxins including aflatoxins, a targeted clean-up (e.g. using multi-toxin IACs) limits the applicability of the method. One commonly used method involves injection of a diluted extract (so-called "dilute-and-shoot") into the HPLC system. Nevertheless, matrix effects are a major challenge in the successful development of reliable, quantitative methods. This "dilute-and-shoot" protocol does not require prior clean-up of the sample because the amount of matrix in the sample being injected is reduced (Mol et al., 2008; Spanjer, Rensen & Scholten, 2008). Quantification is mostly achieved using matrix-matched standards to account for signal enhancement or suppression due to the coinjected matrix.

3.3.4 Capillary electrophoresis

In capillary electrophoresis, molecules are separated based upon charge and mass-dependent migration under an electric field. The separation is done using an aqueous buffer solution rather than organic solvents as with HPLC. Capillary electrophoresis is ideal for separating closely related molecules. This highly sensitive technique is best used when combined with immunoassays and fluorescence-based detectors, as described for aflatoxins by Pena et al. (2002). The main drawback is that the instrumentation is very cumbersome.

3.4 Semiquantitative methods

Semiquantitative methods, mostly based on immunological principles, are rapid and can either act as a preliminary screen or be used in place of chromatographic

methods. Ever since the development of specific antibodies against aflatoxins, many immunoassays that rely on the recognition of an aflatoxin epitope by the antibody have been described (reviewed in Li, Zhang & Zhang, 2009).

3.4.1 ELISA

ELISA is one of the most established and widely used assays, and a large number of commercial ELISA kits are now available. ELISAs are routinely used because they are simple to use and cost-effective, and are ideal for screening purposes or for sensitive quantification of aflatoxins in various samples. The process of detection is rapid with no clean-up requirements. The principle of this technique is the immobilization of either the antibody or the antigen on a suitable substrate, allowing for a competitive assay, followed by an interaction with a chromogenic substrate to provide a visual or optical measurable result. The disadvantage of this technique is the potential for cross-reactivity and dependence on the specific matrix for which the assay was validated. In addition, the range of detection is limited by antibody sensitivity (Turner, Subrahmanyam & Piletsky, 2009).

ELISAs continue to be developed to reduce costs and to facilitate application in remote areas, predominantly in developing countries (Shephard, 2009), such as the one developed by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) for use in Africa (Mutegi et al., 2009).

3.4.2 Lateral flow tests

Immuno-dipsticks are immunochromatographic assays based on high sensitivity and specificity of antigen–antibody reaction for a rapid analysis. These are the latest in immunoassays, using polyvinylidene difluoride (PVDF), nitrocellulose or nylon as immobilization surfaces (Delmulle et al., 2005; Xiulan et al., 2006). Lateral flow devices contain a rigid backing for support, a porous membrane for the flow, an absorbent pad for increasing the flow and a sample pad (containing colloidal gold coated with antibody) for contact between the liquid sample and the membrane (Wacoo et al., 2014). After sample extract application, "aflatoxin if present will interact with the gold conjugated anti-aflatoxin antibodies at the base of the stick. Both bound and unbound antibodies move along the stick membrane, passing a test line, composed of immobilized mycotoxin which will bind free antibody to form a visible line indicating a level of aflatoxin contamination below the test cut-off value" (Shephard, 2009). Further movement along the strip causes the solution to pass a control line of anti-antibodies. The presence of a visible control line is required for a valid test. The test cut-off value is determined by the manufacturer, which could be a limitation of this method. In addition, these tests are valid for some matrices only, and there should be few false negatives. The number of false positives is not an issue because these samples are usually also

tested using other quantitative methods. Photometric strip readers are used for semiquantification of toxin levels using this method (Delmulle et al., 2005; Salter et al., 2006).

3.4.3 Direct fluorescence

The sample is cleaned-up by IAC (as described in section 3.2). Aflatoxin quantification is done by derivatization of the sample and measurement of its fluorescence directly using a commercial fluorometer. Depending on the IAC used, the method can be applied to AFB_1, AFM_1 or AFT.

3.4.4 Fluorescence polarization immunoassay (FPIA)

The advantage of FPIA is that it is performed as a homogenous assay, and the requirement to separate free and bound tracer is not needed compared with ELISA (Maragos, 2009). The principle involved in FPIA is the measurement of the rate of rotation of a fluorescent molecule. Free fluorescent-labelled aflatoxin molecules (smaller molecules) rotate faster than antibody-bound aflatoxins (larger molecules). This rotation has an effect on the extent of depolarization of plane-polarized light; the more the rotation, the more the depolarization. The results of FPIA analysis for aflatoxins in maize, sorghum, peanut butter and peanut paste (Nasir & Jolley, 2002) compared well with HPLC results, but were lower in value.

3.4.5 Biosensors

Biosensors are simple, easy-to-handle instruments that convert a biological interaction between an analyte and a biological element or bioreceptor into an electrical signal via a transducer detector element. The signals can be further amplified. These low-cost and multiple-use sensors are highly sensitive and selective and easily transportable (Tothill & Turner, 2003; Tothill, 2011). Most biosensors do not require much sample preparation or clean-up. However, solid samples may require extraction and clean-up procedures similar to those used for HPLC techniques.

There are many types of biosensors based on the basic components of the sensor: (1) optical (fibre-optics or fluorescence polarization device; Maragos & Thompson, 1999; Nasir & Jolley, 2002); (2) electrochemical (with transducers of gold electrodes, which are more specific binders than carbon electrodes (Piermarini et al., 2007; Li et al., 2010; Tothill, 2011; Vidal et al., 2013; Malhotra et al., 2014a); or (3) piezoelectric (for example, quartz crystal microbalance based on a change in mass; Jin et al., 2009). Optical biosensors include interferometric, fluorometric, refractometric biosensors and ones based on surface plasmon resonance platform as transducers (Hodnik & Anderluh, 2009; Liu et al., 2012).

Electrochemical biosensors use potentiometric, amperometric, capacitative and conductometric transducers, whereas physical biosensors use magnetic, piezoelectric, calorimetric and surface acoustic wave transducers (Malhotra et al., 2014a).

The most significant part of the biosensor is the bioreceptor that recognizes the analyte, which can be an enzyme, antibody, nucleic acid, cell receptor protein, whole cell or tissue. These recognition elements are immobilized in a matrix of various materials (Logrieco et al., 2005; Malhotra et al., 2014a), which is the second part of the biosensor. The third part of the biosensor is a transducer (optical, electrochemical or physical) that converts the biochemical reaction into an electronically recognized output signal.

Sensors can be highly sensitive. For example, with a biocatalysed deposition amplification system, a sensor detected aflatoxin in milk in the range of 0.01–10.0 µg/L (Jin et al., 2009). Xu et al. (2013) developed a biosensor for AFB_1 with detection in the range of 0.5–20 µg/L and a limit of 0.16 µg/L. Although not stated by the authors, the detection limit for peanuts was extrapolated to approximately 1.25 µg/kg by Yao, Hruska & Di Mavungu (2015).

3.5 Indirect methods

These methods do not directly measure the presence or quantity of aflatoxins, but measure the presence of an indicator that is associated with production of aflatoxins.

3.5.1 Spectroscopy

Mid- or near-infrared (NIR) spectroscopy has been used to detect aflatoxins in maize kernels (Pearson et al., 2001) or *A. flavus* infection assessment (Gordon et al., 1999). Fungus-infected maize kernels were also identified with NIR spectroscopy (850–1650 nm) and colour imaging with a 99% accuracy (Tallada et al., 2011); and this combined spectroscopic analysis was better than colour sorting by itself. Aflatoxins in maize kernels were also detected using both transmission (500–950 nm) and reflectance spectra (550–1700 nm; Pearson et al., 2001). The accuracy for detection for mid-level aflatoxin concentration (10–100 µg/kg) was only 25%, but it was 95% for low (<10 µg/kg) and high (>100 µg/kg) aflatoxin content.

3.6 Emerging technologies

3.6.1 Hyperspectral imaging

Hyperspectral imaging involves fluorescence emission imaging of naturally fluorescing material when these substances are excited with shortwave radiation from a UV light source or a laser. This method offers rapid, non-invasive, non-

destructive inspection of contaminated material. The methodology has been successfully used to detect aflatoxin contamination in ground red chilli pepper flakes (Ataş, Yardimci & Temizel, 2012) and in maize kernels (Yao et al., 2010) with a correlation coefficient of 0.72 (as compared to actual aflatoxin determination by HPLC), and as high as 0.87. Classification accuracy under a two-class scheme ranged from 0.84 to 0.91 when a threshold of either 20 or 100 µg/kg was used (Yao et al., 2010).

3.6.2 Electronic nose

An electronic nose mimics the human sense of smell using an array of sensors capable of recognizing complex odours (volatiles as sensory indicators), which the instrument then identifies or quantifies through a pattern recognition system (reviewed in Logrieco et al., 2005; Yao, Hruska & Di Mavungu, 2015). This methodology has yet to be fully explored, and it is not even clear if this will offer any quantification capability (Cheli et al., 2007). Aflatoxigenic fungi and atoxigenic (non-aflatoxigenic) fungi produce different volatile compounds (Zeringue et al., 1993; De Lucca et al., 2012) or may produce the same volatile compound at a different time point. For example, furans are produced by both types of *A. flavus*, but the toxigenic strains release them several days after the non-toxigenic strains (De Lucca et al., 2012).

3.6.3 Aptamer-based biosensors

This emerging technology is based on using single-stranded oligonucleotides, called aptamers, as molecular recognition probes instead of antibodies to detect aflatoxins in a biosensor. Ruscito et al. (2016) reviewed the current status and future prospects of this novel technology for detection of mycotoxins. Aptamers have the advantage in that they can fold into distinct three-dimensional conformations providing for both high-affinity and specific binding to the target molecule comparable to that of monoclonal antibodies. The interaction of aptamers with the target molecule is through non-covalent as well as hydrogen bonding. Aptamers have been developed for recognition of AFB_1 (Le et al., 2011; Ma et al., 2014), AFB_2 (Ma et al., 2015) and AFM_1 (Malhotra et al., 2014b). Apart from their speed, aptamer-based biosensors have several advantages: (1) they are extremely sensitive and can detect target molecules on the basis of just one functional group or even minor structural differences; (2) because they are nucleic acid sequences, they can be synthesized relatively easily, at a very low cost and with no batch-to-batch variability, and are stable for longer; and (3) their chemical stability allows their use in even harsh environmental conditions, including remote and low-resource areas.

3.6.4 Molecularly imprinted polymers (MIPs)

Various analytical methods based on molecular recognition components (e.g. antibodies, functionalized surfaces and aptamers) require adhesion or chemical linking of the recognition component on a platform or a detection material. Molecular-imprinted technology, however, incorporates molecular recognition material within the matrix of the sensor. These sensors can be used for both aflatoxin analysis and sample clean-up. "The selective recognition components of imprinted polymers are formed during polymer synthesis through the interaction of the functional groups of the polymer reagents with a template molecule. After the polymer is formed, the template is removed to leave a polymeric material with specific binding sites" (Appell & Meuller, 2016). MIPs offer a high degree of sensitivity, ease of handling, stability (thermal and mechanical), tolerance to solvents and a greater ease of customization (De Middeleer, Dubruel & De Saeger, 2016). Several imprinted polymers have been developed to detect aflatoxins in various matrices, with different detection techniques such as fluorescence-based sensors (Sergeyeva et al., 2008) or capillary liquid chromatography method (Szumski et al., 2014). Aflatoxins in the range of 1–1000 μg/kg were detected using one MIP (Serheeva et al., 2007) and 1–500 μg/L using another (Wyszomirski & Prus, 2012). The imprinting effect in an MIP may be as high as a 10-fold increase in detection level compared with a non-imprinted polymer.

3.7 Special considerations

Aflatoxin detection methodology has received significant attention, particularly in the last two decades or so, as health risk assessment analysis has become commonplace worldwide. The knowledge of the great extent of human and animal exposure has increased the urgency for mitigating this exposure using highly accurate and rapid detection methods. Several of the detection technologies have had significant practical impact, whereas others are highly sensitive research tools. The availability of detection tools that can identify even traces of aflatoxins (at ng/kg and less) puts significant pressure on producers to find a way to completely or almost completely eliminate these toxins from food and feed chains.

Several methods that have been developed never met validation standards or are unsuitable for the conditions for which they were developed, and therefore remain as academic exercises. Trucksess & Zhang (2016) argued that for analytical methods to be practical they should meet the basic guideline of reproducibility in different laboratory settings. Taking this into consideration, the United States Food and Drug Administration and the European Union have issued stringent guidelines on accuracy, precision, selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, range, uncertainty and ruggedness as criteria for acceptance of quantitative analytical methods. For semiquantitative or qualitative

methods, "the acceptability criteria include (1) false-negative rates of less than 5% for analytical results at target level; (2) false-positive rates of less than 10–15% at target level; (3) a known threshold level (cut-off level) for an intended matrix; and (4) a confirmation method for positive results (against a validated reference method)" (Trucksess & Zhang, 2016).

WHO has challenged the scientific community concerned with mycotoxins to develop low-cost, low-technology, accurate detection methods for preventing human and animal exposure to aflatoxins and avoid episodes of aflatoxicosis such as those that occurred in Kenya in 2004. Shephard (2016) indicated that sampling remains a problem in many developing countries because subsistence farmers in these countries do not produce enough grain to spare the large quantities needed for testing. However, organizations such as the Partnership for Aflatoxin Control in Africa and the World Food Programme are addressing these issues. The World Food Programme has instituted the Purchase for Progress programme to ensure grain quality by creating the Blue Box, which contains test kits for grain quality, including aflatoxins (World Food Programme, 2011, 2014). Even though such steps are few and far between, the awareness of the need to create rapid, low-cost aflatoxin detection methods and devices has grown.

4. Sampling protocols

The presence and extent of fungal infection of agricultural products are sporadic in grain and nut products. Consequently, mycotoxin contamination of these commodities is inherently non-homogeneous in nature. This problem is especially acute for aflatoxin contamination in nuts and large grain cereals and can be related to the nature of the fungal infection and the counts (kernels) per unit mass of the commodity. This inhomogeneity in contamination means no two samples taken from a batch of agricultural product will be the same and that the true contamination level can only be known by analysing the complete batch. This problem in determining the true contamination level has been known for a long time; particular note was made of it at the fifty-sixth JECFA meeting (Annex 1, reference *152*).

The problem of sampling for aflatoxins (as indeed for all mycotoxins) has been addressed by statistical means and the drawing up of sampling plans. A manual aimed at addressing sampling procedures and written for both food analysts and regulatory officials and explaining some of the statistical issues was produced as part of the Joint FAO/IAEA Programme, Nuclear Techniques in Food and Agriculture (Whitaker et al., 2010). The Food and Agriculture Organization of the United Nations (FAO), in collaboration with the Italian National Institute

of Health, produced a training video showing practical applications (http://www.soluzionepa.it/produzioneaudiovisivi.html; Brera, Miraglia & Pineiro, 2007). The Italian Ministry of Health, with the Istituto Superiore di Sanità and the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, has produced a general video on sampling from the perspective of the European Union (www.iss.it/myconews/?lang=2&id=86&tipo=16; ISS, 2015).

In general, sampling plans specify, among various items, the size of lots, sublots, incremental samples, aggregate samples, laboratory samples and test portions. In some instances, such as that for regulatory control of aflatoxins in the European Union (European Union, 2006, 2010), the analytical performance of the test method is also specified. The best known and easily accessible plans are those from the Codex Alimentarius Commission (FAO/WHO, 2001, 2004) and from the European Union.

In generating contamination data for regulatory control purposes, official laboratories are required to ensure their samples are obtained by official sampling plans. The sampling plans developed in the USA are based on the work of Whitaker and co-workers and, in turn, form the basis of the Origin Certificate Program in the USA (Adams & Whitaker, 2004). The setting of maximum levels for aflatoxins in various foods by the Codex Alimentarius Commission has been accompanied by relevant sampling plans. These plans for peanuts, almonds, Brazil nuts, hazelnuts and pistachios intended for further processing and for ready-to-eat almonds, Brazil nuts, hazelnuts, pistachios and dried figs can be accessed online (FAO/WHO, 2016).

This sampling problem should be kept in mind whenever data on aflatoxin occurrence need to be considered. Survey data in the scientific literature are frequently obtained using small sample sizes, whereas official data are usually more representative, provided sampling plans have been correctly implemented. Consequently, official data may be more reliable as an indicator of average contamination across a batch of product, but literature data certainly indicate the instantaneous levels to which consumers might be exposed.

Statistical analysis of analytical data can be represented in an operating characteristics curve. This curve describes, for a given set of sampling parameters, the statistical probability of commodity batches being accepted or rejected when their true contamination levels are, respectively, above or below the specified regulatory limit. Much of this statistical research has recently been placed in the public domain and consolidated by FAO in their development of a mycotoxin sampling tool, containing information on 26 mycotoxin–commodity combinations (http://www.fstools.org/mycotoxins/; FAO, 2013). In certain cases, the sampling plans approved by the Codex Alimentarius Commission have operating characteristics attached.

Apart from regulatory compliance, testing of mycotoxin contamination in food stores of rural subsistence farmers, who may be the population most at risk from mycotoxin exposure, remains a challenge as the use of large samples decreases the available food supply. Recommendations in this regard are contained in a recent IARC publication (IARC, 2012).

The problem of obtaining a representative sample is reduced (but not eliminated) in processed products, which include a ground form of the raw material. The aflatoxin contamination of milk may be considered to be reasonably homogeneous and most of the uncertainty in the determination probably relates to the analytical variability (Annex 1, reference *152*).

5. Effects of processing

Sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalization and extrusion are food processes that can potentially reduce mycotoxin levels (Bullerman & Bianchini, 2007). Therefore, these processing and decontamination procedures can be useful for reducing the level of human exposure to aflatoxins (Phillips, Clement & Park, 1994; Lopez-Garcia, Park & Phillips, 1999).

5.1 Physical methods

Unit operations like cleaning, sorting and handpicking (Dickens & Whitaker, 1975) are management strategies (Park & Liang, 1993) that remove aflatoxin from the entire lot and prevent the spread of fungal contamination to undamaged kernels during storage. In the peanut industry in the USA, for example, separation, blanching and sorting are combined at the processing level, resulting in cumulative reductions of aflatoxins (Table 9). Methods used are electronic sorting based on colour, seed size and density (Dorner, 2008), which can reduce aflatoxin levels by 70% in stored lots. Blanching has been reported to be an effective method of reducing aflatoxins in raw shelled peanuts (Whitaker, 1997). Thus, colour sorting after blanching (rather than at shelling) is more effective for detecting discoloration of peanuts (Whitaker, Giesbrecht & Slate, 2002; Dorner, 2008). Blanching (according to Whitaker, Giesbrecht & Slate, 2002) is a two-stage process where the skins of the peanuts are removed from the kernels and then the kernels are passed through a colour sorter that facilitates removal of discoloured and damaged kernels.

Mutungi et al. (2008) found that dehulling maize during its processing into muthokoi (a traditional Kenyan dish) decreased aflatoxin levels by an average

Table 9
Effectiveness of aflatoxin management strategies at the processing level on peanut products

Technology	Aflatoxin concentration (µg/kg)	Reduction (%)	Cumulative reduction (%)
Farmers' stock	217	–	–
Belt separator	140	35	35
Shelling plans	100	29	54
Colour sorting	30	70	86
Gravity table	25	16	88
Blanching / Colour sorting	2.2	91	99.0
Recolour sorting	1.6	27	99.3

Source: Park & Liang (1993)

of 47% (starting concentrations 10.7–270 µg/kg), while soaking and boiling further decreased the levels in muthokoi.

5.1.1 Sorting

Basic sanitary measures such as removal of dust and other debris prevents fungal growth and toxin production. The distribution of aflatoxin or the fungal infection in a stored maize, peanut or even pistachio lot is not uniform and may be concentrated in a small percentage of kernels. These damaged kernels can be distinguished based on discoloration, reduced size and altered shape (shrivelled). Sorting of these kernels not only removes the aflatoxin contamination in the entire lot, but also prevents the spread of fungal contamination to undamaged kernels during storage.

In addition to colour sorting by hand or machine, electronic sorting based on colour, seed size and density (Dorner, 2008) can reduce aflatoxin levels by 70% in stored lots. A 40–80% reduction in aflatoxin levels is possible when mould-damaged kernels, seeds or nuts are physically removed by cleaning and separation from intact raw samples (Park, 2002), as a major portion of aflatoxins has been associated with small and shrivelled (Davidson, Whitaker & Dickens, 1982) and mouldy or stained (Fandohan et al., 2005) peanuts. The disadvantage of this method is that not all shrivelled, discoloured or damaged kernels are contaminated with aflatoxins, and their removal results in yield losses (Waliyar et al., 2008).

Blanching of peanut kernels followed by photo-electric colour sorting and subsequent manual removal has been shown to be effective at reducing contaminated peanuts (Dorner, 2008). Because bright green-yellow fluorescence is a characteristic of aflatoxin-contaminated maize, cottonseed and pistachios,

fluorescence sorting was tried as a way of discarding contaminated peanuts (Pelletier & Reizmer, 1992); however, manual colour sorting was found to be more effective than either machine or fluorescence sorting.

Sorting has also been done by infrared reflectance (De Mello & Scussel, 2009). Flotation and density segregation in tap water or salt solution are effective methods and can remove over 90% of contaminated kernels of maize or peanuts (Phillips, Clement & Park, 1994; Matumba et al., 2015a). Currently available technologies used for sorting large-scale lots are infrared and UV sorting coupled with colour-detection technology (Womack, Brown & Sparks, 2014).

5.1.2 Wet milling and dry milling

During the wet milling of cereals such as maize, overall aflatoxins are not reduced but rather they are partitioned between the milling fractions. Bennett & Anderson (1978) and Wood (1982) found that aflatoxin was partitioned as follows: steep water, 39–42%; fibre, 30–38%; gluten, 13–17%; germ, 6–10%; and starch, 1%. Thus wet milling results in the loss of aflatoxins in steep water and the benefit of low levels in the main product, starch.

Dry milling can be a cost-effective industrial method of reducing aflatoxin levels in flour. Park (2002) found that after dry milling of maize in the USA, whole grits, low-fat meal and low-fat flour contained only 6–10% of the original aflatoxins, whereas the germ and hull fractions had the highest levels. Schroder, Boller & Hein (1968) found that dry milling and processing of rice caused the partitioning of aflatoxins, with the bran containing the highest concentration of the toxin compared with unprocessed grains; Scott (1984) found the same with wheat. Njapau, Muzungaile & Changa (1998) reported that when maize kernels were dehulled and soaked for 24 hours before grinding into maize flour, aflatoxin was reduced by 85–90%.

5.1.3 Heat treatments

Camoou-Arriola & Price (1989) noted reductions in aflatoxin levels when autoclaving at 121 °C and alkaline treatment of naturally contaminated maize, a process called nixtamalization, were carried out before frying this maize to make a snack. Méndez-Albores et al. (2004) reported that traditional non-alkaline toasting and boiling processes used to produce a meal of ground maize and beans (pinole) reduced chemically detectable aflatoxin in contaminated maize. Nixtamalization, which has been reported to reduce aflatoxin in maize, is a traditional process of cooking maize in lime water to produce nixtamal, which is then ground to form "masa" in Mexico (Torres, Guzman-Ortiz & Ramirez-Wong, 2001). These authors reported that traditional nixtamalization, which

involves both steeping and cooking maize, removed 52%, 84% and 79% of the aflatoxins in tortilla, tortilla chips and maize chips, respectively.

During processing, notwithstanding the relative stability of mycotoxins, there is a moderate reduction of aflatoxin at 150 °C (Bullerman & Bianchini, 2007). Conway et al. (1978) found that heating and roasting of maize reduced aflatoxin levels. For the processing of cassava bread, Adegoke, Akinnuoye & Akanni (1993) found that unit operations, such as milling, pre-gelling, battering and baking at 215 °C for 40 minutes, led to reductions in aflatoxin from 1.91 µg/kg in the raw cassava tuber to 0.11, 0.06, 0.06 and 0.03 µg/kg, respectively. During the production of "tuwo", a West African sorghum-based product, boiling for 30 and 60 minutes reduced aflatoxin levels by 68% and 81%, respectively (Adegoke, Otumu & Akanni, 1994).

Darman (2013) noted that unit operations of artisanal processing of sorghum in Cameroon reduced the levels of mycotoxin contamination as follows: winnowing, 15%; laying and threshing, 10%; husking and polishing, 38%; and cleaning of coarse paddy grains, 20%.

5.2 Fermentation

Scott (1991) reported a reduction of about 50% in detectable aflatoxin levels during the fermentation of wheat flour dough; baking of the dough alone resulted in reductions of 0–25%. Fandohan et al. (2005) noted that during the preparation of solid fermented maize dough in Benin, aflatoxin levels decreased during processing from 15.28 µg/kg in the raw maize to a non-detectable level in the final product (Table 10). These authors noted that sorting, winnowing, washing and crushing combined with dehulling were unit operations that were effective in removing aflatoxins from maize grain. During the production of "ogi", a West African maize product, the processes of steeping and fermentation caused a 73% reduction in aflatoxin from an initial level of 150 µg/kg to 41.25 µg/kg (Adegoke, Otumu & Akanni, 1994). Kpodo, Sorensen & Jakobsen (1996) found that cooking fermented maize dough for 3 hours resulted in a reduction in aflatoxin levels of up to 80%.

5.3 Extrusion

Extrusion involves applying a high temperature and pressure over a short time. Grehaigne et al. (1983) used extrusion to decontaminate peanuts. Cazzaniga et al. (2001) found that extrusion (at up to 180 °C) of maize did not substantially destroy aflatoxins, but Camargo, Fonseca & Ciacco (1989) observed a 75% reduction in aflatoxin levels during the extrusion of rice flour with 17–20% moisture content, a screw speed of 130 rpm and a temperature of 140–200 °C. Saalia & Phillips

Table 10
Mean total aflatoxin levels in maize products at each processing stage during the preparation of "mawe" (dry basis)

Maize product	pH	Mean total aflatoxin (µg/kg)
Raw maize	–	15.28
Washed maize	–	1.42
Washed grit	–	<1
Mawe	5.90	<1
Fermented mawe at 24 hours	4.14	<1
Fermented mawe at 48 hours	3.70	<1

Source: Fandohan et al. (2005)

(2011) obtained a 91% reduction in aflatoxins in artificially contaminated peanut meal using extrusion at 140 °C and moisture content of 20%.

Extruder design, moisture content and applied pressure are important variables that could have been responsible for differences in the findings of these studies. Also, it must be noted that there are reports of extrusion causing adverse physical and chemical transformations that affect the nutritional profiles of extruded materials (Phillips, 1989; Friedman, 1999). In looking for processes to reduce aflatoxins, the nutritive, physicochemical and sensory qualities of foods should not be negatively affected.

6. Prevention and control

6.1 Introduction

Aflatoxin contamination poses an annual global economic burden in the order of hundreds of millions of dollars as a result of discarding contaminated commodities and because of the health impacts from consumption of contaminated food. For example, for the entire value chain for farmers in the USA, the economic burden is estimated to be over $200 million in bad years (CAST, 2003; Mitchell et al., 2016b). Most countries have established maximum permitted residue limits for aflatoxin in food and feed. In spite of these regulations, human and animal populations continue to be at risk. In developing countries, particularly in Africa as well as in parts of Latin America and East Asia, exposures to aflatoxin are a serious health hazard (Wild, Miller & Groopman, 2015). Even if the regulatory capacity exists, large-scale sampling plans may deprive food-scarce farmers of both food and livelihood. Because of the stringency of regulatory limits imposed by developed countries, farmers are able to sell only the best grains and nuts

and consume the more contaminated material (e.g. Matumba et al., 2015b). Crop genotypes with greater tolerance to the relevant toxigenic fungi may be too expensive, and poor storage facilities coupled with poor weather may result in postharvest contamination (Trucksess & Zhang, 2016).

Contamination remains a significant food safety issue. A reduction in risk requires an integrated systems approach that includes targeted agronomic cultural practices, biological control methods and enhancement of host plant resistance, coupled with postharvest technologies such as proper drying, storage and, most of all, sorting of affected crop products with the development of appropriate alternative uses so that affected crops retain at least some economic value (Wild, Miller & Groopman, 2015).

6.2 Preharvest control

Interest in understanding the dynamics of aflatoxin contamination during the growth and harvesting seasons has increased in the last couple of decades because aflatoxins and toxigenic fungi come from the field, even though their content increases in harvested crops or crop products because of poor harvest management. Strategies for preharvest mitigation are designed to limit fungal invasion of crops by aflatoxigenic fungi and subsequent aflatoxin production.

6.2.1 Management practices

Strategies to minimize aflatoxin contamination in crops begin prior to planting. Decisions must be made about the cultivar to be planted, planting and harvesting dates, plant density, co-cropping and crop rotation as well soil treatments, irrigation and pest management (reviewed in Hell, Cardwell & Poehling, 2003; Wagacha & Muthomi, 2008; Waliyar et al., 2008; Abbas et al., 2009; Munkvold, 2014; Torres et al., 2014; Alberts et al., 2017). Although the contribution of each of these management practices may vary from location to location, they are considered to have a significant effect on reducing aflatoxin contamination when practised together. Environmental effects such as temperature and rainfall may negate the effect of all the management practices, however (Payne, 1998).

(a) Soil amendments and type

As the likelihood of aflatoxin contamination is lower in soils with higher water-holding capacity, the addition of soil conditioners that enhance water retention affects fungal infection (Torres et al., 2014). Waliyar et al. (2008) suggested that application of lime with natural fertilizers and conditioners can significantly reduce fungal infection and aflatoxin production in peanuts.

(b) **Fertilizer use**

Susceptibility of the maize or peanut crop to fungal contamination can be significantly affected by the nitrogen levels in soil, and addition of fertilizer to avoid plant stress can markedly reduce aflatoxin levels. Subsistence farmers can add manure to improve the nutritional status of the field and the condition of the soil, such as the microbiome and water-holding capacity.

(c) **Crop rotation**

To limit contamination by *Fusarium*-produced mycotoxins, wheat and maize should not be planted in rotation. However, rotations that include potato or vegetable crops are recommended. Continuous cultivation of peanuts in the same field can increase the population of *A. flavus*. The likelihood of aflatoxin contamination increased when maize was intercropped with cowpea (Hell et al., 2008).

(d) **Tillage**

Tillage can be beneficial or detrimental, depending on the soil type and location, because it alters the soil permeability (Payne, Cassel & Adkins, 1986). Tilled soils may dry more quickly, which may create drought-like conditions. In other conditions, tillage may improve the soil permeability by breaking up compacted layers. What is more important during tillage is to bury previous crops that may serve as a source of aflatoxigenic fungi.

(e) **Seed or variety selection**

Varieties of plants with seeds that are resistant to fungal infection should be used. In some cases, fungicide treatment of seeds prior to planting can achieve the same goal. In maize, hybrid selection for thicker kernel pericarp and tighter and longer husk coverage and adaptations to local conditions of abiotic stress may provide an advantage with respect to aflatoxin contamination (Munkvold, 2014).

(f) **Planting and harvesting dates**

Most studies have concluded that early planting reduces the risk of aflatoxin contamination in maize and peanuts, probably because of reduced insect activity (Waliyar et al., 2008; Abbas et al., 2009). Similarly, early harvest is preferred in all crops to prevent high aflatoxin levels. However, harvesting the crop at optimum maturity is recommended. For example, harvesting of overmature peanuts tends to be associated with higher toxin concentrations (Munkvold, 2014).

(g) Planting density

If there is excessive crowding of plants, there is increased competition for water and nutrition, resulting in severe plant stress. A reasonable distance between plants also prevents mechanical damage to the plant that could provide a port of entry for the fungus. The recommendations of the seed supplier and extension personnel and other information on agronomic characteristics should be considered to determine the ideal plant density for a particular crop or variety.

(h) Weed, fungal and insect control

Mechanical removal of weeds or by using herbicides has been found to be very beneficial in reducing levels of aflatoxins by reducing competition for resources. Use of fungicides has, however, provided very little relief against *A. flavus* infection (Abbas et al., 2009). Insect damage is one of the most significant sources of fungal infection because the damaged seed is more susceptible to fungal colonization. Use of registered insecticides could be useful in reducing this risk. A number of studies suggest that *Bt* (*Bacillus thuringiensis*) maize hybrids have lower aflatoxin concentrations than their isogenic counterparts, although this depends on environmental conditions and insect pressure (Wiatrak et al., 2005; Abbas et al., 2009; Ostrý, Malíř & Pfohl-Leszkowicz, 2015). The effect is not as consistent as for fumonisin.

(i) Irrigation management

Under ideal situations, adequate watering to avoid drought stress reduces aflatoxin contamination. Conversely, excessive watering can raise humidity in the field and promote fungal infection. Prolonged water deficit during the seed filling period and higher soil temperatures promote aflatoxin formation in peanuts (reviewed in Torres et al., 2014). It has been suggested that maintaining high kernel water activity until harvest has the potential for preserving the plant's natural defence mechanisms against aflatoxigenic fungi. In some cases irrigation during flowering and crop maturation needs to be avoided (Alberts et al., 2017). This cultural practice may be impractical in many areas where added cost of irrigation may not be profitable or water supplies may be limited (in arid and semi-arid areas).

6.2.2 Biological control

Among the many strategies that have been recommended for control of aflatoxins prior to harvest, biological control using non-toxigenic *A. flavus* has received significant attention. Other microorganisms, such as bacteria and yeast, have also been investigated for their ability to reduce this contamination. Methods to reduce aflatoxin contamination in food and feed using microbes have been reviewed by

Dorner (2004), Yin et al. (2009), Guan et al. (2011) and Bandyopadhyay et al. (2016).

(a) Bacteria

Several bacterial species have shown promise for reducing toxin contamination in various crops, but their efficacy in the field has not been clearly established (reviewed in Dorner, 2004; Yin et al., 2008). Bacterial species such as *Bacillus subtilis*, *Lactobacillus* spp., *Pseudomonas* spp., *Ralstonia* spp. and *Burkholderia* spp. isolated from almond samples (Palumbo, Baker & Mahoney, 2006) and non-rhizophere of maize soil (Nesci, Bluma & Etcheverry, 2005) may have some use in biocontrol application. Maize grain or walnut and pistachio seeds could also be protected by treatment with various bacterial strains (Haggag, Abd-El-Kareem & Saleh, 2014; Haggag, El-Habbasha & Mekhail, 2014).

Some recent studies (Qing et al., 2015; Al-Saad et al., 2016) have demonstrated that *Bacillus* and *Pseudomonas* spp. can significantly reduce the relative expression of aflatoxin biosynthetic pathway regulatory and structural genes under various conditions. Yet another study demonstrated that gamma-irradiated mutant strains of *B. subtilis* significantly reduced *A. flavus* sporulation and toxin production in treated pistachio nuts.

These organisms show some promise, but their practical application has yet to be determined. One recent study has shown, in greenhouse and field studies, that seed treatment or soil application of a powder-formulation of *B. subtilis* reduces the soil population of *A. flavus*, fungal infection and aflatoxin content in pods (Shifa et al., 2016).

(b) Yeasts

Saprophytic yeast species such as *Candida krusei* and *Pichia anomala* isolated from the fruits of almond, pistachio and walnut trees were shown to have the ability to inhibit *Aspergillus* growth under laboratory conditions (Hua, Baker & Flores-Espiritu, 1999; Masoud & Kaltoft, 2006). *Saccharomyces cerevisiae* (Rahaie et al., 2010; Prado et al., 2011; Pizzolitto et al., 2013) and *Candida parapsilosis* (Niknejad et al., 2012), as well as many other species (Afsah-Hejri, 2013), have also been shown to have some potential for controlling aflatoxigenic fungi during food and feed storage. Some field experiments have demonstrated that yeast sprayed onto pistachio trees can reduce *A. flavus* populations in orchards (Hua, 2006) and reduce aflatoxin levels in corn fields (Isakeit et al., 2005). However, the preparation of stable formulations with prolonged shelf-lives remains a work in progress (Hua et al., 2015).

(c) Non-toxigenic *Aspergillus flavus*

The species implicated as the causal agent of aflatoxin contamination events is *A. flavus*, which is composed of individual strains that vary widely in many characteristics. This diversity within *A. flavus* has been extensively studied to gain insights into the etiology, epidemiology and management of aflatoxin contamination. Certain lineages of *A. flavus* demonstrate relatively high virulence to plants and produce very large concentrations of aflatoxins, whereas other lineages are atoxigenic (non-aflatoxigenic) and produce no aflatoxins. As a result, these lineages are important etiological agents of contamination and important targets of management practices. Several of the atoxigenic *A. flavus* strains have been developed into biopesticides for the management of aflatoxin contamination. With the complete characterization of the genetics of the aflatoxin biosynthesis and the whole genome sequencing of *A. flavus* completed (Payne et al., 2006; Bhatnagar et al., 2008), it is easy to determine the reason for a strain's atoxigenicity. Almost all of the strains studied have mutations or deletions in various genes in the aflatoxin biosynthetic cluster. For example, the atoxigenic strain used for biocontrol in cotton in the USA (AF36) contains a mutation in *pksA*, a gene responsible for a very early step in aflatoxin biosynthesis, whereas the biocontrol agent NRRL21882 has a deletion of the entire biosynthetic cluster (strain registered as AflaGuard by Syngenta; Abbas et al., 2009).

The assumption with using atoxigenic strains as a biological control strategy is that the atoxigenic strain occupies the same niche as the toxigenic strain when applied to soil in the field, and that the naturally present toxigenic wild-type strain is physically displaced or competitively excluded when the crop is infected. For this strategy to work the following conditions need to be met: (1) atoxigenic strains must be selected from local environments because they would be well adapted to the agro-ecological system of the field of application, and will not cause any change in the native population of the location; (2) the reason for the atoxigenicity (with respect to the aflatoxin biosynthetic pathway) must be clearly established; (3) the ability of the selected strain to make other known toxic metabolites must be well characterized; (4) the applied atoxigenic strain must be highly competitive (Shieh et al., 1997); and (5) the applied atoxigenic strain should be delivered in such a way that its conidial population is the dominant one relative to the toxigenic strain during the crop infection process and, consequently, the timing and mode of application are crucial factors in achieving success (Wild, Miller & Groopman, 2015; Bandyopadhyay et al., 2016). Environmental factors, such as moisture levels (for example, the presence of dew or very dry soils or heavy rain) can affect the efficacy of this biocontrol approach (Bock & Cotty, 1999).

Biological control using atoxigenic strains of *A. flavus* in field conditions has been successfully used in parts of the USA where it has been found to achieve 70–90% reductions in aflatoxin levels in various crops, especially cotton, peanuts, maize and pistachios (Cotty, 1990, 1994; Dorner & Cole, 2002; Abbas et al., 2006; Dorner, 2008). Excessive rainfall can wash away the applied biocontrol formulation, reducing its effectiveness (Wild, Miller & Groopman, 2015). Various products and formulations have been registered for application in different parts of the world (reviewed in Dorner, 2004; Yin et al., 2009; Torres et al., 2014; Chulze et al., 2015; Bandyopadhyay et al., 2016). After the success of the biocontrol strategy in the USA in cotton, peanuts and maize, this technology has been improved for use in areas of sub-Saharan Africa where high levels of aflatoxin contamination in maize and peanuts are a chronic problem (Bandyopadhyay et al., 2016). With international funding of various projects with the International Institute of Tropical Agriculture (IITA) in Nigeria, and in cooperation with the Agricultural Research Service of the United States Department of Agriculture (USDA) and other partners, adaptation of the biocontrol technology for maize and peanuts was developed for use in some African nations (e.g. the Gambia, Kenya and Senegal) (Cardwell & Henry, 2004). Significant effort to implement the biocontrol approach in these countries is underway, while registration is being sought for biocontrol products specific to Burundi, Ghana, Malawi, the United Republic of Tanzania, Uganda and Zambia, among others (Bandyopadhyay et al., 2016). The number of participating countries is expected to increase. Unlike the biocontrol products in the USA, which contain only one fungal strain as the active ingredient, the IITA-developed product, under the trade name Aflasafe, contains at least four native strains to provide long-term stability and additive beneficial effects in diverse environments (even though applications need to be repeated year after year). This strategy has also been tested and incorporated for peanuts in Australia (Pitt & Hocking, 2006), Argentina (Chulze et al., 2015) and China (Yin et al., 2009). Research carried out in Thailand for control of aflatoxin contamination in maize showed promising results for both pre- and postharvest control. However, the results were inconsistent (Pitt et al., 2015).

The mode of application of the biocontrol formulation has been experimented with, and the carrier or substrate for the atoxigenic strain has been more or less established based on cost and efficacy. Aerial sprays of homogenized fungal cultures showed inconsistent results and were not cost-effective. Soil application prior to planting or in the early stages of crop growth was effective in controlling aflatoxin contamination. For these applications, solid matrices such as wheat or rice grains (more recently sorghum) are sterilized and mixed with fungal inoculum under agitation for a complete coating of the matrix, without clumping and fungal sporulation. The grains are then scattered over the field, and the fungus sporulates rapidly, producing numerous spores on the surface of

the grain. This inoculum, growing rapidly on the nutritional source provided, gets dispersed in the soil and competes with toxigenic strains in the field, which have to compete with other microflora in the soil for their nutrition. Inoculum rates and time of application are very important components for this strategy to be effective. Every application parameter has to be worked out for each location and crop.

(d) Special considerations on biocontrol

Biopesticides formulated with atoxigenic *A. flavus* strains are used over very large acreages of maize, cotton, peanuts and pistachios each year in the USA (Bandyopadhyay et al., 2016). A number of countries in Africa are evaluating formulations tailored to local environments for control of aflatoxin in maize. However, as with any new technology, several uncertainties remain about this approach with respect to the potential for any residual minimal risks to humans, animals or the agro-ecosystem (King et al., 2011; Ehrlich, 2014; Moore, 2014; Ehrlich et al., 2015; Alberts et al., 2017).

Bandyopadhyay et al. (2016) and Wild, Miller & Groopman (2015) have also reviewed these uncertainties, particularly in the context of the use of this technology in Africa. The potential for reducing or increasing levels of other mycotoxins (such as fumonisins) as a result of these applications is still under investigation. Studies are also needed to assess the impact of the addition of biocontrol strains on the population dynamics of *A. flavus*, especially in the context of climate change. Similarly, with the discovery of the sexual stage of this fungus, the probability of these atoxigenic strains reverting back to toxin producers through recombination is not known. This is of particular concern when there is only a single mutation in one gene that results in atoxigenicity. The cost, especially to subsistence farmers, of the biocontrol product and the cost of its application in the field year after year must be addressed. Bandyopadhayay et al. (2016) have responded to the apprehensions about the use of atoxigenic strains in biocontrol formulations. These responses are related to concerns such as "use of sorghum as a carrier, distribution costs, aflatoxin-conscious markets, efficacy during drought, postharvest benefits, risk of allergies and/or aspergillosis, influence of Aflasafe [the IITA-developed biocontrol product], on other mycotoxins and on soil microenvironment, dynamics of *Aspergillus* genotypes, and recombination between atoxigenic and toxigenic genotypes in natural conditions".

The efficacy of the biocontrol agent declines during subsequent years unless reapplied. This indicates that the composition of the fungal population is dynamic in natural conditions. The diversity of the vegetative compatibility groups after application of biocontrol agents suggests that the *Aspergillus*

population changes as a result of application of the biocontrol strain. At the time of writing, the available data suggest that the strains used in the formulations have high genetic stability (Bandyopadhyay et al., 2016). Nonetheless, the potential for sexual recombination in field conditions, even at very low frequency, is real (Horn et al., 2014, 2016), and its impact on the atoxigenic population must continue to be studied in detail (Wild, Miller & Groopman, 2015). Long-term sampling of soil populations using extensive genetic analysis provided by newer DNA sequencing technologies is underway to look carefully at genetic shifts after long-term applications of biocontrol agents (Grubisha & Cotty, 2015).

6.2.3 Host resistance

The most long-term, stable solution to control preharvest aflatoxin contamination is by enhancing the ability of the host crop to prevent fungal infection and/or the production of aflatoxins by the invading fungus. This can be achieved through plant breeding or genetic engineering of crops of interest. These processes are laborious and extremely time consuming, but with the advent of new technologies such as genomics, proteomics and transcriptomics, the process of understanding and utilizing host–pathogen interactions to enhance host plant resistance has been significantly speeded up.

(a) Plant breeding

Developing host plant resistance against fungal infection or aflatoxin production through traditional methods is a highly desirable strategy. However, plant breeding is very time consuming and labour intensive, taking many years and many cropping cycles to arrive at the desired result. Breeding efforts to obtain germplasm resistant to aflatoxin accumulation is particularly challenging because of strong environmental pressures on infection and aflatoxin production by *A. flavus* (reviewed in Payne, 1998; Brown et al., 2013a,b; Warburton & Williams, 2014; Bhatnagar-Mathur et al., 2015; Fountain et al., 2015).

Maize

Maize ear inoculation techniques (such as silk inoculation or pin-bar inoculation through husks) have been used to screen for resistance. As early as 1988, five lines were identified in Mississippi, USA, that exhibited resistance to *A. flavus* kernel infection. Two out of these five lines, Mp313E and MP420, were the first real sources of resistance against fungal infection (reviewed in Williams et al., 2014). In Georgia, USA, another germplasm, GT-Mas:gk, was generated that demonstrated resistance.

The inbred lines Tex6, Y7 and Mp420 were shown to be highly resistant in midwestern USA (Betran, Isakeit & Odvody, 2002). Whether the lines

generated in the Midwest demonstrate resistance in Mississippi is unknown; the environmental conditions in the southern USA are more conducive to fungal growth and toxin formation. Several maize lines developed by the International Maize and Wheat Improvement Center (CIMMYT) have shown resistance to aflatoxin contamination in studies in Texas, USA (Betran, Isakeit & Odvody, 2002).

Germplasm showing resistance against aflatoxin contamination was developed in Georgia, USA (GT601, GT602 and GT603; Guo et al., 2007, 2011). From this, four lines were developed in Mississippi as sources of resistance – Mp715, Mp717, Mp718 and Mp719 (Williams & Windham, 2001, 2006, 2012).

Menkir et al. (2008) released six tropical maize germplasm lines with resistance to aflatoxin accumulation developed by a plant breeding collaboration of the Agricultural Research Service of the USDA and the IITA (Menkir et al., 2006). These lines have been developed by combining resistant traits of inbred lines in the USA with those of African lines; they have shown resistance in different geographical regions of both Africa and the USA. These developments were speeded up by the development of a kernel screening assay (Brown et al., 1997; Du et al., 1999), making it possible to rapidly assess fungal infection levels in individual kernels and to predict the corresponding aflatoxin levels in the field. The Germplasm Enhancement of Maize (GEM) project has identified resistant germplasm that is being further tested (Li et al., 2002; Henry et al., 2013). Plant breeding and varietal selection have provided significant maize genetic material that demonstrates resistance; however, no commercial lines have yet been marketed.

Peanuts

Several early efforts to breed peanut lines resistant to aflatoxin formation were undertaken in the USA and at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) (Mehan, McDonald & Rajagopalan, 1987; Cole, Dorner & Holbrook, 1995) with very limited results (Waliyar et al., 1994). Some of these genotypes were used to develop elite lines that showed resistance against aflatoxin production that have been released as improved germplasm but do not show resistance under all agro-ecosystems (Waliyar et al., 2008). Similarly, the peanut breeding programme in the USA has not yielded many aflatoxin-resistant lines (Guo et al., 2009; Holbrook et al., 2009; Fountain et al., 2015). Some of these lines are drought resistant and have been shown to accumulate reduced levels of aflatoxins. Six accessions have shown over 90% reduction in aflatoxin levels over multiple years of testing (Holbrook, Wilson & Matheron, 2002), and these have been hybridized to combine resistance with desirable agronomic characteristics.

(b) Molecular breeding

The difficulty in finding resistant lines through traditional breeding is that the phenotypic or agronomic characteristics that the breeder needs to look for are difficult to define. Moreover, resistance to *A. flavus* is not conferred by a single gene but is a quantitative trait needing the combined effect of multiple genes (Kelley et al., 2012). Therefore, identification of markers to facilitate the transfer of resistance traits into desirable genetic backgrounds is essential. Marker-assisted breeding or selection is a process where traits of interest (in this case, disease resistance or toxin production) are linked to specific morphological, biochemical or genetic markers rather than to the trait itself. The assumption is that the marker gene or quantitative trait locus (QTL) of interest associates at high frequency with disease resistance due to genetic linkage (i.e. close proximity on the chromosome of both traits, namely the marker locus and the disease resistance–determining locus).

Various techniques such as quantitive real-time polymerase chain reaction (qRT-PCR) analysis, microarray analysis and QTL mapping have been used to identify chromosomal regions associated with resistance to *Aspergillus* ear rot and aflatoxin inhibition (reviewed in Bhatnagar-Mathur et al., 2015, and Fountain et al., 2015). Many QTLs have been reported for resistance to fungal infection and aflatoxin production in maize (Brooks et al., 2005; Warburton & Williams, 2014) located on chromosomes 2, 3 and 7 (Paul et al., 2003) and 4 (Busboom & White, 2004). Recently, researchers have linked genome-wide association studies with metabolic pathways analysis to better understand the mechanism of host defence (Tang et al., 2015). "In peanut, molecular marker assays for aflatoxin resistance have detected very little variation at the nucleic acid level, even though considerable variation has been detected in cultivated peanut varieties for agronomic and morphological traits" (Bhatnagar-Mathur et al., 2015). However, several DNA markers associated with reduced aflatoxin levels were identified from interspecific hybrids (Milla-Lewis et al., 2007), suggesting the possibility of using molecular marker-assisted breeding for resistance in peanuts.

With the tools of proteomics and genomics for rapid screening of the entire protein or gene profile, respectively, of maize, several resistance-associated proteins (RAPs) have been identified from kernel, rachis and silk tissues that have been shown to be associated with resistance to fungal infection or toxin production (Brown et al., 2013a,b; Chen et al., 2015). A comparison of protein profiles of resistant and susceptible germplasm provided evidence of the higher presence of specific proteins in resistant germplasm. The maize proteins contributing to resistance can be divided into four categories: (1) antifungals; (2) stress-related;

(3) storage; and (4) other. A few examples are shown in Table 11. (Additional information is available in Brown et al., 2013a,b and Chen et al., 2015.)

The genes for several of these RAPs are located in the chromosomal regions associated with resistance as identified in QTL mapping studies. The maize kernel resistance is associated with both constitutive and induced proteins, and resistant lines constitutively express higher levels of antifungal proteins. The reason for this may be that the constitutive proteins delay fungal invasion for long enough for the induced proteins to be synthesized by the plant to fend off the infection. To verify if these proteins do indeed play a role in resistance, RNA interference (RNAi) studies have been undertaken to genetically engineer maize in order to silence the genes responsible for producing these proteins and measuring the impact on fungal growth or toxin production (reviewed in Brown et al., 2013a,b; Chen et al., 2015).

With the difficulties in breeding for resistance, developing transgenic lines with antifungal traits could be extremely valuable, especially in crops like cotton where no known varieties have shown resistance to *A. flavus* infection. Several antifungal proteins and peptides of plant and insect origin have been identified and have been assayed for their effect on *Aspergillus* spp. (Cary et al., 2009; Rajasekaran et al., 2012), with promising results. Transformation of crops such as peanuts, maize and cotton with antifungal proteins and lytic peptides has demonstrated inhibitory activity against *A. flavus* infection (reviewed in Rajasekaran et al., 2012; Bhatnagar-Mathur et al., 2015).

More recently, the effectiveness of RNAi technology in silencing five aflatoxin biosynthetic pathway genes in the fungus was demonstrated when transgenic peanuts containing the RNAi vectors were inoculated with *A. flavus*; the result was a lack of aflatoxin contamination in these seeds (Arias, Dang & Sobolev, 2015). With insect control in transgenic *Bt* corn, peanuts and cotton, reduced levels of aflatoxin contamination have been recorded (reviewed in Guo et al., 2009).

In spite of the promising results with this approach, success with the genetic engineering technology will depend on (1) identification of desirable resistance genes; (2) development of the right and efficient genetic transformation methods for each crop; (3) design of gene constructs that will allow the expression of the desired genes in the right plant tissue, at the right time and in enough quantities of the antifungal peptide or protein for maximal effect; and (4) consumer acceptance of transgenic crops.

Table 11
Examples of identified RAPs in maize

Antifungals	Stress-related	Other
Zeamatin	Aldose reductase (ALD)	Serine/threonine protein kinase
Trypsin inhibitor 14 kDa (TI)	Pathogenesis-related protein 10 (PR-10)	Translation initiation factor 5A
Glyoxalase I (GLX I)	Peroxiredoxin 1 (Per1)	
TI-10 (10 kDa)	Cold-regulated protein (COR)	
β-1,3-glucanase	Water stress inducible (WSI)	
Ribosome-inactivating protein (RIP)	Anionic peroxidase	
	Heat Shock 16.9	
	Globulin I	
	Globulin II	
	Late embryogenesis abundant protein (LEA) III	
	Late embryogenesis abundant protein (LEA) 14	
	Cupin domain containing protein (Zmcup)	

RAP: resistance-associated protein

6.3 Postharvest control

The preharvest contamination of commodities with aflatoxins is generally limited to maize, cottonseed, peanuts and tree nuts. But postharvest contamination can be found in a variety of other agricultural crops, for example, coffee, rice and spices. This contamination during storage can be influenced by factors such as moisture, temperature, mechanical or insect damage to commodities, aeration and the level of fungal inoculum. Measures against postharvest aflatoxin contamination must address these conditions. Other measures can be taken to remove aflatoxins from already-contaminated commodities (Hell et al., 2008; Waliyar et al., 2015).

6.3.1 Moisture control at storage

Grain moisture content greater than 10% results in fungal growth and aflatoxin production in peanuts. Similarly, holding *A. flavus*–contaminated maize at kernel moisture content above 18% for more than 4–6 hours can rapidly increase aflatoxin contamination. Once fungal growth starts, the moisture from the metabolism is sufficient for further growth and aflatoxin production. If environmental relative humidity is maintained at 70% and temperature at 25–27 °C, peanuts can be stored safely for a year or longer (Waliyar et al., 2008; Torres et al., 2014).

Drying of harvested products is the most effective way to prevent toxin accumulation. Rapid drying of agricultural products reduces moisture content and prevents conditions favourable for fungal growth (reviewed in Chiewchan, Mujumdar & Devahastin, 2015). Sun or solar drying is a traditional method that has practically no cost associated with it. However, it takes a very long time

to achieve the right moisture content, and the availability of sunlight can vary during drying periods.

Solar drying should be done on a clean surface other than the ground to prevent fungal contamination from the soil. Mechanical drying using electric dryers adds operating cost, but is more consistent and reliable. Convective dryers have been used in superheated steam-drying to provide heat from superheated steam. The end result is a product that is better in appearance and in nutritive value than the products of the other drying methods. In addition, convective drying may cause some microbial inactivation.

6.3.2 Storage conditions

Basic sanitary measures are essential to prevent a build-up of aflatoxin contamination in storage conditions. A thorough cleaning of harvesting equipment and storage facilities with water and/or compressed air is recommended. The area surrounding the storage facility should be kept clear of any items that could increase insect infestation. The storage facilities must be dry and well-ventilated. Care must be taken to routinely monitor temperature and fungal growth (Hell et al., 2000; Alberts et al., 2017).

Drying and adequate storage do not reduce the aflatoxin already present in a commodity but they help to avoid further toxin production. Turner et al. (2005) found a 60% reduction in the mean levels of aflatoxin in groundnuts when the following intervention strategies were used as postharvest measures in Guinea: (1) initial hand sorting to remove mouldy peanuts and damaged shells; (2) proper drying using the sun; (3) use of natural-fibre jute bags and wooden pallets on which the bags of ground nuts can be stored to prevent build-up of humidity; and (4) prevention of insect damage. Aflatoxin development in stored maize grains can be stopped through thorough cleaning, proper cooling and keeping moisture levels below 12–13% (Sumner & Lee, 2012).

Hell et al. (2000) found that cleaning the stores before loading new produce correlated with the reduction of aflatoxin levels in maize in four agro-ecological zones in Benin.

Adoption of food safety practices like hazard analysis and critical control point (HACCP) systems can therefore be useful in reducing levels of aflatoxins (FAO/IAEA, 2001; Aldred & Magai, 2004).

Aflatoxin contamination can be prevented or reduced by altering the air gas composition – of carbon dioxide, nitrogen and sulfur dioxide – in silos (Kabak, Dobson & Var, 2006). An increase in carbon dioxide levels resulted in significant reduction in aflatoxin production (Heathcote & Hibbert, 1978; Magan & Aldred, 2007). Smoking grains during storage can reduce moisture content

and fungal infestation (Hell & Mutegi, 2011). Use of desiccants such as calcium chloride and silica gel during storage is also considered good practice.

6.4 Decontamination

Chemical methods to decontaminate feed of aflatoxins using chemicals such as ammonia, calcium hydroxide and ozone have been examined. Aflatoxins in animal feed made up of peanut meal, maize and cottonseed have been inactivated in various industrial processes with ammonia or sodium bisulfite (Rustom, 1997; Méndez-Albores, Del Río-García & Moreno-Martínez, 2007; Kolosova & Stroka, 2011). France, Mexico, Senegal, the United Kingdom and the USA are among the countries that have experimented with the ammoniation process to decontaminate feed (Proctor, 1994; Huwig et al., 2001; Park, 2002). This process is used in some parts of the USA to decontaminate cottonseed for cattle feed. The drawback of this method is the added cost and possible discoloration of the product. In addition, the transformation products are not readily known and could potentially be toxic.

Ozone has been approved for use as a disinfectant in the food industry. Ozone gas has been used to detoxify aflatoxins in red pepper (Inan, Pala & Doymaz, 2007), maize (Prudente & King, 2002), peanuts (Proctor et al., 2004), pistachios (Akbas & Ozdemir, 2006), dried figs (Zorlugenc et al., 2008) and other dried foods (Karaca, Velioglu & Nas, 2010).

Because the use of chemicals for detoxification can lead to harsh reactions, biological detoxification methods have been examined for minimal reduction in nutritive value, discoloration or palatability. A large number of microorganisms have been examined for their efficacy in microbial degradation of AFB_1 (reviewed in Wu Q et al., 2009; McCormick, 2013; Kong et al., 2015; Verheecke, Liboz & Mathieu, 2016). An example of bacteria used in aflatoxin risk mitigation is the well-studied *Lactobacillus* spp. (Ahlberg, Joutsjoki & Korhonen, 2015). Fungal growth and toxin production can be reduced by competition between the bacteria and the fungi for nutrition, and even dead bacterial cells have been reported to bind aflatoxins, reducing the risk.

Enzymes of microbial origin have also been examined for their ability to detoxify aflatoxins, and their mode of action has also been examined. Extracellular enzymes from an edible fungus *Armillariella tabescens* convert aflatoxin to a less toxic or mutagenic compound (Liu et al., 1998). A polyphenol oxidase enzyme (laccase) from the white rot fungus, *Pleurotus ostreatus*, as well as the mushroom *Trametes versicolor* degraded aflatoxins (Motomura et al., 2003; Alberts et al., 2009). It appears that microbial detoxification of aflatoxins could become a viable strategy in the future as a result of all the advances in molecular technology in

biology and chemistry (1) to identify the active ingredient (enzyme) produced by the microorganisms; (2) to improve the efficacy of the enzymes; (3) to mass produce the enzymes; and more importantly (4) to understand the by-products of the detoxification to ensure that the transformation does not cause further toxicity.

Ever since the discovery of aflatoxins, extensive research has been undertaken to examine the possibility of using natural products, primarily of plant origin, to reduce aflatoxin contamination. Numerous compounds of diverse chemistry and various extracts containing activity inhibitory to aflatoxin biosynthesis have been reported; these are too numerous to list here. A few reviews (for example, Holmes, Boston & Payne, 2008; Razzaghi-Abyaneh, Shams-Ghahfarokhi & Chang, 2011; Friedman & Rasooly, 2013) provide insight into several of the inhibitory compounds and their potential modes of action. Many of these compounds could be used effectively as protectants during storage. Some, being of plant origin, could be pathway-engineered for expression in specific crop tissues to prevent aflatoxin biosynthesis (Holmes, Boston & Payne, 2008).

6.5 Enteroabsorbents

A relatively recent approach to reducing exposure to aflatoxins is the use of "mycotoxin binders" in the diet to prevent absorption of aflatoxins in the gastrointestinal tract and subsequent distribution in the bloodstream to target organs (reviewed extensively in Kolosova & Stroka, 2011; Kensler et al., 2012; Di Gregorio et al., 2014; Miller et al., 2014; Womack, Brown & Sparks, 2014).

Clay-based products are in use as enteroabsorbents in animals to reduce exposure to aflatoxins (Phillips, Clement & Park, 1994). With the issuance of European Union Regulation (EC) No. 386/2009 on additives for use in animal feed, a new category was added: "substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action". This action, in May 2009, has resulted in a thorough examination of clays such as bentonites, zeolites and hydrated sodium calcium aluminosilicate (HSCAS) as feed additives for binding aflatoxins effectively. Calcium montmorillonite clay (also known as NovaSil clay) is one of the most commonly used because it is an extremely effective enterosorbent that tightly and selectively binds aflatoxins in the animal gut (reviewed extensively in Kolosova & Stroka, 2011; Di Gregorio et al., 2014).

The main problem with clays is that they absorb several micronutrients including trace elements. In addition, natural clays are sometimes contaminated with heavy metals (e.g. lead, cadmium) or dioxins (Marroquin-Cardona, 2011).

Similarly, the problem with silica-based polymers is that they need to be in the feed at a high inclusion level (5–20 g/kg feed) to be effective.

6.6 Predictive modelling

Over the last few decades, a number of research groups have attempted to correlate various environmental factors with the potential for *A. flavus* growth and consequently aflatoxin production, both in preharvest and postharvest situations. "Predictive analytics" is an emerging discipline where large volumes of data are mined to extract information and use it to predict outbreaks. In contrast to models for *Fusarium* head blight and deoxynivalenol, for which government and commercial predictive models are widely used by farmers in Canada and the USA, there are no commercially successful models to predict aflatoxin in any commodity. An attempt was made to commercialize one model in Australia (Afloman), but this was ultimately discontinued, an important reason likely being the very large investment needed to collect weather, agronomic and climate data in each new region over many years, and with new cultivars each year. Two widely used predictive models for *Fusarium* head blight and deoxynivalenol were developed over 10 years and also required considerable public and private investment, with data obtained on deoxynivalenol in the crop at final harvest each year (Schaafsma & Hooker, 2007; Bianchini et al., 2015).

The reliability and predictive power of the results of successful models depend entirely on the quality and number of data points from farmers' fields coupled with about a decade of field experience to refine the model. However, with the significant negative impact of aflatoxin contamination of field crops and stored commodities on food safety, economics and sustainability of the food supply, methods to forecast aflatoxin contamination have received more attention in largely academic studies.

6.6.1 Field application of models

(a) Peanuts

Some early predictive models (regression models) used parameters such as soil temperature, soil moisture stress, crop stage, planting dates, etc., to determine and establish the potential for fungal infection and aflatoxin contamination as well as crop yield in some instances (Thai et al., 1990; Parmar et al., 1997; Henderson et al., 2000). These studies concluded that "these relationships could form the basis of a decision-support system to predict the risk of aflatoxin contamination in peanuts in similar environments" (Craufurd et al., 2006). However, the models were incapable of predicting all but extreme concentrations of aflatoxin (Henderson et al., 2000).

Subsequent models to assess aflatoxin risks in peanuts were developed in Australia as part of the Agricultural Production Systems Simulator (APSIM) peanut module, which calculated the aflatoxin risk index (ARI) using temperature and soil water as variables in the last 4–6 weeks of the pod-filling stage of peanut growth (Chauhan et al., 2010). The ARI model, with data from eight peanut grower fields, showed a significant correlation ($r = 0.95$) with the level of aflatoxin contamination. The authors of the study concluded that under the conditions in Queensland, Australia, "ARI simulated by the model is a reliable indicator of aflatoxin contamination that can be used in aflatoxin research as well as a decision-support tool to monitor preharvest aflatoxin risk in peanuts" (Chauhan et al., 2010). An attempt was made to commercialize this model, but it did not prove to be useful in practice and was discontinued.

(b) Maize

Chauhan, Wright & Rachaputi (2008), using primarily temperature and soil moisture during the grain-filling period of the maize crop and aflatoxin, developed the ARI for maize. This model had a moderate correlation ($R^2 = 0.69$; $P < 0.01$) between the prediction and the actual aflatoxin contamination levels in rain-irrigated areas of Australia and a lower correlation ($R^2 = 0.62$; $P < 0.01$) when irrigated locations were included in the analysis. This study also showed that factors such as sowing time, length of growing season, plant density and, of note, the maize hybrid grown affected the ARI.

The same group developed a soil moisture stress model for maize in Queensland and northern New South Wales, Australia (Chauhan et al., 2013). An attempt was made to apply this model to maize production conditions in Kenya using five test sites. Not surprisingly, soil moisture stress (drought) explained 98% of the variance of aflatoxin concentration at harvest. However, the relationship between soil moisture stress was dependent on the hybrid planted. Further, variation in insect damage, which is a major driver of crop aflatoxin contamination, rendered the model less useful (Chauhan et al., 2015).

Battilani & Leggieri (2015) reviewed the status of development and validity of models for predicting preharvest aflatoxin contamination in maize. Battalani, Barbano & Piva (2008) developed an aridity index in response to a severe aflatoxin outbreak in Italy in 2003. This predictive model was developed using meteorological data and predicts aflatoxin contamination risk during maize development stages between July and September (Fig. 3). The model had moderate accuracy (64%) and was more suited to making predictions for larger areas than for individual farms. Battilani et al. (2013) developed a model to predict aflatoxin concentrations in maize resulting from *A. flavus* infection. The model was weather-driven based on the infection cycle of the fungus in maize from silk

Fig. 3
Risk maps for aflatoxin contamination in maize at harvest in different climate scenarios[a]

[a] Three different climate scenarios: (a) present; (b) +2 °C; (c) +5 °C. Mean daily data used as input result from 100-year run of the predictive model "AFLA-maize" in 2254 geo-referenced points throughout Europe, in the three scenarios. The scale 0–200 refers to the aflatoxin risk index (ARI) output from the predictive model; increasing the number, the risk of contamination increases.
Source: Battilani et al. (2016).

emergence to the harvest. Model inputs included hourly temperature, relative humidity and rainfall readings along with field data on aflatoxin contamination levels in Italy. The main purpose of this model was to create a probability index for exceeding the European legal limit for aflatoxin contamination of 5 µg/kg of unprocessed maize. It was determined that the model prediction for such a probability was correct for 73% of the fields, and 68% when using data not included in model development. The model developers have suggested that this model has the capability "to support decision-making for (1) crop management, (2) harvest timing, (3) maize lots cleaning and logistic, and (4) maize sampling for aflatoxin analysis at consignment". These models are inherently maize hybrid–specific and not responsive to unmanaged insect pressure. This kind of model seems mainly to be suited for long-term predictions of aflatoxin risk rather than each crop season (Battilani et al., 2016).

Masuoka, Chamberlin & Elias (2010) proposed a model to draw probability maps for Africa using several parameters from the environmental data and the geo-referenced locations of aflatoxin contamination occurrence. Water stress is also an important risk factor for fumonisin accumulation in maize, although modelling of this factor has not been reported. Modelling of drought and vegetation indices are provided by the Famine Early Warning System (FEWS), an information service funded by the United States Agency for International Development (USAID) and designed to help decision-makers prevent famine in Africa. FEWS assesses remotely sensed data, ground-based

sources and other factors that affect local food availability. Data produced include weather and crops reports and remote sensing images (http://www.fews.net/), as well as 1-day estimates of accumulated precipitation for Africa also prepared by United States government agencies with support from USAID (http://www.cpc.ncep.noaa.gov/products/international/). Riley & Miller (2003) and Boken et al. (2008) advocated the development of algorithms that would give early warnings of mycotoxin risk.

6.6.2 Storage application of models

(a) Maize

A number of in vitro studies have correlated abiotic factors such as temperature, water activity and pH with growth of *A. flavus* on various media in the laboratory (Pitt, 1993; Gibson et al., 1994, 1997; Sautour et al., 2001a,b; Samapundo et al., 2007). Samapundo et al. (2007) attempted to verify the validity of some of these models on autoclaved maize, and concluded that in vitro models evaluated appeared to be suitable predictors of growth rates and lag phases of *A. flavus* and *A. parasiticus* on autoclaved maize.

(b) Pistachios

Marín, Ramos & Sanchis (2012) investigated *A. flavus* growth and aflatoxin production at various moisture contents and temperatures in pistachios post-harvest, during storage and transport. They suggested that the predictive power of their model was high (89%).

Aldars-García et al. (2015) considered temperature and humidity fluctuations during transport and retail storage of pistachios. They suggest that the model was able to correctly predict the presence of aflatoxin in 70–81% of cases.

These models would be beneficial if enough temperature and moisture sensors were placed in transport and storage containers.

(c) Rice

Mousa et al. (2011, 2013) compared several models to be able to predict *A. flavus* growth and aflatoxin production in stored paddy as well as in polished and brown rice. The researchers were able to successfully characterize the influences of water activity and temperature on the growth of the fungus and toxin production, and concluded that the models were capable of providing "good, related estimates of growth rates". In another study, a kinetic model of AFB_1 conversion was plotted and successfully validated in aflatoxin-contaminated rice during thermal treatments.

(d) Chilli powder

Mathematical models were applied to predict aflatoxigenic fungal growth in powdered red chillies as a function of water availability (Marín et al., 2009). This study for risk assessment purposes suggests that "careful hazard analysis and critical control point (HACCP) techniques during raw material production and the subsequent stages of drying, transportation, elaboration and storage are indispensable". Similarly, based on predictive mycology in Sri Lankan spices (chilli powder and black pepper), using temperature and water activity as storage parameters, suitable storage conditions for these spices was suggested to minimize the risk from aflatoxin contamination.

6.6.3 Neural networks

The use of artificial neural networks has provided avenues for developing predictive tools for addressing the aflatoxin problem. These models measure the influence of certain factors on the effect of specific biological phenomena mediated through a cascade of networks within the organism's system. These models can be difficult in that they are initialized with randomly chosen weights. The gradient of error is computed using an optimization method, to correct the initial weights. However, these biological neural networks, identified through genomic studies, have provided useful information on approximating functions that can depend on a large number of inputs that are generally known. Henderson et al. (1998, 2000) used a genetic algorithm/neural network hybrid in which the genetic algorithm was used to assign weights for a neural network for predicting aflatoxin contamination levels in peanuts based on available environmental data.

6.6.4 Special considerations on modelling

The ability to predict reasonably accurately the potential for aflatoxin contamination prior to harvest, after harvest or following food processing is very important to the entire food chain – farmers, extension personnel, consumers, commodity groups, politicians, various institutions and researchers – as well as for sustainable agriculture. However, model prediction will never be 100% accurate only because aflatoxin contamination is dependent on a multitude of factors (Payne, Hagler & Adkins, 1988). Quantitative data on interactions between the invading fungus and the crop and the interaction of microbes in the soil environment or plant surface will be very difficult to get, even if good data are available on factors such as environmental conditions, soil types, plant growth parameters and chemical additives. However, as researchers across the globe are attempting to obtain such information to as high a degree of accuracy as possible, predictive modelling to determine aflatoxin contamination may turn out to be an

important part of protecting the human and animal population against risk from aflatoxin contamination.

7. Levels and patterns of contamination of food commodities

7.1 Surveillance data

The Committee discussion on the occurrence of aflatoxins was restricted to AFB_1 and AFT in cereals, nuts, spices and other foodstuffs liable to be contaminated with aflatoxins to any significant degree, as well as the mammalian hydroxylated metabolite AFM_1 in milk. The starting point for data search was taken as the beginning of 2007 to avoid duplicating occurrence data covered by the sixty-eighth JECFA meeting (Annex 1, reference *188*) and the end-point was data from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) at the end of June 2016. The data presented were obtained from two sources, the open literature and the WHO GEMS/Food contaminants database. Specific submissions from individual countries and the European Union in response to the JECFA call for data were incorporated with data from the GEMS/Food contaminants database so as to obtain a holistic dataset.

7.1.1 GEMS/Food contaminants database

Data on AFB_1 and AFT for cereals, nuts, dairy products (AFM_1) and other foods, including figs, herbs, soybeans and spices, are presented in Tables A1-1 to A1-4 in Appendix 1. The spices category in the GEMS/Food contaminants database is presented in Table A1-5 and consists largely of European Union data. These data were subsequently separated into component spices, and these are presented in Table A1-6. The tables give numbers of samples, per cent of samples that were contaminated (occurrence), ranges of LOD and LOQ, means of all samples (lower-bound mean with uncontaminated samples taken as zero) and maximums and medians (in those cases where per cent contamination exceeds 50%). The 90th percentile column gives an estimate that shows an indication of the skewed distribution of results. It was taken from the ordered parameters and represents the value below which 90% of results occur or above which 10% of samples are found to be contaminated.

Although the database records AFT values, differences may exist in what congeners are included in this term. In addition, the per cent contaminated

value is entirely a function of the LOD or LOQ, and the ranges recorded in the tables for these two parameters vary considerably. Another factor influencing the values in the tables is large outliers, which can raise the mean values calculated. For this reason, data from Finland for the year 2012 were excluded, as values were, in general, 3 orders of magnitude above similar data from other European countries. The effect was also apparent in calculated means for AFB_1 and AFT for Brazil nuts. A number of targeted samples from Germany were recorded such that their inclusion (Table A1-5) gave means of 6.48 and 67.3 µg/kg, respectively, whereas their exclusion would have given 0.98 and 1.18 µg/kg, respectively (see Table 13 and Table 12, respectively, in section 8).

In general, it is also apparent that the number of samples tested for any individual item is a reflection of both the importance of that item in the market and concerns over its susceptibility to *Aspergillus* infection and consequent aflatoxin contamination. Thus, barley, buckwheat, millet and oats are represented by few data points, and little to no contamination is reported. Similarly, cashews and chestnuts are represented by far fewer samples than peanuts and pistachios and show very little contamination. For dairy products, cows' milk was both the item tested to the largest extent and the one that shows the highest contamination levels. Most of the other foods listed in Table A1-5 have low sample numbers, except for figs, nutmeg, pepper and the general category "spices". These four items have moderate mean contamination (maximum mean level being 3.23 µg/kg for AFT in nutmeg; Table 12). The maximum levels reported, however, are very high and range up to 202 µg/kg for AFB_1 in spices, 30 µg/kg for AFT in pepper, 106.5 µg/kg for AFB_1 in nutmeg and 1329 µg/kg for AFT in figs (Table A1-5).

The most tested items shown in Table A1-6 are chilli powder and paprika, of which AFB_1 in chilli powder has the highest overall mean of 2.04 µg/kg. Both spices can be highly contaminated; the highest level was 150.9 µg/kg for AFT in paprika. The only maximum higher than this was a maximum of 202 µg/kg for AFB_1 in a sample of curry.

7.1.2 Open literature search: FAO scoping review

An open literature search for data published between January 2007 and the end of 2015 was conducted by the JECFA Secretariat in three databases, Scopus, PubMed and Ovid. Results were tabulated for AFB_1 in cereals and cereal products (Table A1-1); nuts (Table A1-2); and other foods but mainly spices (Table A1-3); and for AFM_1 in dairy products (mainly milk, cheese and yoghurt; Table A1-4). The data for cereals (Table A1-1) were subdivided into barley, buckwheat, maize, millet, oats, rice, rye, sorghum, sweet corn, wheat and other. The data for nuts (Table A1-2) were subdivided into almonds, Brazil nuts, cashew nuts, chestnuts, hazelnuts, groundnuts (peanuts), pistachios, walnuts and other (legumes and oilseeds).

Data were from a number of developed and developing countries. Sample numbers varied widely between papers. The tabulated methods clearly show that HPLC is more commonly used as an analytical technique than TLC or ELISA. As a consequence, LOD/LOQ values are low and reported methods are capable of determining aflatoxins at or below the 1 µg/kg (ppb) level. However, there is no clear consensus on reporting of mean contamination levels, with some studies reporting mean of all samples, others mean of positive samples only and yet others not clearly stating which was applied.

For AFB_1 in cereals, the major cereals studied were maize, rice and wheat (Table A1-1). The few studies in barley, buckwheat, oats and sorghum reported low contamination levels. The highest levels are generally found in reports from developing countries, for example, maximum levels of AFB_1 in maize from Burkina Faso of 636 µg/kg (median 23.6 µg/kg); in maize from Nigeria of 6738 µg/kg (median 74 µg/kg); in maize from Pakistan of 850 µg/kg (mean of positives 192 µg/kg); in rice from Brazil of 1707 µg/kg (median 4.2 µg/kg); in rice from Nigeria of 309 µg/kg; and in wheat from Iraq of 254 µg/kg.

For AFB_1 in nuts, a few studies found individual samples or batches with high contamination levels (Table A1-2). This was particularly the case for groundnuts (peanuts) and pistachios, although a few high results were also reported for almonds, chestnuts, hazelnuts and walnuts; little was found for Brazil nuts and cashews. The highest mean values were found for groundnuts (peanuts) in local markets in the Democratic Republic of the Congo, where the mean of positive samples was as high as 229 µg/kg (maximum 937 µg/kg; 72% of samples contaminated). Both Tables A1-1 and A1-2 show that the contamination distribution is skewed in that maximum levels lie well above mean or median figures, which can be an order of magnitude lower.

For AFB_1 in foods other than cereals, nuts and dairy products (i.e. mainly spices), chilli has the highest incidences (up to 100%) combined with high levels of contamination (maximum of 687 µg/kg, median 22.9 µg/kg in one study in Sri Lanka; Table A1-3). In general, the contamination has a skewed distribution, with mean or median figures well below the maxima.

Data shown in Table A1-4 (dairy products) were originally reported in a variety of units, such as parts per trillion (ppt), ng/L, µg/L, ng/kg and µg/kg, which makes comparison of the data points difficult. Nevertheless, a number of reports from developing countries found maximum levels of AFM_1 that exceeded the Codex standard of 0.5 µg/kg (500 ng/kg) (FAO/WHO, 1995). Examples include 0.760 µg/L for milk in Brazil; 1.135 µg/L (mean of all samples 0.0466 µg/L) for milk in Croatia; 113 µg/L for milk in the Islamic Republic of Iran; 92.3 µg/kg (median 16.2) for milk in Mexico; 845.4 µg/kg (mean of all samples 150.7 µg/kg) for milk in Pakistan; 0.7854 µg/kg (mean of all samples 0.1664 µg/kg) for cheese in the Islamic Republic of Iran; 4.100 µg/kg for cheese in Turkey; and 615.8 µg/kg

(mean of all samples 90.4 µg/kg) for yoghurt in Pakistan. Other than the highly contaminated batches described above, the distribution of contamination within the milk batches tested appears in general to be less skewed than for the other commodities.

7.1.3 Open literature search: aflatoxin in human breast milk

The population most sensitive to mycotoxins in general and aflatoxins in particular are neonates. Given that WHO advocates exclusive breastfeeding for up to 6 months of age (WHO, 2016), a further survey of the open literature was conducted to investigate the occurrence of aflatoxins in human breast milk. Both PubMed and Scopus were searched using the terms "aflatoxin" and "breast milk" over the same period as the FAO scoping study. Besides individual studies, the search retrieved two review articles of mycotoxins, including aflatoxins, in human breast milk, a generalized review of exposure in children and the potential health implications (Tonon, Reiter & Scussel, 2013), and a systematic review of available data for the period 1984–2015 (Cherkani-Hassani, Mojemmi & Mouane, 2016). The Cherkani-Hassani, Mojemmi & Mouane (2016) review included comprehensive data tabulations. The studies summarized mainly determined AFM_1. The presence of this aflatoxin in human milk is a consequence of maternal exposure; as such, low levels may be expected in countries with significant enforcement of food safety regulations, whereas the situation may be more complex in developing countries, and especially in rural areas. Indeed, of the various studies from Europe included in the reviews, AFM_1 levels were low, whereas those studies from Africa and Asia frequently had samples exceeding the Codex standard of 0.5 µg/kg set for dairy milk.

7.2 Distribution curves

No distribution curves were considered, but, where possible, mean, median, estimated 90th percentile and maximum values are given in the occurrence tables to indicate the skewed nature of most aflatoxin contamination levels.

7.3 Data on annual variation in contaminant levels

As for all mycotoxins, aflatoxin levels can show considerable annual variation related to weather conditions that influence fungal growth and toxin production. Correlations between these conditions and toxin production are used to generate predictive models and are dealt with in section 6.5.

8. Food consumption and dietary exposure assessment

8.1 Concentrations in food used in the dietary exposure estimates

For national and international estimates of dietary exposure derived by the Committee, concentrations of aflatoxins in food commodities and in some processed foods were derived from the WHO GEMS/Food contaminants database (see section 7 for a full description of these data including the quality and reporting). Comparison of the aflatoxin concentration data from the scientific literature with those from the GEMS/Food contaminants database (see section 7) suggests that the GEMS/Food contaminants database contains information on all currently identified significant food sources of aflatoxins. Where possible, occurrence data were classified according to the food groups used in the GEMS/Food cluster diets, which include information on consumption of raw or minimally processed foods.

The GEMS/Food contaminants database was queried for the period 2007–2016 for records relating to AFT, AFB_1 or AFM_1 in any food. It was assumed that records for AFT in the GEMS/Food contaminants database would have included analysis for AFB_1, AFB_2, AFG_1 and AFG_2. However, in some cases AFT may represent summation of results for a narrower range of aflatoxins. Summing of individual aflatoxins may have an additive effect of LOD and LOQ, resulting in elevated upper-bound estimates of the mean concentration compared with a single aflatoxin. Data extracted originated from 37 countries, representing nine of the 17 GEMS/Food cluster diets.

Two scenarios were considered when calculating the mean aflatoxin concentrations: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario). Records in the GEMS/Food contaminants database usually report a numerical value for results between the LOD and LOQ. Such values were included in the calculation of mean values, and the limit value used for deriving upper-bound estimates of the mean was usually the LOD.

The concentration data for AFT, AFB_1 and AFM_1 in foods for the GEMS/Food cluster diets are shown in Tables 12–14. Individual data points on the concentration of the contaminant in foods from the GEMS/Food contaminants database for each cluster were pooled for each commodity to derive summary representative (lower- and upper-bound) concentrations for use in the dietary exposure calculations. For each commodity, when concentration data were not available for a cluster, the global total lower- and upper-bound mean concentrations, obtained by pooling the data across all countries, were used to assess exposure. By doing this, it is assumed that food is traded in a global market

and that concentrations from commodities grown in one area of the world are representative of other areas of the world. No weightings were applied to the concentrations as insufficient information about much of the data was available to allow for this.

Data from random sampling were compared to data from targeted surveys, on a country-by-country basis, before pooling, to determine that there was no major bias in the results from targeted surveys. When mean results from targeted surveys differed from those from random sampling by at least an order of magnitude (>10 times), the results from the targeted survey were excluded from calculation of the lower- and upper-bound mean. This measure was applied to exclude data related to specific investigation of heavily contaminated samples, as it is likely that the food products related to such contamination incidents would have been excluded from the food supply.

8.2 Food consumption data used in the dietary exposure estimates

In addition to national estimates of dietary exposure published in the literature, the Committee derived additional national estimates of dietary exposure using food consumption information from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss), in combination with summary concentration data from the GEMS/Food contaminants database (see section 7.1.1). CIFOCOss total mean food consumption information (in g/kg bw per day) was used for all additional national dietary exposure assessments.

The Committee calculated international estimates of dietary exposure to AFT, AFB_1 and AFM_1 using the GEMS/Food cluster diets. The consumption cluster diets provide mean per capita consumption values based on FAO food balance sheet data for raw commodities and some semiprocessed commodities for 17 clusters of countries (Sy et al., 2013). Clusters G01 and G06 include primarily Middle Eastern, central Asian and north African countries; clusters G03, G13 and G16 include primarily African countries; cluster G02 includes countries in West Asia and the Balkan region of Europe; cluster G04 includes Middle Eastern and Caribbean countries; cluster G09 includes countries in Asia and Africa; clusters G07, G08, G10, G11 and G15 include European and North American countries and developed countries from Asia and the Pacific (Australia, Japan, New Zealand, the Republic of Korea); clusters G05 and G12 consist mainly of South and Central American countries; and clusters 14 and 17 include Caribbean/Asia/Pacific island states.

Relevant food consumption data for each of the 17 GEMS/Food cluster diets, including a description of category aggregation, are shown in Table 15.

Table 12

Summary of data from the GEMS/Food contaminants database on concentrations of AFT in commodities in GEMS/Food cluster diets

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Cereals and pseudocereals																			
Barley	No. of individual samples	500	–	–	–	–	–	–	8	5	18	321	–	–	–	–	65	–	–
	% samples <LOD or LOQ	93.2	–	–	–	–	–	–	100	100	100	87.7	–	–	–	–	87.8	–	–
	Mean (LB; µg/kg)	0.08	–	–	–	–	–	–	0.00	0.00	0.00	0.07	–	–	–	–	0.22	–	–
	Mean (UB; µg/kg)	0.33	–	–	–	–	–	–	0.45	0.33	0.20	0.30	–	–	–	–	0.81	–	–
Buckwheat	No. of individual samples	80	–	–	–	–	–	–	11	31	–	–	–	–	–	–	36	–	–
	% samples <LOD or LOQ	83.7	–	–	–	–	–	–	36.4	80.6	–	–	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.81	–	–	–	–	–	–	0.18	2.02	–	–	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	1.97	–	–	–	–	–	–	0.29	2.27	–	–	–	–	–	–	2.33	–	–
Maize	No. of individual samples	1 855[b]	–	–	–	–	–	–	39	122	269	962	1	–	–	–	209[b]	–	–
	% samples <LOD or LOQ	72.7	–	–	–	–	–	–	69.2	92.6	34.9	77.8	100	–	–	–	71.8	–	–
	Mean (LB; µg/kg)	2.07	–	–	–	–	–	–	0.15	0.04	9.12	1.21	0.00	–	–	–	0.62	–	–
	Mean (UB; µg/kg)	2.71	–	–	–	–	–	–	0.46	0.48	9.85	1.75	0.40	–	–	–	1.60	–	–
Millet	No. of individual samples	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	80	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.088	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	0.78	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oats	No. of individual samples	78	–	–	–	–	–	–	2	3	–	40	–	–	–	–	32	–	–
	% samples <LOD or LOQ	100	–	–	–	–	–	–	100	100	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.00	–	–	–	–	–	–	0.00	0.00	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.40	–	–	–	–	–	–	0.40	0.16	–	0.35	–	–	–	–	0.49	–	–
Rice	No. of individual samples	2 483	–	–	–	–	–	–	440	589	–	405	–	–	98	–	410	–	–
	% samples <LOD or LOQ	74.3	–	–	–	–	–	–	33.9	77.2	94.2	99.5	–	–	14.3	–	77.8	–	–
	Mean (LB; µg/kg)	1.72	–	–	–	–	–	–	0.80	0.29	0.13	0.01	–	–	35.17	–	0.63	–	–

Table 12 (continued)

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
	Mean (UB; µg/kg)	2.21	–	–	–	–	–	–	0.93	0.43	0.54	1.46	–	–	35.19	–	1.44	–	–
Rye	No. of individual samples	61	–	–	–	–	–	–	4	14	2	14	–	–	–	–	27	–	–
	% samples <LOD or LOQ	95.1	–	–	–	–	–	–	50	100	100	92.9	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.02	–	–	–	–	–	–	0.10	0.00	0.00	0.05	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.90	–	–	–	–	–	–	0.35	0.40	0.20	0.32	–	–	–	–	1.59	–	–
Sorghum	No. of individual samples	96	–	–	–	–	–	–	–	–	29	24	–	–	–	–	–	–	–
	% samples <LOD or LOQ	81.2	–	–	–	–	–	–	–	–	58.6	83.3	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.99	–	–	–	–	–	–	–	–	1.86	1.03	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	1.56	–	–	–	–	–	–	–	–	3.62	1.25	–	–	–	–	–	–	–
Sweet corn	No. of individual samples	51	–	–	–	–	–	–	2	–	–	–	–	–	–	–	13	–	–
	% samples <LOD or LOQ	96.1	–	–	–	–	–	–	0	–	–	–	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.007 8	–	–	–	–	–	–	0.20	–	–	–	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.16	–	–	–	–	–	–	0.20	–	–	–	–	–	–	–	0.38	–	–
Wheat	No. of individual samples	1 486	–	–	–	–	–	–	17	100	25	344	–	–	–	–	428	–	–
	% samples <LOD or LOQ	88.3	–	–	–	–	–	–	11.8	100	96.0	100	–	–	–	–	85.0	–	–
	Mean (LB; µg/kg)	0.18	–	–	–	–	–	–	0.25	0.00	0.18	0.00	–	–	–	–	0.25	–	–
	Mean (UB; µg/kg)	0.91	–	–	–	–	–	–	0.34	0.19	0.51	0.34	–	–	–	–	1.53	–	–
Tree and groundnuts																			
Almonds	No. of individual samples	2 218	–	–	–	–	–	–	406	75	–	241	–	–	–	–	1477	–	–
	% samples <LOD or LOQ	76.9	–	–	–	–	–	–	39.7	97.3	–	94.6	–	–	–	–	82.9	–	–
	Mean (LB; µg/kg)	1.14	–	–	–	–	–	–	0.81	1.31	–	3.19	–	–	–	–	0.13	–	–
	Mean (UB; µg/kg)	1.36	–	–	–	–	–	–	1.07	1.43	–	3.82	–	–	–	–	0.76	–	–
Brazil nuts	No. of individual samples	75[c]	–	–	–	–	–	–	24	24[c]	–	3	–	–	–	–	24	–	–
	% samples <LOD or LOQ	57.3	–	–	–	–	–	–	0	83.3	–	100	–	–	–	–	83.3	–	–
	Mean (LB; µg/kg)	1.18	–	–	–	–	–	–	0.65	1.97	–	0.00	–	–	–	–	0.98	–	–
	Mean (UB; µg/kg)	1.40	–	–	–	–	–	–	0.65	2.07	–	0.43	–	–	–	–	1.51	–	–

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Cashew nuts	No. of individual samples	399	–	–	–	–	–	–	243	36	–	10	–	–	–	–	85	–	–
	% samples <LOD or LOQ	77.4	–	–	–	–	–	–	63.8	100	–	100	–	–	–	–	97.6	–	–
	Mean (LB; µg/kg)	0.109	–	–	–	–	–	–	0.18	0.00	–	0.00	–	–	–	–	0.01	–	–
	Mean (UB; µg/kg)	0.48	–	–	–	–	–	–	0.52	0.10	–	0.66	–	–	–	–	0.62	–	–
Chestnuts	No. of individual samples	42	–	–	–	–	–	–	2	10	–	25	–	–	–	–	5	–	–
	% samples <LOD or LOQ	92.9	–	–	–	–	–	–	100	80	–	100	–	–	–	–	80	–	–
	Mean (LB; µg/kg)	0.07	–	–	–	–	–	–	0.00	0.18	–	0.00	–	–	–	–	0.23	–	–
	Mean (UB; µg/kg)	0.61	–	–	–	–	–	–	0.30	0.50	–	0.66	–	–	–	–	0.73	–	–
Hazelnuts	No. of individual samples	4 741	–	–	–	–	–	–	746	2 435	–	62	–	–	–	–	253	–	–
	% samples <LOD or LOQ	59.5	–	–	–	–	–	–	62.1	50	–	74.2	–	–	–	–	90.1	–	–
	Mean (LB; µg/kg)	1.50	–	–	–	–	–	–	0.70	1.74	–	0.50	–	–	–	–	3.12	–	–
	Mean (UB; µg/kg)	1.96	–	–	–	–	–	–	0.98	1.84	–	0.76	–	–	–	–	3.56	–	–
Peanuts	No. of individual samples	20 870	–	–	–	–	1 423	–	622	1 922	69	15 394	–	–	–	–	553	–	–
	% samples <LOD or LOQ	64.6	–	–	–	–	67.4	–	29.4	84.6	62.3	61.1	–	–	–	–	90.1	–	–
	Mean (LB; µg/kg)	9.45	–	–	–	–	19.44	–	2.48	3.23	2.28	10.39	–	–	–	–	2.48	–	–
	Mean (UB; µg/kg)	9.76	–	–	–	–	20.39	–	2.66	3.37	3.16	10.57	–	–	–	–	3.52	–	–
Pistachios	No. of individual samples	9 949	–	–	–	–	–	–	274	9 393	–	36	–	–	–	–	245	–	–
	% samples <LOD or LOQ	81	–	–	–	–	–	–	61.7	81.6	–	77.8	–	–	–	–	79.6	–	–
	Mean (LB; µg/kg)	2.54	–	–	–	–	–	–	6.19	2.27	–	3.82	–	–	–	–	8.81	–	–
	Mean (UB; µg/kg)	2.66	–	–	–	–	–	–	6.57	2.36	–	4.17	–	–	–	–	9.63	–	–
Walnuts	No. of individual samples	411	–	–	–	–	–	–	79	66	–	43	–	–	–	–	223	–	–
	% samples <LOD or LOQ	76.4	–	–	–	–	–	–	32.9	100	–	97.7	–	–	–	–	80.7	–	–
	Mean (LB; µg/kg)	0.20	–	–	–	–	–	–	0.27	0.00	–	0.06	–	–	–	–	0.26	–	–
	Mean (UB; µg/kg)	0.66	–	–	–	–	–	–	0.38	0.41	–	0.62	–	–	–	–	0.85	–	–
Spices																			
Anise, fennel, coriander[d]	No. of individual samples	27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	88.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 12 (continued)

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
	Mean (UB; µg/kg)	0.23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cinnamon[d]	No. of individual samples	29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	93.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	1.21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cloves[d]	No. of individual samples	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	85.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	4.14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	5.12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ginger	No. of individual samples	271	–	–	–	–	–	–	62	114	–	–	–	–	–	–	22	–	–
	% samples <LOD or LOQ	56.5	–	–	–	–	–	–	54.8	53.5	–	–	–	–	–	–	59.1	–	–
	Mean (LB; µg/kg)	2.19	–	–	–	–	–	–	2.25	2.57	–	–	–	–	–	–	1.46	–	–
	Mean (UB; µg/kg)	2.57	–	–	–	–	–	–	2.73	2.77	–	–	–	–	–	–	1.89	–	–
Nutmeg, mace, cardamon	No. of individual samples	541	–	–	–	–	–	–	38	402	–	2	–	–	–	–	15	–	–
	% samples <LOD or LOQ	27.9	–	–	–	–	–	–	28.9	23.6	–	0	–	–	–	–	66.7	–	–
	Mean (LB; µg/kg)	3.16	–	–	–	–	–	–	5.63	3.13	–	5.45	–	–	–	–	2.03	–	–
	Mean (UB; µg/kg)	3.37	–	–	–	–	–	–	5.93	3.23	–	5.45	–	–	–	–	3.11	–	–
Pepper (black, white)	No. of individual samples	633	–	–	–	–	–	–	82	238	–	6	–	–	–	–	109	–	–
	% samples <LOD or LOQ	70.3	–	–	–	–	–	–	43.9	77.2	–	66.7	–	–	–	–	78	–	–
	Mean (LB; µg/kg)	0.57	–	–	–	–	–	–	0.75	0.12	–	2.74	–	–	–	–	1.01	–	–
	Mean (UB; µg/kg)	1.10	–	–	–	–	–	–	0.98	0.61	–	3.32	–	–	–	–	1.53	–	–
Spices, other	No. of individual samples	2 646	–	–	–	–	–	–	358	1032	–	25	–	–	–	–	430	–	–
	% samples <LOD or LOQ	52.6	–	–	–	–	–	–	26.8	45.3	–	68	–	–	–	–	66.7	–	–
	Mean (LB; µg/kg)	1.27	–	–	–	–	–	–	2.33	1.00	–	0.71	–	–	–	–	1.48	–	–
	Mean (UB; µg/kg)	1.93	–	–	–	–	–	–	2.59	1.23	–	1.08	–	–	–	–	2.38	–	–

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Other foods																			
Figs	No. of individual samples	2 135	–	–	–	–	–	–	230	1 846	–	–	–	–	–	–	58	–	–
	% samples <LOD or LOQ	68	–	–	–	–	–	–	91.3	64.8	–	–	–	–	–	–	74.1	–	–
	Mean (LB; µg/kg)	2.47	–	–	–	–	–	–	0.78	2.68	–	–	–	–	–	–	2.41	–	–
	Mean (UB; µg/kg)	2.58	–	–	–	–	–	–	1.13	2.76	–	–	–	–	–	–	2.61	–	–
Soy	No. of individual samples	24	–	–	–	–	–	–	3	11	–	–	–	–	–	–	9	–	–
	% samples <LOD or LOQ	87.5	–	–	–	–	–	–	0	100	–	–	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.08	–	–	–	–	–	–	0.60	0.00	–	–	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.18	–	–	–	–	–	–	0.60	0.11	–	–	–	–	–	–	0.14	–	–

AFT: total aflatoxins; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; mean LB: mean value of contamination (LB approach); mean UB: mean value of contamination (UB approach); No.: number: UB: upper bound

[a] In some cases the number of samples contributing to the global total is greater than the sum of the samples for the individual clusters. This is because some countries have not been assigned to any cluster (e.g. Singapore), but have contributed monitoring data. Similarly, some monitoring data are identified with a region, but not a particular country. All of these data were used in the calculation of global total LB and UB means.

[b] Five samples from a targeted survey were excluded. The samples had an LB mean AFT concentration of 19.1 µg/kg, compared to an LB mean AFT concentration of 0.9 µg/kg for random samples from the same country.

[c] Nine samples from a targeted survey were excluded. The samples had an LB mean AFT concentration of 618 µg/kg, compared to an LB mean AFT concentration of 1.97 µg/kg for random samples from the same country.

[d] Insufficient samples of some foods were available to calculate meaningful cluster-specific means and only global LB and UB means were calculated for these foods.

Table 13

Summary of data from the GEMS/Food contaminants database on concentrations of AFB_1 in commodities in GEMS/Food cluster diets

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Cereals and pseudocereals																			
Barley	No. of individual samples	577	–	–	–	–	–	–	6	17	–	358	1	–	–	–	85	–	–
	% samples <LOD or LOQ	96.2	–	–	–	–	–	–	100	100	–	98	100	–	–	–	89.4	–	–
	Mean (LB; µg/kg)	0.055	–	–	–	–	–	–	0.00	0.00	–	0.060	0.00	–	–	–	0.11	–	–
	Mean (UB; µg/kg)	0.28	–	–	–	–	–	–	0.13	0.15	–	0.34	0.50	–	–	–	0.35	–	–
Buckwheat	No. of individual samples	210	–	–	–	–	–	–	11	67	–	73	–	–	–	–	46	–	–
	% samples <LOD or LOQ	91	–	–	–	–	–	–	36.4	89.6	–	100	–	–	–	–	97.8	–	–
	Mean (LB; µg/kg)	0.28	–	–	–	–	–	–	0.13	0.82	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.59	–	–	–	–	–	–	0.18	0.94	–	0.35	–	–	–	–	0.67	–	–
Maize	No. of individual samples	2 566[b]	–	–	–	–	–	–	47	146	3[b]	1 159	61	–	–	–	284[c]	–	–
	% samples <LOD or LOQ	78.8	–	–	–	–	–	–	74.5	98.6	100	78.7	96.7	–	–	–	84.5	–	–
	Mean (LB; µg/kg)	1.68	–	–	–	–	–	–	0.05	0.02	0.00	1.10	0.07	–	–	–	0.39	–	–
	Mean (UB; µg/kg)	2.10	–	–	–	–	–	–	0.14	0.27	0.07	1.59	0.59	–	–	–	0.74	–	–
Millet	No. of individual samples	59	–	–	–	–	–	–	5	28	–	13	–	–	–	–	11	–	–
	% samples <LOD or LOQ	89.8	–	–	–	–	–	–	0	96.4	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.021	–	–	–	–	–	–	0.20	0.01	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.75	–	–	–	–	–	–	0.20	1.05	–	0.50	–	–	–	–	0.68	–	–
Oats	No. of individual samples	378	–	–	–	–	–	–	2	34	–	235	–	–	–	–	70	–	–
	% samples <LOD or LOQ	100	–	–	–	–	–	–	100	100	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.00	–	–	–	–	–	–	0.00	0.00	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.60	–	–	–	–	–	–	0.10	0.07	–	0.88	–	–	–	–	0.23	–	–
Rice	No. of individual samples	3 666	–	–	–	–	–	–	389	1 078	80	800	80	–	–	–	679	–	–
	% samples <LOD or LOQ	77.5	–	–	–	–	–	–	28	81	73.8	82	92.5	–	–	–	83.5	–	–
	Mean (LB; µg/kg)	0.24	–	–	–	–	–	–	0.58	0.18	0.15	0.16	0.12	–	–	–	0.37	–	–

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
	Mean (UB; µg/kg)	0.51	–	–	–	–	–	–	0.62	0.35	0.19	0.88	0.58	–	–	–	0.64	–	–
Rye	No. of individual samples	206	–	–	–	–	–	–	4	100	–	38	2	–	–	–	54	–	–
	% samples <LOD or LOQ	97.1	–	–	–	–	–	–	50	98	–	97.4	100	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.011	–	–	–	–	–	–	0.10	0.01	–	0.02	0.00	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.32	–	–	–	–	–	–	0.20	0.25	–	0.46	1.00	–	–	–	0.36	–	–
Sorghum	No. of individual samples	1 613	–	–	–	–	–	–	–	–	–	24	–	–	1 533	–	–	–	–
	% samples <LOD or LOQ	93	–	–	–	–	–	–	–	–	–	91.7	–	–	92.9	–	–	–	–
	Mean (LB; µg/kg)	2.62	–	–	–	–	–	–	–	–	–	0.03	–	–	2.75	–	–	–	–
	Mean (UB; µg/kg)	5.94	–	–	–	–	–	–	–	–	–	0.43	–	–	6.23	–	–	–	–
Sweet corn	No. of individual samples	67	–	–	–	–	–	–	2	–	–	2	–	–	–	–	15	–	–
	% samples <LOD or LOQ	94	–	–	–	–	–	–	0	–	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.035	–	–	–	–	–	–	0.20	–	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.18	–	–	–	–	–	–	0.20	–	–	0.50	–	–	–	–	0.37	–	–
Wheat	No. of individual samples	2 443	–	–	–	–	–	–	27	225	–	877	25	–	–	–	485	–	–
	% samples <LOD or LOQ	96.4	–	–	–	–	–	–	37	100	–	99.5	100	–	–	–	93.6	–	–
	Mean (LB; µg/kg)	0.026	–	–	–	–	–	–	0.11	0.00	–	0.01	0.00	–	–	–	0.06	–	–
	Mean (UB; µg/kg)	0.52	–	–	–	–	–	–	0.15	0.17	–	0.50	0.64	–	–	–	0.64	–	–
Tree and groundnuts																			
Almonds	No. of individual samples	3 727	–	–	–	–	–	–	406	1821	–	672	233	–	–	–	528	–	–
	% samples <LOD or LOQ	80	–	–	–	–	–	–	38.9	80	–	89.7	97.4	–	–	–	90.3	–	–
	Mean (LB; µg/kg)	1.10	–	–	–	–	–	–	0.61	0.95	–	2.97	0.03	–	–	–	0.13	–	–
	Mean (UB; µg/kg)	1.31	–	–	–	–	–	–	0.72	1.04	–	3.48	0.48	–	–	–	0.33	–	–
Brazil nuts	No. of individual samples	387[b]	–	–	–	–	–	–	24	64b	–	220	29	–	–	–	49	–	–
	% samples <LOD or LOQ	75.2	–	–	–	–	–	–	0	75	–	80.5	100	–	–	–	73.5	–	–
	Mean (LB; µg/kg)	0.98	–	–	–	–	–	–	0.39	0.68	–	1.32	0.00	–	–	–	0.73	–	–
	Mean (UB; µg/kg)	1.37	–	–	–	–	–	–	0.39	0.76	–	1.89	0.50	–	–	–	0.85	–	–

Table 13 (continued)

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Cashew nuts	No. of individual samples	933	–	–	–	–	–	–	243	125	–	267	43	–	–	–	149	–	–
	% samples <LOD or LOQ	88.4	–	–	–	–	–	–	63.4	98.4	–	97.4	95.3	–	–	–	99.3	–	–
	Mean (LB; µg/kg)	0.064	–	–	–	–	–	–	0.09	0.00	–	0.07	0.02	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.45	–	–	–	–	–	–	0.22	0.09	–	1.05	0.50	–	–	–	0.23	–	–
Chestnuts	No. of individual samples	109	–	–	–	–	–	–	2	47	–	50	–	–	–	–	3	–	–
	% samples <LOD or LOQ	93.6	–	–	–	–	–	–	100	91.5	–	98	–	–	–	–	66.7	–	–
	Mean (LB; µg/kg)	0.048	–	–	–	–	–	–	0.00	0.04	–	0.05	–	–	–	–	0.35	–	–
	Mean (UB; µg/kg)	0.15	–	–	–	–	–	–	0.10	0.13	–	0.17	–	–	–	–	0.45	–	–
Hazelnuts	No. of individual samples	7 025	–	–	–	–	–	–	747	4129	–	125	251	–	–	–	379	–	–
	% samples <LOD or LOQ	69.1	–	–	–	–	–	–	61.6	65.1	–	93.6	87.3	–	–	–	91.3	–	–
	Mean (LB; µg/kg)	0.58	–	–	–	–	–	–	0.33	0.57	–	0.18	0.19	–	–	–	1.82	–	–
	Mean (UB; µg/kg)	0.71	–	–	–	–	–	–	0.43	0.65	–	0.57	0.62	–	–	–	2.02	–	–
Peanuts	No. of individual samples	7 907	–	–	–	–	1 318	–	622	2 226	–	1 421	198	–	–	–	824	–	–
	% samples <LOD or LOQ	63.4	–	–	–	–	0.1	–	28.9	86.3	–	68.1	91.4	–	–	–	90.7	–	–
	Mean (LB; µg/kg)	4.69	–	–	–	–	16.78	–	1.86	2.08	–	3.14	1.98	–	–	–	1.73	–	–
	Mean (UB; µg/kg)	4.88	–	–	–	–	16.78	–	1.92	2.17	–	3.54	2.36	–	–	–	2.12	–	–
Pistachios	No. of individual samples	11 612	–	–	–	–	–	3	274	9 827	–	445	371	–	–	–	489	–	–
	% samples <LOD or LOQ	81.3	–	–	–	–	–	66.7	55.8	81.8	–	78.2	91.1	–	–	–	82.6	–	–
	Mean (LB; µg/kg)	2.21	–	–	–	–	–	1.07	5.43	1.91	–	3.32	4.08	–	–	–	5.04	–	–
	Mean (UB; µg/kg)	2.33	–	–	–	–	–	1.20	5.56	1.99	–	3.76	4.49	–	–	–	5.31	–	–
Walnuts	No. of individual samples	940	–	–	–	–	–	–	79	146	–	330	47	–	–	–	327	–	–
	% samples <LOD or LOQ	89.3	–	–	–	–	–	–	32.9	100	–	94.5	100	–	–	–	91.4	–	–
	Mean (LB; µg/kg)	0.09	–	–	–	–	–	–	0.13	0.00	–	0.12	0.00	–	–	–	0.10	–	–
	Mean (UB; µg/kg)	0.41	–	–	–	–	–	–	0.18	0.13	–	0.59	0.50	–	–	–	0.41	–	–

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Spices																			
Anise, fennel, coriander	No. of individual samples	113	–	–	–	–	–	–	3	11	–	29	13	–	–	–	4.00	–	–
	% samples <LOD or LOQ	96.5	–	–	–	–	–	–	66.7	90.9	–	100	100	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.03	–	–	–	–	–	–	0.07	0.01	–	0.00	0.00	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.35	–	–	–	–	–	–	0.17	0.11	–	0.84	0.54	–	–	–	0.20	–	–
Cinnamon	No. of individual samples	63	–	–	–	–	–	–	1	10	–	31	–	–	–	–	5	–	–
	% samples <LOD or LOQ	95.2	–	–	–	–	–	–	0	100	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.035	–	–	–	–	–	–	1.00	0.00	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.62	–	–	–	–	–	–	1.00	0.22	–	0.97	–	–	–	–	0.20	–	–
Cloves	No. of individual samples	32	–	–	–	–	–	–	–	3	–	15	–	–	–	–	–	–	–
	% samples <LOD or LOQ	78.1	–	–	–	–	–	–	–	100	–	100	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.70	–	–	–	–	–	–	–	0.00	–	0.00	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	1.19	–	–	–	–	–	–	–	0.27	–	0.95	–	–	–	–	–	–	–
Ginger	No. of individual samples	328	–	–	–	–	–	–	62	129	–	7	11	–	–	–	28	–	–
	% samples <LOD or LOQ	54.9	–	–	–	–	–	–	41.9	56.6	–	42.9	63.6	–	–	–	57.1	–	–
	Mean (LB; µg/kg)	1.00	–	–	–	–	–	–	1.09	1.22	–	0.81	0.96	–	–	–	0.84	–	–
	Mean (UB; µg/kg)	1.14	–	–	–	–	–	–	1.21	1.30	–	0.92	1.28	–	–	–	1.01	–	–
Nutmeg, mace, cardamon	No. of individual samples	744	–	–	–	–	–	–	38	494	–	60	27	–	–	–	23	–	–
	% samples <LOD or LOQ	33.3	–	–	–	–	–	–	23.7	26.5	–	70	51.9	–	–	–	69.6	–	–
	Mean (LB; µg/kg)	2.57	–	–	–	–	–	–	4.89	2.69	–	1.85	1.55	–	–	–	0.92	–	–
	Mean (UB; µg/kg)	2.73	–	–	–	–	–	–	4.96	2.77	–	2.55	1.81	–	–	–	1.34	–	–
Pepper (black, white)	No. of individual samples	1 022	–	–	–	–	–	–	83	412	–	33	36	–	–	–	144	–	–
	% samples <LOD or LOQ	74.9	–	–	–	–	–	–	39.8	76.9	–	75.8	88.9	–	–	–	81.9	–	–
	Mean (LB; µg/kg)	0.22	–	–	–	–	–	–	0.41	0.10	–	1.09	0.12	–	–	–	0.39	–	–
	Mean (UB; µg/kg)	0.43	–	–	–	–	–	–	0.51	0.29	–	1.49	0.56	–	–	–	0.61	–	–
Spices, other	No. of individual samples	3 974	–	–	–	–	–	–	369	1 620	–	314	119	–	–	–	579	–	–
	% samples <LOD or LOQ	48.6	–	–	–	–	–	–	19.2	39.8	–	73.9	63.9	–	–	–	63.2	–	–

Table 13 (continued)

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
	Mean (LB; µg/kg)	1.18	–	–	–	–	–	–	1.83	0.94	–	1.94	1.80	–	–	–	1.17	–	–
	Mean (UB; µg/kg)	1.39	–	–	–	–	–	–	1.89	1.03	–	2.51	2.12	–	–	–	1.47	–	–
Other foods																			
Figs	No. of individual samples	2 858	–	–	–	–	–	–	230	2 402	–	148	5	–	–	–	62	–	–
	% samples <LOD or LOQ	73.4	–	–	–	–	–	–	87	70.8	–	91.2	100	–	–	–	79	–	–
	Mean (LB; µg/kg)	1.44	–	–	–	–	–	–	0.42	1.54	–	1.26	0.00	–	–	–	2.08	–	–
	Mean (UB; µg/kg)	1.53	–	–	–	–	–	–	0.53	1.62	–	1.59	0.50	–	–	–	2.21	–	–
Soy	No. of individual samples	55	–	–	–	–	–	–	3	15	–	3	–	–	–	–	32	–	–
	% samples <LOD or LOQ	94.5	–	–	–	–	–	–	0	100	–	100	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.01	–	–	–	–	–	–	0.20	0.00	–	0.00	–	–	–	–	0.00	–	–
	Mean (UB; µg/kg)	0.22	–	–	–	–	–	–	0.20	0.21	–	1.31	–	–	–	–	0.13	–	–

AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] In some cases the number of samples contributing to the global total is greater than the sum of the samples for the individual clusters. This is because some countries have not been assigned to any cluster (e.g. Singapore), but have contributed monitoring data. Similarly, some monitoring data are identified to a region, but not a particular country. All of these data were used in the calculation of global total LB and UB means.

[b] Five samples from a targeted survey were excluded. The samples had an LB mean AFB_1 concentration of 12.0 µg/kg, compared with an LB mean AFB_1 concentration of 0.7 µg/kg for random samples from the same country. A further 42 samples were excluded as they were described as "corn/maize for feed". The samples had an LB mean AFB_1 concentration of 10.0 µg/kg, while AFB_1 was not detected in any random samples from the same country.

[c] Eight samples from a targeted survey were excluded. The samples had an LB mean AFB_1 concentration of 273 µg/kg, compared with an LB mean AFB_1 concentration of 0.8 µg/kg for random samples from the same country.

Table 14

Summary of data from the GEMS/Food contaminants database on concentrations of AFM_1 in commodities in GEMS/Food cluster diets

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Butter[b]	No. of individual samples	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB; µg/kg)	0.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (UB; µg/kg)	0.019	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cattle milk	No. of individual samples	3 441[c]	–	–	–	–	–	–	350	944[c]	–	990	16	–	–	–	1 083	–	–
	% samples <LOD or LOQ	88.6	–	–	–	–	–	–	98.6	91.1	–	80.8	100	–	–	–	90.8	–	–
	Mean (LB; µg/kg)	0.021	–	–	–	–	–	–	0.000	0.002	–	0.040	0.000	–	–	–	0.027	–	–
	Mean (UB; µg/kg)	0.063	–	–	–	–	–	–	0.039	0.008	–	0.133	0.011	–	–	–	0.056	–	–
Cheese	No. of individual samples	563	–	–	–	–	–	2	27	217	–	294	1	–	–	–	21	–	–
	% samples <LOD or LOQ	77.4	–	–	–	–	–	50	100	94.5	–	62.2	0	–	–	–	95.2	–	–
	Mean (LB; µg/kg)	0.014	–	–	–	–	–	0.012	0.000	0.005	–	0.023	0.012	–	–	–	0.003	–	–
	Mean (UB; µg/kg)	0.046	–	–	–	–	–	0.016	0.377	0.034	–	0.024	0.012	–	–	–	0.043	–	–
Fermented milk products	No. of individual samples	322	–	–	–	–	–	1	16	110	–	149	–	–	–	–	10	–	–
	% samples <LOD or LOQ	92.2	–	–	–	–	–	0	100	100	–	84.6	–	–	–	–	100	–	–
	Mean (LB; µg/kg)	0.003	–	–	–	–	–	0.025	0.000	0.000	–	0.006	–	–	–	–	0.000	–	–
	Mean (UB; µg/kg)	0.032	–	–	–	–	–	0.025	0.317	0.026	–	0.008	–	–	–	–	0.036	–	–
Goat milk	No. of individual samples	287	–	–	–	–	–	–	78	19	–	134	–	–	–	–	56	–	–
	% samples <LOD or LOQ	90.6	–	–	–	–	–	–	98.7	94.7	–	87.3	–	–	–	–	85.7	–	–
	Mean (LB; µg/kg)	0.009	–	–	–	–	–	–	0.000	0.000	–	0.014	–	–	–	–	0.013	–	–
	Mean (UB; µg/kg)	0.013	–	–	–	–	–	–	0.004	0.005	–	0.016	–	–	–	–	0.020	–	–

Table 14 (continued)

Food	Statistic	Global total[a]	Data per GEMS/Food cluster diet																
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Sheep milk	No. of individual samples	220	–	–	–	–	–	–	39	73	–	82	–	–	–	–	26	–	–
	% samples <LOD or LOQ	95.5	–	–	–	–	–	–	100	90.4	–	97.6	–	–	–	–	96.2	–	–
	Mean (LB; µg/kg)	0.001	–	–	–	–	–	–	0.000	0.000	–	0.001	–	–	–	–	0.001	–	–
	Mean (UB; µg/kg)	0.089	–	–	–	–	–	–	0.005	0.004	–	0.229	–	–	–	–	0.010	–	–

AFM_1: aflatoxin M_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LOD: limit of detection; LOQ: limit of quantification; No. : number; UB: upper bound

[a] In some cases the number of samples contributing to the global total is greater than the sum of the samples for the individual clusters. This is because some countries have not been assigned to any cluster (Singapore), but have contributed monitoring data. Similarly, some monitoring data are identified to a region, but not a particular country. All of these data were used in the calculation of global total LB and UB means.

[b] Insufficient samples of some foods were available to calculate meaningful cluster-specific means and only global LB and UB means were calculated for these foods.

[c] One sample from a targeted survey was excluded. The sample had an AFM_1 concentration of 50 µg/kg, compared to an LB mean AFM_1 concentration of 0.002 µg/kg for random samples from the same country.

Table 15

Summary of the consumption data from 17 GEMS/Food cluster diets used for estimates of international dietary exposure to AFT, AFB_1 and AFM_1

	Food consumption per GEMS/Food cluster diet in g/person per day																
Food commodities	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Cereals and pseudocereals																	
Barley, including flour and grits	12.5	7.6	0.1	0.1	1.3	0.6	0.6	2.6	0.4	2.0	1.0	0.1	5.5	0.0	1.9	0.0	0.0
Buckwheat	0.0	0.3	0.0	0.0	0.1	0.1	0.0	0.6	0.1	0.3	0.0	0.0	0.0	2.8	0.0	0.0	0.0
Maize, including maize flour	23.3	35.6	87.8	34.9	47.9	61.5	14.3	12.9	21.1	12.6	4.2	52.3	94.3	8.1	28.1	55.9	28.1
Millet, including millet flour	1.3	2.1	4.6	0.8	14.6	0.0	0.0	0.2	1.6	0.6	0.0	0.0	54.0	0.7	0.0	26.3	0.0
Oats, including rolled oats	0.0	3.9	0.1	1.1	0.5	0.0	4.1	3.4	0.1	2.7	1.7	1.7	0.2	0.0	1.5	0.1	0.0
Rice, including milled, husked, broken and flour	35.4	11.9	72.8	87.7	150.7	72.6	16.8	12.8	263.2	58.3	13.1	67.9	43.8	222.0	14.9	15.2	60.2
Rye, including rye flour	0.1	15.5	0.1	0.1	0.0	1.7	2.6	28.3	0.2	5.2	1.2	0.0	0.0	0.0	11.2	0.0	0.7
Sorghum, including sorghum flour	3.9	0.0	11.6	14.2	9.9	2.6	0.0	0.0	1.3	1.2	0.0	7.1	76.0	1.8	0.0	19.8	0.0
Sweet corn, including frozen or preserved	0.0	0.5	0.0	1.4	0.1	0.1	3.5	1.9	0.1	4.5	1.6	0.8	0.0	0.0	1.5	0.0	0.0
Wheat, including flour, macaroni and bread	300.5	268.0	29.4	221.4	136.0	341.9	190.7	192.7	106.1	182.8	169.8	124.1	57.2	86.9	211.0	19.8	99.2
Tree and groundnuts																	
Almonds, shelled and in shell	2.0	0.1	0.0	1.3	0.1	1.3	0.9	2.2	0.0	1.0	1.5	0.0	0.0	0.0	0.6	0.0	0.0
Brazil nuts, shelled and in shell	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Cashew nuts, shelled and in shell	0.0	0.0	0.4	0.6	0.3	0.1	0.6	0.2	0.3	0.5	1.8	11.1	3.6	0.2	0.1	0.0	0.0
Chestnuts	0.0	0.0	0.0	0.3	0.1	0.7	0.3	0.2	1.1	0.5	0.1	0.0	0.0	0.0	0.7	0.0	0.0
Hazelnuts, shelled and in shell	0.0	0.2	0.0	0.1	0.0	1.2	0.5	1.2	0.0	0.3	1.6	0.0	0.0	0.0	0.2	0.0	0.0
Peanuts, shelled and in shell	0.4	1.0	6.9	1.5	1.5	1.8	2.4	2.1	6.8	4.4	1.3	0.9	7.7	0.4	1.4	8.5	0.5
Pistachios	0.4	0.1	0.0	0.9	0.0	1.1	0.4	0.5	0.1	0.4	0.2	0.0	0.0	0.0	0.2	0.0	0.0
Walnuts, shelled and in shell	0.3	2.6	0.0	0.4	0.1	3.0	0.4	1.0	0.3	0.4	0.5	0.0	0.0	0.0	1.1	0.0	0.0

Table 15 (continued)

	Food consumption per GEMS/Food cluster diet in g/person per day																
Food commodities	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Spices																	
Anise, fennel, coriander	0.5	0.0	0.0	1.1	0.3	0.3	0.2	0.2	0.1	0.1	0.4	0.1	0.0	1.5	0.2	0.0	0.1
Cinnamon	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.4	0.0	0.1	0.1	0.0	0.3
Cloves	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Ginger	0.3	0.0	0.2	1.2	0.6	0.0	0.3	0.1	0.5	0.7	0.6	0.6	0.7	0.7	0.1	0.0	0.0
Nutmeg, mace, cardamon	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0
Pepper (black, white)	0.1	0.1	0.0	0.4	0.2	0.1	0.3	0.4	0.1	0.3	0.7	0.9	0.0	1.1	0.2	0.1	0.2
Spices, all spices not elsewhere specified	0.4	0.1	0.1	1.4	1.4	0.8	0.2	0.1	0.2	0.1	0.7	0.2	0.1	1.0	0.3	0.1	0.9
Milk and dairy products																	
Butter, including ghee, all species	5.3	4.5	0.1	3.9	4.9	5.9	14.4	12.5	0.3	5.5	10.9	0.8	0.5	0.6	4.9	0.1	3.1
Cattle milk, all fat and moisture levels, all species not elsewhere specified	243.3	448.4	19.3	102.8	168.7	131.6	283.1	246.1	36.8	248.1	277.3	95.1	81.0	22.4	348.1	57.4	27.7
Cheese, all fat contents, all species	2.3	7.2	0.1	10.7	1.6	14.9	44.1	42.2	0.4	27.8	50.7	5.0	1.3	0.1	24.7	0.0	0.5
Fermented milk products	0.2	1.0	0.1	0.3	0.0	0.0	2.8	10.0	0.0	0.6	10.5	0.9	0.0	0.0	6.5	0.0	0.1
Goat milk, whole or skimmed	8.0	2.6	0.9	18.0	6.3	6.1	0.4	0.4	2.6	1.5	0.0	0.0	11.2	0.5	0.8	1.5	0.0
Sheep milk, whole or skimmed	7.0	6.1	0.2	7.0	0.0	9.5	0.1	0.0	0.9	0.1	0.0	0.0	6.2	0.0	13.1	0.1	0.0
Other foods																	
Figs, dried and fresh	1.3	0.5	0.0	0.3	0.1	3.5	0.4	0.4	0.0	0.3	0.2	0.0	0.0	0.0	0.6	0.0	0.0
Soybean, including soy paste and curd	0.6	0.0	0.4	0.0	1.6	0.3	0.0	0.3	7.3	6.7	0.0	5.8	2.8	0.1	0.3	3.2	0.0

AFB_1: aflatoxin B_1; AFM_1: aflatoxin M_1; AFT: total aflatoxins; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme

8.3 Assessments of dietary exposure

8.3.1 National estimates of dietary exposure from the scientific literature

Since the evaluation of aflatoxins at the sixty-eighth meeting of the Committee in 2007, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations from a number of countries in Africa as well as Argentina, Brazil, China, Egypt, France, Greece, the Islamic Republic of Iran, Ireland, Japan, Kenya, Lebanon, Malaysia, Mexico, Morocco, the Netherlands, New Zealand, Pakistan, Portugal, the Republic of Korea, Serbia, Spain, Sri Lanka, Tunisia, Turkey and the United Republic of Tanzania (Table 16). These reports include dietary exposure assessments for AFT (27 studies); AFB_1 only (29 studies); AFB_2 only (six studies); AFG_1 only (five studies); AFG_2 only (five studies); and AFM_1 (19 studies).

(a) African Region

AFT exposures for several foods (maize, kenkey, peanut butter, yam chips, beer, millet, sorghum, rice and peanuts) in several African countries (Benin, Botswana, the Gambia, Ghana, Kenya, the United Republic of Tanzania) were determined using information on food contamination and food consumption levels in the literature and a standard body weight of 60 kg (Shephard, 2008). AFT dietary exposure estimates ranged from 1.4 ng/kg bw per day for consumption of sorghum in the Gambia to 850 ng/kg bw per day from consumption of kenkey, a fermented maize product, in Ghana.

(b) Argentina

A stochastic model was developed to predict dietary exposure to AFM_1 based on AFB_1 contamination levels in animal feed, rates of carry-over into bovine milk, consumption of milk and milk products and consumer body weights (Signorini et al., 2012). Food consumption information was obtained from the Argentinian Ministry of Agriculture and modelled as normal distributions. A standard body weight of 60 kg was assumed. The predicted mean concentration of AFM_1 in bovine milk was 0.059 µg/kg, equating to a mean dietary exposure to AFM_1 from consumption of milk and milk products of 0.12 ng/kg bw per day.

(c) Brazil

AFT dietary exposure for the Brazilian population was estimated based on individual food consumption information from a 2008–2009 survey conducted by the Brazilian Institute of Geography and Statistics (IBGE; n = 34 003 people aged 10 years or older, surveyed on 2 non-consecutive days) and aflatoxin concentration data from various states in Brazil for samples analysed between 2002 and 2011 (Andrade et al., 2013a). A high consumption estimate was derived

Table 16
Summary of national estimates of dietary exposure to aflatoxin from the literature

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
AFT						
Benin	Published studies	Food balance sheets	Adults	105	Only yam chips included	Shephard (2008)
Botswana	Published studies	Assumption	Children	23	Only peanut butter included	Shephard (2008)
Brazil	Published studies	National survey, 2 non-consecutive days	Population (≥10)	6.6–6.8 (16–28)[c]	Rice	Andrade et al. (2013a)
Brazil, Federal District	Data from Central Public Health Laboratory 2002–2011	Household budget survey	Population (≥10)	0.06–0.08 (33–47)[c]	Brazil nuts, peanuts	Andrade et al. (2013a)
Brazil, São Paolo	Foods from volunteers' homes, mean of positive samples	Food frequency questionnaire	Volunteers, $n = 34$	1.6	Peanut products	Jager et al. (2013)
China, Yangtze delta region	Survey mean	Per capita intake, with high (95th percentile) defined as twice per capita intake	Children Adults	25 (50) 8.3 (16.6)	Cereals and plant-derived oils	Li et al. (2014)
Egypt	Survey mean	Assumption	Children	20	Maize-based snacks	El-Sawi & El-Sawi (2012)
			Children	7.3	Cereal-based infant food	
			Adolescents	8.4	Maize-based snacks	
			Adults	5.6	Maize-based snacks	
Gambia	Published studies or maximum limit (peanuts)	Food balance sheets	Adults	1.4–30	Maize, millet, sorghum, rice, peanuts as alternative staples	Shephard (2008)
Ghana	Published studies	Estimated possible	Adults	850	Only kenkey included	Shephard (2008)
Japan	LB–UB survey mean	National survey, represented by log-normal distributions	Children (1–6)	(0.013–0.014)		Sugita-Konishi et al. (2010)
			Children (7–14)	(0.011–0.012)		
			Adolescents (15–19)	(0.006–0.007)		
			Adults (20+)	(0.003)		

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
Japan	Survey results fitted to log-normal distributions	National survey data fitted to log-normal distributions	Children (1–6)	(0.006–0.007)[d]		Kumagai et al. (2008)
			Children (7–14)	(0.005–0.006)		
			Adolescents (15–19)	(0.004–0.005)		
			Adults (>19)	(0.000 6–0.001)		
Kenya	Published studies	Not stated	Adults	133	Only commercial maize included	Shephard (2008)
Kenya	Mean from rural areas affected by outbreaks	Not stated	Adults	353	Only maize from rural markets included	Shephard (2008)
Kenya	Survey results fitted to log-normal or pert distributions	Household survey	Household inhabitants	292	Only maize and maize products included	Kilonzo et al. (2014)
Kenya	Household food concentrations	Four-pass 24-hour dietary recall	Children (1–3)	21.3 (range 0–197)[e]	Maize	Kiarie et al. (2016)
Lebanon	LB–UB survey mean	Single day 24-hour dietary recall and 1-year food frequency survey	Children (8–13)	1.5–4.4 (3.5–7.7)	Cereal and cereal products	Soubra et al. (2009)
			Teenagers (14–18)	1.3–3.8 (3.1–6.5)		
Malaysia	LB survey mean	National consumption rate	Adults	10.7	Only raw peanuts included	Arzandeh, Selamat & Lioe (2010)
Malaysia	LB–UB survey mean	National 15-month food frequency survey	Adults (18–59)	29–58 (160–200)[f]	Peanuts	Chin, Abdullah & Sugita-Konishi (2012)
Malaysia	Survey mean (ND = LOQ/2)	Food frequency survey	Adults (18–59)	10	Raw peanuts	Othman & Keat (2006)
Mexico	Published studies	3-day diary for tortilla consumption and food frequency questionnaire for maize-based foods	*Males*		Tortilla (only maize-based foods included)	Wall-Martinez et al. (2014)
			With tortilla	14–16 (64–66)		
			Without tortilla	3.1–3.2 (14)		
			Females			
			With tortilla	12–13 (52–55)		
			Without tortilla	3.6 (16)		
New Zealand	LB–UB survey mean	Individual food consumption (24-hour dietary recall) and use of typical recipes for spice consumption	Children (5–10)	0.32–0.39 (0.77–1.2)	Spices, nuts	Cressey & Reeve (2013)
			Teenage males (11–14)	0.20–0.25 (0.51–0.88)		
			Teenage females (11–14)	0.23–0.27 (0.73–0.94)		

Table 16 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
			Males (15–24)	0.13–0.15 (0.48–0.59)		
			Females (15–24)	0.15–0.16 (0.41–0.56)		
			Adult males (25+)	0.12–0.14 (0.54–0.63)		
			Adult females (25+)	0.09–0.11 (0.35–0.44)		
Spain, Catalonia	Survey mean, ND either substituted with LOD/2 or Kaplan–Meier method used	Food frequency questionnaire (1 393 respondents)		Substitution / Kaplan–Meier	Children, adolescents: breakfast cereals, corn snacks Adults: peanuts, pistachios	Cano-Sancho et al. (2013)
			Children	0.11 / 0.033		
			Adolescents	0.18 / 0.25		
			Adult males	0.072 / 0.098		
			Adult females	0.077 / 0.094		
			People with coeliac disease	0.086 / 0.086		
			Immigrants	0.079 / 0.28		
Spain, Valencia	LB survey mean	Spanish Agency for Food Safety Survey for longitudinal exposure (chronic) food consumption	Adolescents	0.008 (0.074)	Only coffee included	García-Moraleja et al. (2015)
			Adults	0.036 (0.17)		
Spain	Survey median	Manufacturers' recommended feeding rates	4–24 months[g]	Ecological infant cereals: 0.17–37 Conventional infant cereals: 0.08–0.94	Cocoa-containing, gluten-free, fruit-containing infant cereals	Hernández-Martínez & Navarro-Blasco (2010)
Sri Lanka	Survey mean of positive samples only	Survey of 15 families	Adults	0.19	Only black pepper included	Yogendrarajah et al. (2014a)
Turkey	Survey mean (positive samples only)	Not stated	Adults	Figs from domestic market: 1.3 Figs destined for export: 0.2	Only figs included	Bircan & Koc (2012)
United Republic of Tanzania	Samples of maize analysed from each household	24-hour dietary recall for each participant	Infants at 3 months eating maize-based complementary foods ($n = 67$)	Median: 3.9 (range: 0.14–120)	Only maize-based complementary foods included	Magoha et al. (2016)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
AFB_1						
Brazil, Paraná State	LB–UB mean of survey samples	Food frequency and serving size questionnaire	Adults (18+)	0.6–0.06 (10.0-10.4)	Only peanut products included	Magrine et al. (2011)
China	LB–UB mean of survey samples	Published study	Children	3.3 (17)	Only peanuts included	Ding et al. (2012)
			Adults	1.3 (6.6)		
China, Yangtze River region	LB–UB mean of survey samples	National survey, methodology not stated	Children (2–6 years)	0.78–0.79 (12)	Only peanuts included	Ding et al. (2015)
			Adults	0.34–0.35 (4.1)		
China, Hebei Province	Survey mean	Per capita wheat consumption	Adults	30	Only wheat included	Liu et al. (2015)
China, Huantai, Huaian and Fusui	Survey mean	Food frequency questionnaire	Adults	6.7–45	Maize, plant oils	Lunn et al. (1977)
China	Survey mean	Published values (other countries)	Adults	Deterministic 0.001 4–0.082 Probabilistic 0.057–1.1	Only spices included	Zhao, Schaffner & Yue (2013)
Egypt	Survey mean	Assumption	Children	13	Maize-based snacks	El-Sawi & El-Sawi (2012)
			Children	4.1	Cereal-based infant food	
			Adolescents	5.5	Maize-based snacks	
			Adults	3.7	Maize-based snacks	
France	LB–UB survey mean	INCA2 – national 7-day food diary	Children (3–17)	0.001–0.39 (0.008–0.74)	Cereals	Sirot, Fremy & Leblanc (2013)
			Adults (18–79)	0.002–0.22 (0.01–0.39)		
Greece	Median survey concentrations	Nominal values	Children	0.07–0.12	Only breakfast cereals included	Villa & Markaki (2009)
			Adolescents	0.05–0.10		
			Adults	0.03–0.07		
Iran (Islamic Republic of)	Survey mean	Publication values and nominal values	Adults	3.6	Rice	Yazdanpanah et al. (2013)
Japan	LB–UB survey mean	National survey, represented by log-normal distributions	Children (1–6)	(0.013–0.014)		Sugita-Konishi et al. (2010)
			Children (7–14)	(0.010–0.012)		
			Adolescents (15–19)	(0.006)		
			Adults (20+)	(0.003)		
Lebanon	LB–UB survey mean	Food frequency survey (444 respondents)	Adults	0.63–0.66 (1.4–1.5)	Bread and toast	Raad et al. (2014)

Table 16 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
Malaysia	LB survey mean	Food frequency questionnaire (364 respondents)	Adults	0.36 (8.89)	Fried peanuts	Leong et al. (2010)
Malaysia	Survey mean (positive samples only)	GEMS/Food regional diets	Adults	0.09	Only spices included	Ali et al. (2015)
Malaysia	LB–UB survey mean	National 18-month food frequency survey	Adults (18–59)	24–34 (140–150)[f]	Peanuts	Chin, Abdullah & Sugita-Konishi (2012)
Morocco	Survey mean	Mean of GEMS/Food regional diets	Not specified	<0.05	Only rice included	Serrano et al. (2012)
Netherlands	Concentrations in duplicate diets	Duplicate diets (*n* = 123)	Children (2–6)	Mode 0.06–0.08 (maximum 0.43)	Overall diets	Bakker et al. (2009)
New Zealand	LB–UB survey mean	Individual food consumption (24-hour dietary recall) and use of typical recipes for spice consumption	Children (5–10)	0.27–0.33 (0.63–1.1)	Spices, nuts	Cressey & Reeve (2013)
			Males (11–14)	0.16–0.22 (0.40–0.78)		
			Females (11–14)	0.19–0.23 (0.59–0.83)		
			Males (15–24)	0.11–0.13 (0.37–0.52)		
			Females (15–24)	0.13–0.14 (0.37–0.48)		
			Males (25+)	0.10–0.12 (0.44–0.55)		
			Females (25+)	0.07–0.09 (0.27–0.37)		
Pakistan	LB–UB survey mean	Not stated	Adults	22 (29–30)	Only rice and rice products included	Iqbal et al. (2016)
Republic of Korea	Survey results fitted to distribution	National survey	Adults	0.64 (2.5)	Soybean paste and soy sauce	Ok et al. (2007)
Spain, Navarra	Survey mean	Mean cereal consumption	Adults	0.44	Only barley included	Ibáñez-Vea et al. (2012)
Spain, Valencia	LB survey mean	Spanish Agency for Food Safety Survey for longitudinal exposure (chronic) food consumption	Adolescents	0.001 (0.005)	Only coffee included	García-Moraleja et al. (2015)
			Adults	0.003 (0.013)		

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
Spain	Survey median	Manufacturers' recommended feeding rates	4–24 months[g]	Ecological infant cereals: 0.12–29 Conventional infant cereals: 0.01–0.62	Cocoa-containing, gluten-free, fruit-containing infant cereals	Hernández-Martínez & Navarro-Blasco (2010)
Sri Lanka	Survey mean of positive samples only	Survey of 15 families	Adults	0.17	Only black pepper included	Yogendrarajah et al. (2014a)
Sri Lanka	Survey mean	Household food frequency questionnaire (249 households). Assumed that spices were consumed equally by all household members	Adults *Deterministic* North South *Probabilistic* North South	Chilli pepper / black pepper 3.7 (6.4) / 0.022 (0.051) 2.2 (4.6) / 0.016 (0.038) 0.12–0.37 / 0.03–0.08 (0.39–0.78) / (0.10–0.20) 0.08–0.23 / 0.02–0.06 (0.26–0.53) / (0.09–0.17)	Only chilli and black pepper included	Yogendrarajah et al. (2014b)
Tunisia	Survey mean	Mean of GEMS/ Food regional diets	Not specified	49	Only barley, sorghum, wheat and maize included	Serrano et al. (2012)
Tunisia	LB–UB survey mean	National food consumption survey	Adults urban Adults rural Adults total Infants (6 months)	0.04 0.08–0.09 0.07 1.4–1.5	Only sorghum included	Oueslati et al. (2014)
Turkey	Survey mean (positive samples only)	Not stated	Adults	Figs from domestic market: 0.78 Figs destined for export: 0.13	Only figs included	Bircan & Koc (2012)
United Republic of Tanzania	Mean of positive samples	Mean for South African beer drinkers	Adults	402	Only beer included	Shephard (2008)
AFB_2						
China, Hebei Province	Survey mean	Per capita wheat consumption	Adults	4	Only wheat included	Liu et al. (2015)

Table 16 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
France	UB survey mean	INCA2 – national 7-day food diary	Children (3–17) Adults (18–79)	0.39 (0.74) 0.2 (0.3)	Cereals	Sirot, Fremy & Leblanc (2013)
Morocco	Survey mean	Mean of GEMS/ Food regional diets	Not specified	<0.05	Only rice included	Serrano et al. (2012)
Spain, Valencia	LB survey mean	Spanish Agency for Food Safety Survey for longitudinal exposure (chronic) food consumption	Adolescents Adults	<0.001 (0.002) 0.001 (0.004)	Only coffee included	García-Moraleja et al. (2015)
Spain	Survey median	Manufacturers' recommended feeding rates	4–24 months[g]	Ecological infant cereals: 0.02–3.8 Conventional infant cereals: 0.02–0.13	Cocoa-containing, gluten-free, fruit-containing infant cereals	Hernández-Martínez & Navarro-Blasco (2010)
Sri Lanka	Survey mean	Household food frequency questionnaire (249 households). Assumed that spices were consumed equally by all household members	Adults Deterministic North South	Chilli pepper 0.12 (0.21) 0.004 (0.055) 0.07 (0.15) 0.003 (0.039)	Only chilli and black pepper included	Yogendrarajah et al. (2014b)
Tunisia	Survey mean	Mean of GEMS/ Food regional diets	Not specified	11	Only barley, sorghum, wheat and maize included	Serrano et al. (2012)
AFG_1						
France	UB survey mean	INCA2 – national 7-day food diary	Children (3–17) Adults (18–79)	0.39 (0.74) 0.2 (0.3)	Cereals	Sirot, Fremy & Leblanc (2013)
Morocco	Survey mean	Mean of GEMS/ Food regional diets	Not specified	<0.05	Only rice included	Serrano et al. (2012)
Spain, Valencia	LB survey mean	Spanish Agency for Food Safety Survey for longitudinal exposure (chronic) food consumption	Adolescents Adults	0.001 (0.013) 0.006 (0.029)	Only coffee included	García-Moraleja et al. (2015)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
Spain	Survey median	Manufacturers' recommended feeding rates	4–24 months[g]	Ecological infant cereals: 0.15–3.9 Conventional infant cereals: 0.005–0.16	Cocoa-containing, gluten-free, fruit-containing infant cereals	Hernández-Martínez & Navarro-Blasco (2010)
Tunisia	Survey mean	Mean of GEMS/Food regional diets	Not specified	9.9	Only barley, sorghum, wheat and maize included	Serrano et al. (2012)
AFG_2						
France	UB survey mean	INCA2 – national 7-day food diary	Children (3–17) Adults (18–79)	0.39 (0.74) 0.2 (0.3)	Cereals	Sirot, Fremy & Leblanc (2013)
Morocco	Survey mean	Mean of GEMS/Food regional diets	Not specified	<0.05	Only rice included	Serrano et al. (2012)
Spain, Valencia	LB survey mean	Spanish Agency for Food Safety Survey for longitudinal exposure (chronic) food consumption	Adolescents Adults	0.003 (0.028) 0.014 (0.063)	Only coffee included	García-Moraleja et al. (2015)
Spain	Survey median	Manufacturers' recommended feeding rates	4–24 months[g]	Ecological infant cereals: 0.05–0.68 Conventional infant cereals: 0.0015–0.05	Cocoa-containing, gluten-free, fruit-containing infant cereals	Hernández-Martínez & Navarro-Blasco (2010)
Tunisia	Survey mean	Mean of GEMS/Food regional diets	Not specified	9.7	Only barley, sorghum, wheat and maize included	Serrano et al. (2012)
AFM_1						
Argentina	Predicted from feed contamination	National data modelled as log-normal distributions	Adults	0.12	Milk and milk products	Signorini et al. (2012)
Brazil, São Paolo	Foods from volunteers' homes, mean of positive samples	Food frequency	Volunteers ($n = 34$)	0.14	Fluid milk	Jager et al. (2013)
Brazil, Minas Gerais State	Mean of raw milk survey	Per capita milk consumption	Adults	0.31	Only raw milk included	Picinin et al. (2013)

Table 16 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
Brazil, São Paolo	Mean of survey results	Not stated	Children Adults	1.0 0.19	Powdered milk Fluid and powdered milk	Shundo et al. (2009)
Brazil, Paraná State	Mean for UHT milk survey	Per capita milk consumption	Adults	0.02–0.07	UHT milk	Silva et al. (2015)
China	Survey data fitted to parametric and nonparametric distributions	National survey, 3-day 24-hour recall	Females (2–4) Males (30–45)	0.087 (0.32) 0.005 (0.019)	Only milk products included	Guo, Yuan & Yue (2013)
Egypt	Survey mean	Mean consumption figures (source not stated)	Infants (0–6 months)	Breast milk: 8.8 Infant formula: 1.4		El-Tras, El-Kady & Tayel (2011)
France	UB survey mean	INCA2 – national 7-day food diary	Children (3–17) Adults (18–79)	0.054 (0.13) 0.03 (0.05)		Sirot, Fremy & Leblanc (2013)
France[h]	Published studies	INCA2 – national 7-day food diary	Adults (18–34) Adults (35–54) Adults (55–79)	0.021–0.032 (0.069–0.086) 0.039–0.033 (0.077–0.097) 0.028–0.030 (0.077–0.085)		Wesolek & Roudot (2012)
Ireland	Predicted from AFB_1 contamination in feed	National survey, represented by log-normal distributions	Adult males Adult females	0.008 6 (0.21) 0.009 4 (0.24)	Milk	Coffey, Cummins & Ward (2009)
Lebanon	Survey mean	Food frequency (200 respondents)	Population	0.14	Milk and dairy products	Hassan & Kassaify (2014)
Lebanon	LB–UB survey mean	Food frequency (444 respondents)	Adults	0.22–0.31 (0.55–0.80)	Milk and milk-based beverages	Raad et al. (2014)
Portugal	Survey mean (positive samples only)	Per capita milk consumption	Adults	0.08	Only commercial milk samples included	Duarte et al. (2013)
Republic of Korea	Survey mean		1 month 1–3 months 3–6 months 6–12 months 12–24 months	0.65 0.49 0.36 0.35 0.22	Only infant formula included	Kang et al. (2013)
Serbia	Survey mean	Questionnaire about milk consumption	Males (1–5) Females (1–5) Males (5–15) Females (5–15) Males (15–25)	6.5 6.3 2.3 1.9 1.3	Only cows' milk included	Kos et al. (2014)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors[b]	Reference
			Females (15–25)	0.42		
			Males (25–55)	0.49		
			Females (25–55)	0.56		
			Males (55+)	0.51		
			Females (55+)	0.69		
Serbia	Survey mean	Serbian market basket	Adults	0.5–1.4[i]	Only commercial milk included	Škrbić et al. (2014)
Serbia	Survey mean	Serbian national food consumption survey	Adults	0.03–0.30[i]	Only milk included	Torovic (2015)
Spain	LB survey mean	Infant formula manufacturers' feeding tables	0–2 weeks	0.099 (S)[j]	Only infant formula included	Gómez-Arranz & Navarro-Blasco (2010)
			2–4 weeks	0.13 (S)		
			2 months	0.13 (S)		
			3 months	0.11 (S)		
			4–5 months	0.11 (S), 0.14 (F)		
			6 months	0.08 (S), 0.11 (F)		
			7–12 months	0.096 (F), 0.44 (T)		
			1 year	0.18 (T)		
			2–3 years	0.14 (T)		
Turkey	LB survey mean	Not stated	Adults	0.008[k]	Only UHT milk included	Kabak & Ozbey (2012)
Turkey	Survey means for starter, follow-on and toddler infant formulas	Manufacturers' feeding tables	5 months	0.080 (S)	Only infant formulas included	Kabak (2012)
			9 months	0.028 (F)		
			12 months	0.021 (T)		

F: follow-on formula; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; ND: not detected; S: starter formula; survey mean: mean of all survey samples; T: toddlers' formula; UB: upper bound; UHT: ultrahigh temperature

[a] High percentiles were 95th percentile, unless otherwise stated. Where dietary exposure estimates are presented as a range, the values are the LB and UB estimates of the mean or high percentile dietary exposure.

[b] Major contributing foods contributed at least 20% of total dietary exposure and in many cases greater than 50%.

[c] High percentile was taken as the mean for consumers only.

[d] The range of 95th percentile dietary exposure estimates relate to different treatments of left-censored data.

[e] The study reported dietary exposures as the aggregate of AFT and AFM_1.

[f] High consumer was defined as 97.5th percentile consumption of peanuts, with mean consumption of all other foods.

[g] Separate dietary exposure estimates were determined for five age groups within this range. However, results were not presented in a manner that allowed the separate estimates to be determined and only overall ranges were specified.

[h] Dietary exposure estimates were derived separately for males and females, while two different methods were used to model food consumption data. The exposure ranges presented here are the ranges across these variables.

[i] Dietary exposure was estimated for each of 3 months. The range represents the minimum and maximum monthly estimate.

[j] The original publication reported weekly dietary exposure estimates. These have been converted to daily estimates by dividing by seven. S, F and T refer to dietary exposure estimates determined for consumption of starter formula, follow-on formula and toddlers' formula, respectively.

[k] The study also determined in vitro bioavailability (86.3%) and incorporated this into the dietary exposure calculation.

from the mean of only those consumers who ate foods containing AFT on the survey day, that is, peanuts, peanut products, shelled Brazil nuts, other tree nuts, maize and rice. Exposure estimates, specific to the Federal District were derived using food consumption information from a household budget survey conducted by IBGE (n = 977 households over 7 days, with all foods entering the household assumed to be consumed equally by household members aged 2 years or older) and AFT analytical data from the Central Public Health Laboratory of the Federal District in 2002–2011. A high consumer food consumption estimate was derived by considering only households where AFT-containing foods were acquired during the survey period. For the Brazilian population, mean dietary exposure (lower–upper bound) was estimated to be 6.6–6.8 ng/kg bw per day, while high consumers' dietary exposures were estimated to be about 16.3–27.6 ng/kg bw per day. Rice contributed virtually all of the AFT exposure for the population mean exposure level, while rice and shelled Brazil nuts were the major contributors to exposure for high consumers. For the Federal District, mean AFT dietary exposures were much lower (0.06–0.08 ng/kg bw per day), while high consumer dietary exposure estimates were higher (33.3–47.1 ng/kg bw per day). Shelled Brazil nuts and peanuts were the major contributors to dietary AFT exposure in the Federal District.

A study in São Paolo determined AFT and AFM_1 dietary exposure based on analysis of food samples (n = 240) collected from households of 34 volunteers and food frequency questionnaires completed by the same volunteers in 2011 and 2012 (Jager et al., 2013). AFT was determined in peanut products, maize products and beans, while AFM_1 was determined in dairy products. All analyses were conducted by HPLC with fluorescence detection (HPLC-FD). Mean AFT dietary exposure was 1.58 ng/kg bw per day (range 0.042–14.0 ng/kg bw per day), while mean AFM_1 dietary exposure was 0.14 ng/kg bw per day (range 0.0058–0.34 ng/kg bw per day). On average, peanut products contributed more than 98% of AFT dietary exposure, while fluid milk contributed approximately 70% of dietary AFM_1 exposure.

A further study by the same group collected food samples (n = 222) from the households of volunteers in each of four seasons (n = 33, 31, 27 and 22 volunteers at the four time points) and determined food consumption using a 24-hour dietary recall instrument (Jager et al., 2016). This study was unusual in that it determined exposure as the sum of AFB_1 and AFM_1. Mean dietary exposure was estimated to range from 0.09 (summer) to 1.35 (spring) ng/kg bw per day. A significant correlation was found between dietary exposure to AFB_1 + AFM_1 and urinary AFM_1.

A study in the Paraná state of Brazil estimated dietary AFB_1 exposure from consumption of peanut products (Magrine et al., 2011). Aflatoxins were determined in commercial peanut products (n = 100) taken from stores, markets

and supermarkets. Food consumption was estimated from a questionnaire eliciting information on food frequency and amount of food consumed, completed by 384 adults (aged 18 years and over). Mean dietary AFB_1 exposure (lower–upper bound) was estimated to be 0.6–0.6 ng/kg bw per day, with 95th percentile dietary exposure estimated to be 10.0–10.4 ng/kg be per day.

Raw milk samples (*n* = 129) taken during three seasons in Minas Gerais state were analysed for AFM_1 (Picinin et al., 2013). Concentrations were highest (mean 0.22 µg/L) during the transitional period between dry and wet seasons. Dietary exposure to AFM_1 was estimated using a per capita milk consumption of 148 mL/person per day and assuming a 60 kg body weight. Mean dietary exposure was 0.31 ng/kg bw per day based on analyses by HPLC-FD and 0.047 ng/kg bw per day based on analyses by ELISA.

AFM_1 was determined in 125 samples of powdered milk, pasteurized milk and ultra-heat treated (UHT) milk from the city of São Paolo and dietary exposure was calculated for children and adults, based on a daily consumption of 400 mL of milk by a 23 kg child or 350 mL of milk by a 60 kg adult (Shundo et al., 2009). The estimated mean dietary exposure to AFM_1 was 1.0 ng/kg bw per day for children and 0.19 ng/kg bw per day for adults.

AFM_1 was determined in samples of UHT milk (*n* = 152) collected from the Paraná state of Brazil in each of the four seasons, and dietary exposure was estimated using the mean consumption of milk for Paraná state (132 mL/person per day) and a body weight of 60 kg (Silva et al., 2015). Mean estimated dietary exposure was approximately 0.02–0.07 ng/kg bw per day, with the highest estimated exposures associated with UHT milk sampled in autumn.

(d) **People's Republic of China**

Raw peanuts (*n* = 1040) were collected from four regions of China and analysed for AFB_1 (Ding et al., 2012). Lower- and upper-bound estimates of the mean AFB_1 concentration were calculated and combined with mean and 97.5th percentile estimates of peanut consumption taken from an earlier survey (methodology not identified). Mean estimated dietary exposures to AFB_1 from consumption of peanuts were 3.3 ng/kg bw per day for children (97.5th percentile: 17.1 ng/kg bw per day) and 1.3 ng/kg bw per day for adults (97.5th percentile: 6.6 ng/kg bw per day).

A further study by the same group determined AFB_1 in peanut samples (*n* = 2983) from the Yangtze River region (Ding et al., 2015). Lower- and upper-bound estimates of the mean AFB_1 concentration were combined with mean and high levels of the peanut consumption (Chinese resident nutrition and health survey, methodology not stated) for children (aged 2–6 years) and adults, and normalized using standard body weights (15.2 kg for children and 60.6 kg for

adults). Mean dietary exposure to AFB_1 from consumption of peanuts was estimated to be 0.78–0.79 ng/kg bw per day (high consumption 11.7–11.9) for children and 0.34–0.35 ng/kg bw per day (high consumption 4.1) for adults.

Dietary exposure to AFT from consumption of cereals and plant-derived oils was determined for the Yangtze delta region of China (Li et al., 2014). AFT (and AFB_1) were determined in 59 cereal samples, eight legume samples and nine oil samples. A mean per capita daily consumption of cereal products (402 g/day) was derived from annual per capita consumption figures for China in general. It should be noted that this level of consumption was applied equally to children and adults. High (95th percentile) consumption was taken as twice the mean consumption level. Calculations were based on child and adult body weights of 20 and 60 kg, respectively. Estimated mean dietary AFT exposure was 24.9 ng/kg bw per day (95th percentile 49.8 ng/kg bw per day) for children and 8.3 ng/kg bw per day (95th percentile 16.6 ng/kg bw per day) for adults.

Wheat samples from Hebei province were analysed for a range of mycotoxins, including the four B and G aflatoxins (Liu et al., 2015). Only AFB_1 and AFB_2 were detected. Based on mean wheat consumption in Hebei province of 216 g/person per day and a body weight of 60 kg, mean dietary exposure to AFB_1 and AFB_2 was estimated to be 0.03 and 0.004 µg/kg bw per day (30 and 4 ng/kg bw per day), respectively.

AFB_1 was determined in food samples (n = 209) from three regions of China, Huantai, Huaian and Fusui (Sun et al., 2011). The food samples included maize, rice, wheat flour, plant oils and peanuts. Food consumption was determined based on a food frequency questionnaire administered in the three study areas (number of respondents not stated). Across the three regions, mean dietary exposure to AFB_1 was reported to be approximately 0.40–2.7 µg/day (6.7–45 ng/kg bw per day for a 60 kg body weight). Maize and plant oils were the main contributors to AFB_1 exposure.

Spice samples (n = 480) were collected from retail outlets in eight Chinese cities in 2009 and analysed for AFB_1 by HPLC-FD (Zhao, Schaffner & Yue, 2013). Both deterministic and probabilistic estimates of dietary exposure were calculated. Chinese data on spice consumption were not available and estimates from other sources (India, Europe, New Zealand, Thailand and USA) were used as surrogates. Lower- and upper-bound mean spice AFB_1 concentrations were determined. The impact of excluding samples containing more than 10 or 20 µg/kg of AFB_1 was also considered. If no spice samples were excluded because of their high AFB_1 concentrations, mean deterministic estimates of dietary AFB_1 exposure from consumption of spices were approximately 0.0014–0.082 ng/kg bw per day. For maximum levels of 20 and 10 µg/kg, mean deterministic dietary AFB_1 exposure estimates were approximately 0.001–0.072 and 0.0009–0.068 ng/kg bw per day, respectively.

Probabilistic estimates of dietary exposure were determined by Monte Carlo simulation, with the concentration of AFB_1 represented by a triangular distribution, with minimum equal to zero or the LOD, mode equal to the mean concentrations used for deterministic calculations and maximum equal to the maximum concentration observed. Due to the asymmetric nature of the triangular distributions, all mean dietary exposure estimates from this approach were higher than the corresponding deterministic estimates, with estimates for the base, maximum level of 20 µg/kg and maximum level of 10 µg/kg in the range of 0.057–1.1, 0.024–0.46 and 0.011–0.24 ng/kg bw per day, respectively.

Milk products (n = 411) were collected throughout China in 2006–2007 and analysed for AFM_1 by ELISA (Guo, Yuan & Yue, 2013). Food consumption (3-day, 24-hour dietary recall) and body weight data for a variety of age–sex groups were taken from the 2002 National Health and Nutrition Survey. AFM_1 concentration data were fitted to parametric and nonparametric distributions and dietary exposure determined by Monte Carlo simulation. Mean dietary exposure estimates ranged from 0.005 (males 30–45 years) to 0.087 ng/kg bw per day (females 2–4 years). High (95th percentile) dietary exposures ranged from 0.019 (males 30–45 years) to 0.32 ng/kg bw per day (females 2–4 years). Exposure was predominantly due to consumption of liquid milk.

(e) Egypt

Fifty samples of cereal-based infant foods and 50 samples of maize-based snacks collected from supermarkets and small shops within the Greater Cairo Governate in 2008 were analysed by HPLC-FD (El-Sawi & El-Sawi, 2012). Dietary exposure estimates for AFB_1 and AFT were calculated by assuming an infant (10 kg bw) would consume 30 g/day of cereal-based infant food or maize-based snacks, whereas adolescents (40 kg bw) and adults (60 kg bw) would consume 50 g/day of maize-based snacks. Mean AFT and AFB_1 concentrations for the two food types (treatment of not detected results for mean calculation not stated) were used to calculated estimated daily intakes (EDIs) from consumption of maize-based snacks of 20, 8.4 and 5.6 ng/kg bw per day for AFT for infants, adolescents and adults, respectively, and 13, 5.5 and 3.7 ng/kg bw per day for AFB_1 for the same age groups. EDIs for infants from consumption of cereal-based infant foods were 7.3 and 4.1 ng/kg bw per day for AFT and AFB_1, respectively.

AFM_1 was determined in powdered infant formula (n = 125) and maternal breast milk (n = 125) by ELISA (El-Tras, El-Kady & Tayel, 2011). Mean dietary exposure from birth to 6 months was estimated using a mean consumption of 708 mL/day for breast milk and 834 mL/day for reconstituted infant formula. Estimated dietary exposure to AFM_1 was significantly greater from consumption of breast milk (52.7 ng/day) than of reconstituted infant formula (8.2 ng/day).

Assuming a mean body weight of 6 kg for infants aged 0–6 months, these exposures equate to 8.8 and 1.4 ng/kg bw per day, respectively.

(f) France

Dietary exposures to AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1 were estimated as part of the second French total diet study (Sirot, Fremy & Leblanc, 2013). Aflatoxin levels were determined by HPLC-FD in 577 food samples collected from mainland France. Individual food consumption data and body weights for 1918 adults (aged 18–79 years) and 1444 children (aged 3–17 years) were taken from the INCA2 study, a 7-day food diary study. Only AFB_1 was detected in any of the food samples analysed. Lower- and upper-bound estimates of mean and 95th percentile dietary exposure were determined for AFB_1, while for the other aflatoxins, only upper-bound estimates were determined. Mean and 95th percentile dietary exposures for adults were 0.002–0.22 and 0.01–0.39 ng/kg bw per day for AFB_1, 0.2 and 0.3 ng/kg bw per day for each of AFB_2, AFG_1 and AFG_2, and 0.03 and 0.05 ng/kg bw per day for AFM_1. For children, dietary aflatoxin exposures (mean and 95th percentile) were 0.001–0.39 and 0.008–0.74 ng/kg bw per day for AFB_1, 0.39 and 0.74 ng/kg bw per day for each of AFB_2, AFG_1 and AFG_2, and 0.054 and 0.13 ng/kg bw per day for AFM_1. For the B and G aflatoxins, the major contributors to dietary exposure were cereal products. However, given the very small number of quantified results, this is more a reflection of the amount of these foods consumed than a measure of aflatoxin exposure.

Dietary exposure to AFM_1 by the adult French population was estimated by Monte Carlo simulation, using @Risk software (Wesolek & Roudot, 2012). Probability density functions were defined for dairy food consumption, based on data from the INCA2 study, AFM_1 contamination of dairy products, based on the Blanco et al. (1988) study and body weights based on the INSEE study (Tanguy, Zeghnoun & Dor, 2007). For AFM_1 concentrations, a log-normal distribution was defined using the upper-bound mean and standard deviation from the Blanco et al. (1988) study. Calculations were also carried out using "raw" food consumption data from the INCA2 study. Dietary exposure estimates were determined for three age ranges (18–34, 35–54 and 55–79 years) for males and females. Across both sexes, all age ranges and both treatments of the food consumption data, mean dietary exposure to AFM_1 was estimated to be 0.021–0.033 ng/kg bw per day, with 95th percentile dietary exposures 0.069–0.097 ng/kg bw per day.

(g) Greece

AFB_1 was determined by HPLC-FD in breakfast cereals (n = 55) purchased from the Athens market (Villa & Markaki, 2009). Dietary exposures to AFB_1 were estimated based on nominal values for consumption of breakfast cereals by

children (30–50 g/day), adolescents (50–100 g/day) and adults (50–100 g/day) and body weights of 20, 50 and 70 kg, respectively, and median concentrations of AFB_1 found in breakfast cereals (0.05 µg/kg). Dietary exposure estimates for children, adolescents and adults were 0.07–0.12, 0.05–0.10 and 0.03–0.07 ng/kg bw per day, respectively.

(h) Islamic Republic of Iran

AFB_1 was determined in samples (n = 90) of rice, bread, a type of puffed corn snack, peanuts and wheat flour collected in Teheran in June 2005 (Yazdanpanah et al., 2013). Figures for consumption of rice and bread (mean 107 and 286 g/day, respectively) were taken from an earlier survey (method not stated), while nominal consumption levels were assigned to the puffed corn snack (65 g/day) and peanuts (1 g/day). An adult body weight of 70 kg was assumed. Mean dietary exposure to AFB_1 was estimated to be 3.6 ng/kg bw per day, with more than 95% of dietary exposure from consumption of rice.

(i) Ireland

A stochastic simulation model was used to predict AFM_1 contamination of milk and dietary exposure due to consumption of AFB_1-contaminated feed by dairy cows (Coffey, Cummins & Ward, 2009). Food consumption information was taken from Irish Universities Nutrition Alliance Survey (IUNA, 2001). Food consumption was represented by log-normal distributions for whole milk, low-fat skimmed milk and processed milk. Mean body weights for adult males (82.9 kg) and females (67.5 kg) were also taken from the survey. The mean simulated AFM_1 concentration in milk was 0.016 µg/kg, with a 95th percentile of 0.083 µg/kg. Mean and 95th percentile estimates of dietary exposures to AFM_1 from consumption of milk were estimated to be 0.0086 and 0.21 ng/kg bw per day for adult males and 0.0094 and 0.24 ng/kg bw per day for adult females, respectively.

(j) Japan

All B and G aflatoxins were analysed by HPLC in bitter chocolate, peanuts (whole, shelled, butter, flour), maize (raw, canned, grits, cornflakes), rice, buckwheat (flour, noodles), popcorn and sesame oil (Kumagai et al., 2008). Aflatoxins were only detected in peanut butter (10 of 21) and bitter chocolate (22 of 42). Food consumption information for cacao and cacao products was taken from the 2005 National Health and Nutrition Survey and fitted to log-normal distributions by age group. Aflatoxin concentration data were fitted to a log-normal distribution for positive results and a uniform distribution for results below the LOQ. Consumption and concentration distributions were combined by Monte Carlo

simulation. High percentile (95th) estimates of dietary AFT exposure were below 0.008 ng/kg bw per day for all age groups.

Dietary exposure to AFT and AFB_1 was estimated using data from a 3-year retail market survey of foods (*n* = 884 samples) and food consumption data from the National Health and Nutrition Survey (Sugita-Konishi et al., 2010). Dietary exposure was estimated by Monte Carlo simulation using log-normal distributions fitted to food consumption data and lower- and upper-bound estimates of the mean AFT or AFB_1 concentration for each food. Lower- to upper-bound estimates of high percentile (95th) dietary exposure ranged from 0.003–0.003 ng/kg bw per day (adults 20+ year, AFT or AFB_1) to 0.013–0.014 ng/kg bw per day (children 1–6 years, AFT or AFB_1). This approach combines consumption of different foods in a completely random manner and the high percentile may not represent a true high consumer.

(k) Kenya

Dietary exposure to AFT from consumption of maize and maize products was investigated in 72 households in eastern Kenya (Kilonzo et al., 2014). Samples of maize and maize products were taken from 24 households and analysed for AFT by HPLC. Information on maize consumption was elicited from 299 individuals (technique not specified). AFT concentrations were fitted to either log-normal or pert distributions and combined with food consumption information by Monte Carlo simulation. Mean dietary exposure to AFT from consumption of maize kernels was 292 ng/kg bw per day, while dietary exposure from consumption of maize meal and *muthokoi* (decorticated maize) was 59 and 27 ng/kg bw per day, respectively.

Aflatoxin exposure in children (aged 1–3 years) was investigated in 204 low-income households in Nairobi, Kenya (Kiarie et al., 2016). Samples of maize, peanuts, sorghum and milk were collected from the households or their usual retailer and analysed for AFT and AFM_1 by ELISA. Food consumption information was collected using a four-pass 24-hour dietary recall. Questionnaires were completed by parents on behalf of their child. The study was unusual in reporting aggregate exposure to AFT and AFM_1. Mean dietary exposure to AFT + AFM_1 was 21.3 ng/kg bw per day (range 0–197 ng/kg bw per day), with about 60% of exposure from maize consumption, on average.

(l) Lebanon

AFM_1 content of milk and dairy products (*n* = 524) was determined by ELISA (Hassan & Kassaify, 2014). Consumption of dairy products by a random cohort of 200 individuals was determined via a food frequency questionnaire. Mean

dietary exposure to AFM_1 from consumption of dairy products was reported to be 0.14 ng/kg bw per day.

A total diet study was used to estimate the dietary exposure of the adult Lebanese population to AFB_1 and AFM_1 (Raad et al., 2014). A total of 705 food samples from 47 food types were prepared ready for consumption, then composited across similar food types to give 33 composite samples for AFB_1 analysis and 12 composite samples for AFM_1 analysis. Due to the high proportion of left-censored data, lower- and upper-bound estimates of the mean concentrations of AFB_1 and AFM_1 were calculated from a food frequency survey of 444 individuals from Beirut, and combined with mean or 95th percentile estimates of food consumption. Mean and 95th percentile dietary exposures to AFB_1 were 0.63–0.66 and 1.40–1.46 ng/kg bw per day, respectively, with more than three quarters of mean exposure due to consumption of bread and toast. Mean and 95th percentile dietary exposures to AFM_1 were 0.22–0.31 and 0.55–0.80 ng/kg bw per day, respectively, with the majority of exposure from consumption of milk and milk-based beverages.

Dietary exposure to AFT was estimated for Lebanese children and teenagers (Soubra et al., 2009). AFT was determined in 1160 food items, including cereals and cereal products, pulses and nuts. Lower- and upper-bound estimates of mean concentration were determined for different food types and combined with individual food consumption information from a 1-day 24-hour dietary recall and a 1-year food frequency survey. For children (aged 8–13 years), mean and 95th percentile estimates of dietary AFT exposure were 1.5–4.4 and 3.5–7.7 ng/kg bw per day, respectively. For teenagers (aged 14–18 years), mean and 95th percentile estimates of dietary AFT exposure were 1.3–3.8 and 3.1–6.5 ng/kg bw per day, respectively. Cereals and cereal products, including bread, biscuits, manakeesh and cakes, were the major contributors to AFT exposure, accounting for approximately 60% of exposure.

(m) Malaysia

The AFT content of 84 samples of raw peanut kernels was determined by HPLC-FD (Arzandeh, Selamat & Lioe, 2010). Mean AFT concentration was determined as the sum of AFB_1, AFB_2, AFG_1 and AFG_2 averaged across all samples, assuming that samples in which these were not detected contained true zero concentrations. A national consumption rate for peanuts of 56.9 g/day and a standard body weight of 60 kg were used to estimate dietary exposure to AFT from consumption of peanuts as 10.7 ng/kg bw per day.

AFB_1 was determined in 128 samples of nuts and nut products, sampled from retail outlets in Penang, Malaysia, by LC-MS/MS (Leong et al., 2011). Mean AFB_1 concentrations were calculated for each nut or nut product type,

assuming that results below the LOD represented true zero concentrations. Food consumption information was derived from an interviewer-assisted food frequency questionnaire administered to 364 adults from the Penang region. Dietary exposure estimates were derived for mean and high (95th percentile) levels of nut consumption. A standard body weight of 60 kg was assumed. Mean and 95th percentile estimates of dietary exposures to AFB_1 from consumption of nuts and nut products were 0.36 and 8.89 ng/kg bw per day, respectively, with the highest individual contributing food being fried peanuts.

Aflatoxins were determined in 34 samples of commercial processed spices, sampled in Penang, Malaysia, by HPLC-FD (Ali, Hashim & Shuib, 2015). Based on the mean AFB_1 concentration in positive samples only (1.38 µg/kg), a spice consumption value of 3 g/day and a body weight of 50 kg, the study reported a dietary exposure to AFB_1 from consumption of spices of 0.09 ng/kg bw per day.

Aflatoxin dietary exposure was estimated for the Malaysian population using a total diet approach (Chin, Abdullah & Sugita-Konishi, 2012). Individual food composites (n = 236), representing 38 foods, were prepared ready for consumption and analysed by HPLC-FD. Lower- and upper-bound estimates of the mean AFT and AFB_1 concentrations were derived for each food. Food consumption information was taken from the Malaysian Food Consumption Survey 2003, a 15-month food frequency survey, covering Malaysians aged 18–59 years. Mean estimated dietary exposure to AFT and AFB_1 were 29–58 and 24–34 ng/kg bw per day, respectively. Greater than 80% of dietary exposure was due to consumption of peanuts. The impact of two different maximum levels (5 and 15 µg/kg) were considered. Removal of samples containing more than 15 µg/kg AFT from the concentration calculation resulted in estimates of dietary exposures of 2.7–32 and 2.3–12 ng/kg bw per day for AFT and AFB_1, respectively. Application of the lower maximum level (5 µg/kg) resulted in estimates of dietary exposures of 0.6–30 and 0.5–10 ng/kg bw per day for AFT and AFB_1, respectively. A high consumer dietary exposure estimate was also derived by assuming a 97.5th percentile consumption of peanuts and a mean consumption of all other foods. Dietary exposure estimates, without application of a maximum level, were 160–200 and 140–150 ng/kg bw per day for AFT and AFB_1, respectively.

AFT was determined in samples of raw peanuts, roasted peanuts and rice (n = 310) by HPLC-FD (Othman & Keat, 2006). Mean AFT concentrations were calculated for each food type by assuming not detected results were equal to half the LOQ. Food consumption data from the adult population (aged 18–59 years) was derived from a food frequency survey. A standard body weight of 63 kg was used. Dietary AFT exposure was estimated to be 10 ng/kg bw per day, with approximately 70% of dietary exposure due to consumption of raw peanuts.

(n) Mexico

AFT contamination data were taken from a previous study of tortilla in Mexico City (Castillo-Urueta et al., 2011) and generalized to a range of maize-based foods (Wall-Martinez et al., 2014). Food consumption information was derived from a 3-day diary of tortilla consumption (n = 172) and a food frequency questionnaire for maize-based foods (n = 122) completed by residents of Veracruz City. The distributions of food consumption and AFT concentration were fitted to log-normal distribution, while the proportions of consumer/non-consumers and contaminated/non-contaminated samples were expressed as binomial distributions. Estimates of dietary AFT exposure were determined by simulation (using @Risk software). Exposures were determined separately for males and females, but were quite similar. Mean and 95th percentile estimates of AFT exposure from consumption of maize-based foods were 12–16 and 52–66 ng/kg bw per day, respectively. Removing tortilla from the exposure calculation reduced estimates of exposure by about 80%.

(o) Morocco

Samples of cereals (n = 70; brown and white rice) were collected from supermarkets and food stores in 2010 and analysed by LC-MS/MS (Serrano et al., 2012). The source of food consumption information and body weights used in this exposure assessment were not given. Dietary exposures to AFB_1, AFB_2, AFG_1 and AFG_2 were all estimated to be less than 0.05 ng/kg bw per day.

(p) The Netherlands

Duplicate diets (24-hour) for children (aged 2–6 years, n = 123), collected in 2006, were analysed for AFB_1 and AFM_1 by HPLC-FD (Bakker et al., 2009). AFM_1 was only detected at trace concentrations (<0.014 µg/kg) in 10% of duplicate diet samples. Approximately half of the duplicate diet samples contained quantifiable concentrations of AFB_1. For dietary exposure estimation, analytical results below the LOD were assigned a value of half the LOD. The highest estimated dietary exposure to AFB_1 was 0.43 ng/kg bw per day. Results were only presented graphically and conformed to a right-skewed distribution with a mode of 0.07 ng/kg bw per day.

(q) New Zealand

AFT and AFB_1 were determined in samples of maize and maize products, nuts and nut products, dried fruit and spices by HPLC-FD (Cressey & Reeve, 2013). Lower- and upper-bound estimates of mean AFT and AFB_1 concentrations were calculated for each of 28 food types. Individual food consumption information and body weights for children (aged 5–14 years) and adults (15+ years) were

taken from 24-hour dietary recalls administered as part of national nutrition surveys. Spice consumption was determined by applying typical recipes to spice-containing foods. Estimated mean dietary AFT exposures ranged from 0.09–0.11 (adult females) to 0.32–0.39 ng/kg bw per day (5–10 years), while mean dietary AFB_1 exposures ranged from 0.07–0.09 (adult females) to 0.27–0.33 ng/kg bw per day (children 5–10 years). The highest 95th percentile dietary exposures were 0.77–1.2 (AFT) and 0.63–1.1 ng/kg bw per day (AFB_1) for children aged 5–10 years. Spices accounted for approximately 50% of dietary exposure (upper-bound AFT). However, this was largely due to a single very high analytical result in one spice sample. Exclusion of this analytical value resulted in estimates of dietary exposure reducing by 30–40%.

(r) Pakistan

AFB_1 was determined in samples of rice and rice products (n = 208) from the Punjab region of Pakistan by HPLC-FD (Iqbal et al., 2016). Lower- and upper-bound estimates of mean AFB_1 concentrations were calculated. It should be noted that the lower-bound estimates were determined substituting a value of LOD/√2 for results less than LOD and a value equal to the LOD for results less than LOQ. Mean and 95th percentile rice consumption levels (source not stated) were combined with mean AFB_1 concentrations and standard body weight of 60 kg giving estimates of dietary AFB_1 exposure from consumption of rice and rice products of 22.2–22.3 ng/kg bw per day (mean) and 29.1–30.2 ng/kg bw per day (95th percentile).

(s) Portugal

Commercial milk samples (n = 40) were analysed for AFM_1 by ELISA (Duarte et al., 2013). Mean AFM_1 concentration was calculated only for samples with concentrations higher than the cut-off limit for the test (5 ng/L). Per capita consumption of milk in Portugal is 87 kg per person per year (238 g/person per day), while the mean body weight for a Portuguese adult is 69 kg. Adult dietary exposure to AFM_1 from consumption of milk was estimated to be 0.08 ng/kg bw per day.

(t) Republic of Korea

AFB_1 was determined in 694 food samples purchased in six cities in the Republic of Korea in 2004 and 2005. The food samples included cereals and cereal products, soybeans and soybean products, peanuts and tree nuts and spices (Ok et al., 2007). Food consumption data were obtained from the 2001 National Health and Nutrition Survey and mean body weights (62 kg) from the Korea Food and Drug Administration. Dietary exposure to AFB_1 was determined by Monte Carlo

simulation, with AFB_1 concentration represented by a distribution. AFB_1 results below the LOD were assigned a value of zero, while results between the LOD and LOQ were assigned a value of LOQ/2. Mean dietary exposure to AFB_1 was estimated to be 0.64 ng/kg bw per day, with a 95th percentile of 2.5 ng/kg bw per day. The major contributors to AFB_1 dietary exposure were soybean paste, soy sauce and peanuts, with soybean paste and soy sauce accounting for 91% of dietary AFB_1 exposure.

AFM_1 was determined in reconstituted powdered infant formula (n = 439) by HPLC-FD (Kang et al., 2013). Based on a mean AFM_1 concentration of 2.6 ng/kg of prepared formula, dietary exposure estimates were determined for infants aged 0–1, 1–3, 3–6, 6–12 and 12–24 months, with estimates in the range of 0.22–0.65 ng/kg bw per day.

(u) Serbia

AFM_1 was determined in samples of cows' milk (n = 150) by ELISA (Kos et al., 2014). Mean milk consumption was determined from a survey of 1500 people (details of methods not provided). Mean body weights were used for a range of age–sex groups, but the source of the body weights was not stated. Mean dietary exposure to AFM_1 from consumption of milk ranged from 0.49 (males 25–55 years) to 6.5 ng/kg bw per day (males 1–5 years).

AFM_1 was determined in commercial milk samples (n = 50) by UHPLC-MS/MS (Škrbić et al., 2014). Mean AFM_1 concentrations were calculated for each of three time points (February, April and May) and combined with mean milk consumption estimates from the Serbian market basket (177.5 g/day) and a standard body weight of 60 kg. Estimates of dietary AFM_1 exposure from consumption of milk ranged from 0.50 (in May) to 1.4 (in February) ng/kg bw per day.

AFM_1 was determined in samples of milk (n = 80) by HPLC-FD (Torovic, 2015). Mean AFM_1 concentrations were combined with mean estimates of milk consumption from the Serbian national food consumption survey (135 g/day) and a standard body weight of 60 kg. Estimates of dietary exposure to AFM_1 from consumption of milk ranged from 0.03 (December 2014) to 0.30 (August 2013) ng/kg bw per day.

(v) Spain

AFT was analysed in 603 food samples, including peanuts, pistachios, dried figs, maize products, red pepper, gluten-free foods, ethnic foods and baby foods by HPLC-FD (Cano-Sancho et al., 2013). Mean AFT concentrations were calculated by two different techniques for treatment of left-censored data: substitution by a value equal to half the LOD and a nonparametric Kaplan–Meier method. Food

consumption estimates for the Catalonian population were derived from the food frequency study involving 1393 respondents and frequency of consumption of 38 food types. Deterministic estimates of dietary exposure to AFT ranged from 0.072 (adult males, substitution method) to 0.276 (immigrants, Kaplan–Meier method) ng/kg bw per day.

All samples of barley (n = 123) from the Navarra region of Spain, analysed by rapid resolution liquid chromatography (RRLC)-FD (Ibáñez-Vea et al., 2012), were positive for AFB_1. The mean concentration of AFB_1 in barley samples was combined with a daily cereal consumption estimate of 239 g/day (source not given) and a standard body weight of 70 kg to give a mean estimate of AFB_1 exposure of 0.44 ng/kg bw per day.

Samples of coffee (n = 169) purchased from supermarkets in Valencia, Spain and prepared ready for consumption were analysed by LC-MS/MS (García-Moraleja et al., 2015). Mean concentrations were calculated by assuming that samples with concentrations below the LOD and LOQ contained zero concentrations of aflatoxins. Coffee consumption data were obtained from the Spanish Agency for Food Safety Survey, a survey of 1067 participants conducted in 2009, for long-term (chronic) exposure via food consumption. The mean (95th percentile) EDIs of AFB_1, AFB_2, AFG_1, AFG_2 and AFT for adults were 0.003 (0.013), 0.001 (0.004), 0.006 (0.029), 0.014 (0.063) and 0.036 (0.17) ng/kg bw per day, respectively. The corresponding EDIs for adolescents were consistently lower, with AFT exposures of 0.008 (0.074) ng/kg bw per day. These dietary exposure estimates are somewhat unusual in finding that exposure to AFG_1 plus AFG_2 was greater than exposure to AFB_1 plus AFB_2.

Samples of infant formula (n = 69) were analysed for AFM_1 by HPLC-FD (Gómez-Arranz & Navarro-Blasco, 2010). Mean AFM_1 concentrations were calculated assuming that samples with analytical results below the LOD contained no AFM_1. Manufacturers' feeding tables were used to estimate dietary exposure. Body weights for infants were derived from standard weight-for-age tables. Mean estimated dietary exposure to AFM_1 from consumption of formula was highest for infants aged 7–12 months consuming toddler formula (3.1 ng/kg bw per week equivalent to 0.44 ng/kg bw per day) and lowest for 6-month-old infants consuming starter formula (0.56 ng/kg bw per week, equivalent to 0.08 ng/kg bw per day). Exposure to AFM_1 for infants receiving specialized preterm infant formula was lower (0.11–0.14 ng/kg bw per week, equivalent to 0.015–0.020 ng/kg bw per day).

Infant cereal samples (n = 91) were analysed for AFT by HPLC-FD (Hernández-Martínez & Navarro-Blasco, 2010). Median AFT concentrations for different types of infant cereals were combined with manufacturers' recommended feeding rates and mean body weights for various ages (4, 5, 6, 7–12 and 13–24 months). Estimated dietary exposures to AFB_1 and AFT from consumption of

conventional infant cereals were 0.01–0.62 and 0.08–0.94 ng/kg bw per day, respectively. Estimated dietary exposures from consumption of "ecological" infant cereals were 0.12–29 and 0.17–37 ng/kg bw per day, respectively. Cocoa-containing cereals were the major contributor the dietary AFB_1 and AFT exposure for infants aged 7 months and older. Gluten-free cereals and fruit-containing cereals were major contributor for younger age groups.

(w) Sri Lanka

Aflatoxin contamination of 82 samples of black pepper (*Piper nigrum* L.) obtained from various points along the supply chain was determined by LC-MS/MS (Yogendrarajah et al., 2014a). Consumption of black pepper was determined from a small survey of 15 families (34 g/person per month or 1.1 g/person per day for a 30-day month). Dietary exposures to AFT and AFB_1 from consumption of black pepper were determined using the mean aflatoxin concentrations in positive black pepper samples only. Although not stated, it appears that a standard body weight of 60 kg was used. Dietary exposures to AFT and AFB_1 from consumption of black pepper were 0.19 and 0.17 ng/kg bw per day, respectively.

A quantitative risk assessment was conducted for AFB_1 in chilli (*Capsicum annum* L.) and black pepper (*Piper nigrum* L.) (Yogendrarajah et al., 2014b). Spice consumption information was obtained from a food frequency survey of households (n = 249) in the north and south of Sri Lanka. Spice samples (n = 168) were collected from different regions of Sri Lanka in 2011–2012 and analysed by UHPLC-MS/MS. Exposure assessments were carried out separately for chilli and black pepper. For chilli, deterministic estimates of dietary exposure to AFB_1 and AFB_2 were determined for various combinations of the mean and high percentiles of chilli consumption and aflatoxin contamination level. Estimated dietary exposure, based on the mean concentrations for AFB_2 and mean and 95th percentile chilli consumption levels, were 0.12 and 0.21 ng/kg bw per day, respectively, in the north of Sri Lanka and 0.07 and 0.15 ng/kg bw per day, respectively, in the south of the country. The equivalent mean and 95th percentile dietary exposure estimates for AFB_1 were 3.7 and 6.4 ng/kg bw per day in the north of Sri Lanka and 2.2 and 4.6 ng/kg bw per day in the south. Contamination levels in black pepper were much lower than those in chilli, and deterministic estimates of dietary exposure to each of the individual aflatoxins were less than 0.05 ng/kg bw per day at mean black pepper consumption levels. Dietary exposure to AFB_1 at the 95th percentile level of black pepper consumption was 0.051 ng/kg bw per day in the north and 0.038 ng/kg bw per day in the south. Parametric distributions were fitted to AFB_1 contamination levels and spice consumption data for both chilli and black pepper and dietary exposure was estimated by Monte Carlo simulation. Lower–upper bound estimates for the

mean and 95th percentile dietary AFB_1 exposure from consumption of chilli were 0.12–0.37 and 0.39–0.78 ng/kg bw per day, respectively, for the north of Sri Lanka and 0.08–0.23 and 0.26–0.53 ng/kg bw per day, respectively, for the south of the country. Dietary exposure to AFB_1 from consumption of black pepper differed little between north and south, with mean and 95th percentile exposure estimates in the range of 0.02–0.08 and 0.09–0.20 ng/kg bw per day, respectively.

(x) Tunisia

Samples of cereals (n = 52; barley, sorghum, wheat and maize) were collected from supermarkets and food stores in 2010 and analysed by LC-MS/MS (Serrano et al., 2012). The source of food consumption information and body weights used in this exposure assessment were not given. Dietary exposures to AFB_1, AFB_2, AFG_1 and AFG_2 were estimated to be 49, 11, 9.9 and 9.7 ng/kg bw per day, respectively.

Sorghum samples from Tunisian markets (n = 60), collected in 2013, were analysed for a range of mycotoxins, including all four B and G aflatoxins by LC-MS/MS (Oueslati et al., 2014). Sorghum consumption information was taken from national consumption data, with mean daily intakes of 7.7, 9.3 and 4.6 g/day for total, urban and rural populations, respectively. A standard body weight of 60 kg was used. Estimates were also derived for a 6-month-old infants consuming 20 g/day of sorghum and weighing 8 kg. Although mean AFB_1 concentrations were calculated using both lower- and upper-bound assumptions, there was little difference between these concentration estimates. Estimates of dietary AFB_1 exposure for rural, urban, total and infant populations were 0.04, 0.09, 0.07 and 1.5 ng/kg bw per day, respectively, for upper-bound estimates of the concentration mean.

(y) Turkey

Dried fig samples were collected from exporting companies (n = 2461) and the domestic market (n = 219) during the 2009 crop year and tested for AFT by HPLC-FD (Bircan & Koc, 2012). Mean AFT and AFB_1 concentrations were calculated for samples that contained detectable aflatoxin concentrations only. Concentration values were combined with a daily dried figs consumption of 2.2 g/day, for the Aegean region of Turkey, and a body weight of 60 kg. Dietary AFT exposures from consumption of dried figs from the domestic market and destined for export were 1.3 and 0.2 ng/kg bw per day, respectively. Corresponding dietary exposures to AFB_1 were 0.78 and 0.13 ng/kg bw per day, respectively.

UHT milk samples (n = 40) from the main processors in Turkey were purchased from supermarkets in Corum, Turkey in 2011 and analysed for AFM_1 by HPLC-FD (Kabak & Ozbey, 2012). The mean AFM_1 concentration was calculated by assuming that analytical results less than the LOD were equal to

zero. Milk consumption was taken as 71 g/day and an adult body weight was assumed to be 60 kg. The in vitro bioavailability of AFM_1 in milk determined in the study, 86.3%, was incorporated into the estimation of dietary exposure. Dietary exposure to AFM_1 from consumption of UHT milk was estimated to be 0.008 ng/kg bw per day.

AFM_1 was analysed in samples of infant formula (n = 62) collected from supermarkets and drug stores in Corum, Turkey in 2011, by HPLC-FD (Kabak, 2012). Samples included starter, follow-on and toddler formulas. Infant formula consumption was based on manufacturers' feeding tables and assumed exclusive formula use. Dietary exposure estimates were 0.080 ng/kg bw per day for 5-month-old infants (mean weight 6.5 kg bw) on starter formula; 0.028 ng/kg bw per day for 9-month-old infants (mean weight 9 kg bw) on follow-on formula; and 0.021 ng/kg bw per day for 12-month-old infants (mean weight 10 kg bw) on toddler formula.

(z) United Republic of Tanzania

A study of dietary exposure to aflatoxins in infants (n = 143) was conducted in Rombo, northern United Republic of Tanzania, in 2011–2012 (Magoha et al., 2016). Following the recruitment of mothers, follow-up visits took place at 1, 3 and 5 months of age for infants. Infant food consumption at each time point was estimated from 24-hour dietary recalls completed by the mother. The infants' weekly frequency of maize consumption after their introduction to complementary food was also recorded, with samples of the maize consumed analysed by HPLC-FD. At 3 months of age, 67 infants were eating maize-based complementary foods. Aflatoxins were detected in maize samples from 58% of these households (39 infants). For infants eating contaminated maize, dietary AFT exposure was 0.14–120 ng/kg bw per day, with a median dietary exposure of 3.9 ng/kg bw per day.

(aa) Summary

National estimates of dietary aflatoxin exposure described in the previous sections are summarized in Table 16. Comparison of different estimates is complicated by the various ranges of food included in the various studies. Mean AFT dietary exposures in developed countries are generally less than 1 ng/kg bw per day, even at high exposure percentiles (e.g. 95th). Dietary exposure estimates for some sub-Saharan African countries exceed 100 ng/kg bw per day. However, it should be noted that these estimates are often based on very minimal data.

AFB_1 dietary exposure estimates also indicate differences between developed and developing countries, with dietary exposures in developed countries usually less than 1 ng/kg bw per day, even at high exposure percentiles

(e.g. 95th). Mean dietary exposures in developing countries range from less than 0.1 to approximately 400 ng/kg bw per day.

Estimates of dietary exposure to AFM_1 rarely exceed 1 ng/kg bw per day in any country. The exception was a study in Egypt that estimated dietary exposures up to 8.8 ng/kg bw per day for breastfed infants (El-Tras, El-Kady & Tayel, 2011) and one in Serbia that estimated dietary exposures up to 6.5 ng/kg bw per day for young children (1–5 years; Kos et al., 2014).

8.3.2 National estimates of dietary exposure derived by the Committee

Additional national estimates of dietary exposure were derived by the Committee. Estimates were derived in instances when:

- National food consumption information was available through CIFOCOss;
- Suitable concentration data have been submitted to the Committee (GEMS/Food contaminants database); and
- No existing recent dietary exposure assessment for AFT, AFB_1 or AFM_1 is available for the country (section 8.3.1).

CIFOCOss total mean (g/kg bw per day) food consumption information was used for all additional national dietary exposure assessments. Lower- and upper-bound mean aflatoxin concentrations were calculated for all Level 3 food descriptors for which aflatoxin concentration data were available for the specific country, setting "not detected" analytical results to zero or the LOD. For each country, concentration data from random sampling were compared with data from targeted surveys to determine that there was no major bias in the results from targeted surveys. When mean results from targeted surveys differed from those from random sampling by at least an order of magnitude (>10 times), the results from the targeted survey were excluded from calculation of the lower- and upper-bound mean. This measure was applied to exclude data related to specific investigation of heavily contaminated samples, as it is likely that the food products related to such contamination incidents would have been excluded from the food supply.

In some cases, only a small number of samples (<10) contributed to the mean calculation for a particular food–country combination. Where such means contributed a high proportion of the estimated dietary exposure, this has been highlighted.

Food names used in the GEMS/Food contaminants database were matched ("mapped") to Level 3 food names in CIFOCOss, after checking that

local food names were appropriate to the GEMS/Food food name. In a small number of cases, local food names were used to reassign individual analytical data to more appropriate GEMS/Food contaminants database food names.

Additional national estimates of dietary exposure to aflatoxins are summarized in Table 17. The structure of the CIFOCOss database did not allow estimation of high exposure percentiles. However, it has been proposed that a crude approximation of intake of a substance at the 90th percentile can be obtained by doubling the calculated mean intake (FAO/WHO, 2009). This approximation has been used to provide indicative high percentile estimates of dietary aflatoxin exposure for the additional national estimates of dietary exposure to aflatoxins.

All mean estimates of dietary exposure to AFT or AFB_1 were less than 10 ng/kg bw per day, at the upper bound, with most less than 5 ng/kg bw per day. Mean estimates of dietary exposure to AFM_1 were mostly less than 0.5 ng/kg bw per day except for estimates for Bulgaria (infants, toddlers and other children) with upper-bound dietary exposure estimates up to 2.5 ng/kg bw per day.

8.3.3 International estimates of dietary exposure

Further details on the concentration and consumption data used to derive international estimates of dietary exposure are included in sections 7.1 and 7.2 of this report. Summaries of the international mean total dietary exposure estimates for AFT, AFB_1 and AFM_1 from all contributing food sources, expressed in nanograms per kilogram body weight per day, for the 17 GEMS/Food cluster diets are presented in Tables 18–20. The structure of the GEMS/Food cluster diets did not allow estimation of high exposure percentiles. However, it has been proposed that a crude approximation of intake of a substance at the 90th percentile can be obtained by doubling the calculated mean intake (FAO/WHO, 2009). This approximation has been used to provide indicative high percentile estimates of dietary aflatoxin exposure for the international estimates of dietary exposure to aflatoxin. It should be noted that high percentile estimates of dietary exposure have been included only for the total dietary exposure estimates, not for the estimates of dietary exposure from individual food commodities.

A standard body weight of 60 kg was used for all GEMS/Food clusters to assess exposure per kilogram of body weight.

Mean dietary exposure to AFT ranged from 0.3–1.3 ng/kg bw per day for cluster G08 to 31.6–34.8 ng/kg bw per day for cluster G13. The high AFT exposure in cluster G13 is largely due to high consumption of rice and elevated AFT concentrations in rice samples from this cluster (see Table 18). For most clusters, dietary exposure is dominated by the contribution of one or more cereals (maize, rice, wheat), with peanuts being the only non-cereal commodity

Table 17
Summary of national estimates of dietary exposure to aflatoxins determined by the Committee

Country	Population group[a]	Estimated dietary exposure, mean (high percentile) (ng/kg bw per day)[b,c]	Major contributors[d]
AFT			
Bulgaria	Infants	1.7–4.1 (3.5–8.2)	Cereal-based products
	Toddlers	4.0–9.6 (8.1–19.2)	
	Other children	4.1–9.3 (8.3–18.7)	
Cyprus	Adolescents	0.1–2.0 (0.2–3.9)	Cereal-based products
Czech Republic	Other children	1.0–3.6 (1.9–7.3)	Rice
	Adolescents	0.7–2.8 (1.3–5.6)	
	Adults	0.5–1.6 (1.0–3.2)	
Germany	Toddlers[e]	0.5–3.9 (1.0–7.8)	Cereal-based products
	Other children[e]	0.8–3.7 (1.7–7.1)	
	Adolescents	0.6–2.4 (1.3–4.8)	
	Adults	0.5–2.1 (1.1–4.2)	
	Elderly adults	0.4–1.9 (0.9–3.7)	
	Very elderly adults	0.5–2.0 (0.9–3.9)	
Hungary	Adults	0.06–3.9 (0.12–7.8)	Spices and condiments
	Elderly adults	0.04–3.8 (0.09–7.5)	
	Very elderly adults	0.04–4.1 (0.08–8.2)	
Philippines	Children	7.4–7.6 (14.8–15.3)	Cereal-based products
Sweden	Other children	0.53–0.58 (1.1–1.2)	Only rice included
	Adolescents	0.40–0.43 (0.79–0.87)	
	Adults	0.21–0.23 (0.43–0.47)	
Thailand	General population	1.0–5.0 (2.0–9.9)	Rice
USA	Children <6 years	0.9–5.5 (1.8–11.1)	Peanuts, maize
	Childbearing women	0.4–1.9 (0.8–3.7)	
	General population	0.6–2.5 (1.1–4.9)	
AFB_1			
Belgium	Toddlers	0.83–2.1 (1.7–4.2)	Food for infants and small children, nes, spices and condiments, peanuts, almonds
	Other children	0.21–1.0 (0.43–1.9)	
	Adolescents	0.03–0.19 (0.06–0.38)	
	Adults	0.03–0.17 (0.06–0.34)	
	Elderly adults	0.04–0.12 (0.07–0.24)	
	Very elderly adults	0.05–0.12 (0.09–0.23)	
Bulgaria	Infants	0.0–1.4 (0.0–2.8)	
	Toddlers	0.0–3.6 (0.0–7.3)	
	Other children	0.03–3.6 (0.06–7.3)	
Burkina Faso	Adult women	2.3–3.9 (4.6–7.8)	Only sorghum included
Cyprus	Adolescents	9.0–9.5 (18.0–19.0)	Cereal-based products[f]
Czech Republic	Other children	1.0–1.4 (1.9–2.8)	Rice
	Adolescents	0.7–1.0 (1.3–2.0)	
	Adults	0.5–0.6 (1.0–1.3)	

Country	Population group[a]	Estimated dietary exposure, mean (high percentile) (ng/kg bw per day)[b,c]	Major contributors[d]
Germany	Toddlers[e]	0.3–2.4 (0.5–4.8)	Cereal-based products
	Other children[e]	0.4–2.2 (0.8–4.5)	
	Adolescents	0.3–1.4 (0.7–2.8)	
	Adults	0.3–1.4 (0.6–2.8)	
	Elderly adults	0.3–1.3 (0.6–2.7)	
	Very elderly adults	0.3–1.3 (0.6–2.6)	
Hungary	Adults	0.09–4.0 (0.19–8.1)	Spices and condiments
	Elderly adults	0.07–3.9 (0.14–7.7)	
	Very elderly adults	0.07–4.3 (0.15–8.5)	
Ireland	Adults	0.04–0.57 (0.07–1.1)	Rice
Italy[g]	Infants	0.44–1.6 (0.87–3.2)	Wheat and wheat products
	Toddlers	2.7–7.9 (5.3–15.7)	
	Other children	2.8–7.8 (5.6–15.6)	
	Adolescents	1.7–4.8 (3.4–9.5)	
	Adults	1.1–3.3 (2.2–6.6)	
	Elderly adults	1.0–3.1 (2.0–6.2)	
	Very elderly adults	1.1–3.2 (2.2–6.4)	
Sweden	Other children	0.49–0.54 (0.98–1.1)	Rice
	Adolescents	0.37–0.40 (0.73–0.81)	
	Adults	0.20–0.22 (0.40–0.44)	
Thailand	General population	1.1–1.4 (2.3–2.9)	Rice
USA	Children <6 years	3.8–10 (7.5–20.4)	Peanuts, maize
	Childbearing women	1.0–2.9 (2.0–5.9)	
	General population	1.4–3.9 (2.7–7.8)	
AFM_1			
Belgium	Toddlers	0.032–0.032 (0.063–0.063)	Cheese[h]
	Other children	0.020–0.020 (0.040–0.040)	
	Adolescents	0.006–0.032 (0.012–0.064)	
	Adults	0.007–0.020 (0.015–0.039)	
	Elderly adults	0.007–0.014 (0.014–0.028)	
	Very elderly adults	0.007–0.016 (0.014–0.033)	
Bulgaria	Infants	0.4–1.4 (0.8–2.8)	Cows' milk
	Toddlers	0.7–2.5 (1.4–5.0)	
	Other children	0.5–1.9 (1.1–3.8)	
Cyprus	Adolescents	0.008–0.016 (0.015–0.032)	Cows' milk
Czech Republic	Other children	<0.001–0.055 (<0.001–0.11)	Cows' milk
	Adolescents	<0.001–0.027 (<0.001–0.056)	
	Adults	<0.001–0.010 (<0.001–0.019)	
Germany	Toddlers[e]	0.002–0.24 (0.003–0.48)	Cheese
	Other children[e]	0.001–0.16 (0.002–0.32)	
	Adolescents	0.001–0.043 (0.001–0.087)	
	Adults	0.001–0.046 (0.001–0.092)	
	Elderly adults	0.001–0.045 (0.001–0.091)	

Table 17 (continued)

Country	Population group[a]	Estimated dietary exposure, mean (high percentile) (ng/kg bw per day)[b,c]	Major contributors[d]
	Very elderly adults	0.001–0.047 (0.002–0.094)	
Hungary	Adults	0.023–0.14 (0.045–0.27)	Cows' milk
	Elderly adults	0.023–0.14 (0.045–0.28)	
	Very elderly adults	0.024–0.13 (0.049–0.27)	
Italy	Infants	0.026–0.23 (0.051–0.46)	Cows' milk
	Toddlers	0.050–0.45 (0.10–0.90)	
	Other children	0.019–0.17 (0.039–0.35)	
	Adolescents	0.007–0.065 (0.014–0.13)	
	Adults	0.004–0.036 (0.008–0.071)	
	Elderly adults	0.004–0.034 (0.008–0.068)	
	Very elderly adults	0.005–0.046 (0.010–0.093)	

CIFOCOss: Chronic Individual Food Consumption Database – Summary statistics; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified

[a] European country population group descriptors have the following definitions: infants, <12 months; toddlers, 12–35 months; other children, 3–9 years; adolescents, 10–17 years; adults, 18–64 years; elderly adults, 65–74 years; very elderly adults, ≥75 years (EFSA, 2011).

[b] The range of dietary exposure estimates refers to lower- and upper-bound estimates of mean dietary exposure. The lower-bound mean estimate was derived by substituting zero for analytical results below the LOD when calculating mean concentration values. The upper-bound estimate was derived by substituting the value of the LOD for analytical results below the LOD or the value of the LOQ for analytical results described as "trace".

[c] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

[d] The major contributing food or food group for at least one of the population groups assessed for the country, based on quantified data.

[e] The CIFOCOss food consumption database contains information from three successive surveys of food consumption by toddlers and other children (DONALD 2006, DONALD 2007 and DONALD 2008). The results presented here are the lower- and upper-bound dietary exposure estimates across the three surveys.

[f] Based on analysis of seven cereal and cereal-based products.

[g] Four analytical results were excluded from this analysis as LODs were 1000-fold higher than other samples of the same food type.

[h] Based on analysis of a single cheese sample.

to contribute more than 10% of the estimated dietary exposure for any GEMS/Food cluster. This pattern of food contributions to dietary AFT exposure differs from previous assessments carried out by the Committee due to the availability for the current assessment of a significant body of information on the AFT content of rice and wheat.

Dietary exposure to AFB_1 ranged from 0.2–1.0 ng/kg bw per day for cluster G08 to 7.0–13.5 ng/kg bw per day for cluster G13. As for AFT, the main contributors to AFB_1 exposure are mainly the cereal commodities, maize, rice, sorghum and wheat, with peanuts the only non-cereal commodity to contribute more than 10% to estimated dietary exposure for any cluster.

Dietary exposure to AFM_1 ranged from 0.01–0.02 ng/kg bw per day for clusters G03 and G14 to 0.18–0.56 ng/kg bw per day for cluster G10. The low exposure to AFM_1 in clusters G03 and G14 is due to the low consumption of bovine milk in these clusters, while the high exposure to AFM_1 in G10 is due to the mean AFM_1 concentration in bovine milk from this cluster being higher than for any other cluster contributing data.

Table 18
Chronic dietary exposure to AFT in commodities in the GEMS/Food clusters

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
All foods																	
Mean	3.0–7.3	2.6–6.7	6.5–8.6	5.1–9.1	7.1–10.8	5.8–11.4	1.3–1.7	0.3–1.3	4.4–7.3	1.2–3.9	1.2–3.5	4.4–7.2	31.6–34.8	7.1–10.2	1.5–7.0	4.1–5.6	3.1–5.1
High (90th) percentile[b]	6.0–14.7	5.2–13.4	13.0–17.2	10.2–18.2	14.2–21.7	11.7–22.7	2.5–3.5	0.7–2.6	8.8–14.5	2.4–7.8	2.5–7.1	8.9–14.5	63.2–69.6	14.2–20.3	2.9–14.1	8.3–11.3	6.2–10.2
Cereals and pseudocereals																	
Barley	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.9	0.6	0.0	0.0	0.1	0.0	0.3	0.9	0.0	0.3	0.2	0.0	0.1	0.0	0.4	0.0	0.0
Buckwheat	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.2	0.0	0.0	0.0	0.0	0.0	1.8	0.1	0.2	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Maize	0.8–1.1	1.2–1.6	3.0–4.0	1.2–1.6	1.7–2.2	2.1–2.8	0.0–0.1	0.0–0.1	3.2–3.5	0.3–0.4	0.0–0.0	1.8–2.4	3.3–4.3	0.3–0.4	0.3–0.7	1.9–2.5	1.0–1.3
% AFT	14.4	24.0	46.2	17.3	20.0	24.4	6.4	7.7	47.6	9.4	0.8	32.6	12.2	3.6	10.6	44.7	24.7
Millet	0.0–0.0	0.0–0.0	0.0–0.0.1	0.0–0.0	0.0–0.2	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.1–0.7	0.0–0.0	0.0–0.0	0.0–0.3	0.0–0.0
% AFT	0.2	0.4	0.7	0.1	1.8	0.0	0.0	0.2	0.3	0.2	0.0	0.0	2.0	0.1	0.0	6.1	0.0
Oats	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.4	0.0	0.1	0.0	0.0	1.6	0.7	0.0	0.4	0.3	0.2	0.0	0.0	0.2	0.0	0.0
Rice	1–1.3	0.3–0.4	2.1–2.7	2.5–3.2	4.3–5.5	2.1–2.7	0.2–0.3	0.1–0.1	0.6–2.4	0.0–1.4	0.4–0.5	1.9–2.5	25.7–25.7	6.4–8.2	0.2–0.4	0.4–0.6	1.7–2.2
% AFT	17.8	6.6	31.2	35.5	51.2	23.5	15.0	7.0	32.4	36.3	13.6	34.5	73.7	80.3	5.1	9.9	43.3
Rye	0.0–0.0	0.0–0.2	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.2	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.3	0.0–0.0	0.0–0.0
% AFT	0.0	3.5	0.0	0.0	0.0	0.2	0.9	14.3	0.0	0.7	0.5	0.0	0.0	0.0	4.2	0.0	0.2
Sorghum	0.1–0.1	0.0–0.0	0.2–0.3	0.2–0.4	0.2–0.3	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.1–0.2	1.2–1.9	0.0–0.0	0.0–0.0	0.3–0.5	0.0–0.0
% AFT	1.4	0.0	3.5	4.0	2.3	0.6	0.0	0.0	1.1	0.6	0.0	2.5	5.6	0.5	0.0	9.0	0.0
Sweet corn	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.4	0.0	0.3	0.1	0.0	0.0	0.0	0.1	0.0	0.0

Table 18 (continued)

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Wheat	0.9–4.6	0.8–4.1	0.1–0.4	0.7–3.4	0.4–2.1	1–5.2	0.8–1.1	0.0–0.6	0.3–0.9	0.0–1.0	0.5–2.6	0.4–1.9	0.2–0.9	0.3–1.3	0.9–5.4	0.1–0.3	0.3–1.5
% AFT	62.2	60.8	5.2	37.0	19.0	45.6	62.6	46.4	12.5	26.7	72.7	26.0	2.5	13.0	76.5	5.3	29.4
Tree and groundnuts																	
Almonds	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.1–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.6	0.0	0.0	0.3	0.0	0.3	0.9	4.0	0.0	1.7	0.9	0.0	0.0	0.0	0.1	0.0	0.0
Brazil nuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Cashew nuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.4	1.2	0.1	0.0	0.0	0.0	0.0
Chestnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Hazelnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.1	0.0	0.0	0.0	0.3	0.4	2.7	0.0	0.1	1.5	0.0	0.0	0.0	0.2	0.0	0.0
Peanuts	0.1–0.1	0.2–0.2	1.1–1.1	0.2–0.2	0.5–0.5	0.3–0.3	0.1–0.1	0.1–0.1	0.3–0.4	0.8–0.8	0.2–0.2	0.1–0.1	1.2–1.3	0.1–0.1	0.1–0.1	1.3–1.4	0.1–0.1
% AFT	1.0	2.5	13.0	2.7	4.7	2.6	6.2	9.0	4.9	19.9	6.0	2.0	3.6	0.7	1.2	24.6	1.7
Pistachios	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.2	0.0	0.0	0.4	0.0	0.4	2.2	1.4	0.0	0.7	0.3	0.0	0.0	0.0	0.3	0.0	0.0
Walnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.1	0.4	0.0	0.0	0.0	0.3	0.1	0.5	0.0	0.1	0.2	0.0	0.0	0.0	0.2	0.0	0.0
Spices																	
Anise, fennel, coriander	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Cinnamon	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Cloves	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0
Ginger	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.1	0.0	0.1	0.5	0.2	0.0	0.7	0.2	0.3	0.8	0.7	0.3	0.1	0.3	0.0	0.0	0.0
Nutmeg, mace, cardamon	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.6	0.0	0.0	0.2	0.2	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0
Pepper (black, white)	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.1	0.0	0.0	0.3	0.3	0.0	0.5	0.4	0.2	0.0	0.2	0.1	0.0	0.1
Spices	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.2	0.1	0.0	0.5	0.4	0.2	0.4	0.1	0.1	0.1	0.6	0.1	0.0	0.3	0.2	0.0	0.5
Other foods																	
Figs	0.1–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.1–0.2	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.8	0.3	0.0	0.2	0.0	1.3	0.4	1.4	0.0	0.3	0.3	0.0	0.0	0.0	0.4	0.0	0.0
Soy	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.0	0.2	0.0	0.0	0.0	0.2	0.0

AFT: total aflatoxins; % AFT: percentage of dietary exposure to AFT that is due to consumption of the associated food commodity, based on the UB estimate of dietary AFT exposure; bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. The LB mean estimate was derived by substituting zero for analytical results below the LOD when calculating mean concentration values. The UB estimate was derived by substituting the value of the LOD for analytical results below the LOD or the value of the LOQ for analytical results described as "trace". All exposure estimates are based on a 60 kg body weight.

[b] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

Table 19
Chronic dietary exposure to AFB_1 in commodities in the GEMS/Food clusters

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
All foods																	
Mean	1.2–4.3	1.3–4.0	3.8–5.7	2.3–5.6	2.9–5.8	2.6–6.3	0.7–0.9	0.2–1.0	1.3–2.5	0.7–3.4	0.1–2.2	2.2–4.4	7.0–13.5	1.3–3.2	0.6–3.0	3.2–5.3	1.1–2.4
High (90th) percentile[b]	2.5–8.6	2.6–7.9	7.6–11.5	4.6–11.3	5.8–11.6	5.2–12.7	1.4–1.7	0.4–1.9	2.3–4.7	1.3–6.7	0.4–4.5	4.4–8.8	13.9–26.9	2.6–6.5	1.2–5.9	6.4–10.5	2.3–4.8
Cereals and pseudocereals																	
Barley	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	1.4	0.9	0.0	0.0	0.1	0.0	0.2	0.7	0.1	0.3	0.4	0.0	0.2	0.0	0.4	0.0	0.0
Buckwheat	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.0	0.1	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Maize	0.7–0.8	1.0–1.2	2.5–3.1	1.0–1.2	1.3–1.7	1.7–2.2	0.0–0.0	0.0–0.1	0.0–0.0	0.2–0.3	0.0–0.0	1.5–1.8	2.6–3.3	0.2–0.3	0.2–0.3	1.6–2.0	0.8–1.0
% AFB_1	19.0	31.5	53.7	21.8	29.0	34.0	3.8	6.0	1.0	10.0	1.8	41.6	24.6	8.8	11.6	37.3	40.6
Millet	0.0–0.0	0.0–0.0	0.0–0.0.1	0.0–0.0	0.0–0.0.2	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0.7	0.0–0.0	0.0–0.0	0.0–0.3	0.0–0.0
% AFB_1	0.4	0.7	1.0	0.2	3.2	0.0	0.0	0.3	0.8	0.2	0.0	0.0	5.0	0.3	0.0	6.3	0.0
Oats	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	1.0	0.0	0.2	0.1	0.0	0.8	0.4	0.0	1.2	0.8	0.4	0.0	0.0	0.2	0.0	0.0
Rice	0.1–0.3	0.0–0.0.1	0.3–0.6	0.3–0.8	0.6–1.3	0.3–0.6	0.2–0.2	0.0–0.1	0.6–0.8	0.2–0.9	0.0–0.1	0.3–0.6	0.2–0.4	0.9–1.9	0.1–0.2	0.1–0.1	0.2–0.5
% AFB_1	7.0	2.6	10.9	13.3	22.3	9.8	20.0	7.6	34.9	25.6	5.6	13.2	2.8	58.7	5.4	2.5	21.3
Rye	0.0–0.0	0.0–0.0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0
% AFB_1	0.0	2.1	0.0	0.0	0.0	0.1	1.0	12.0	0.0	1.2	0.9	0.0	0.0	0.0	2.3	0.0	0.2
Sorghum	0.2–0.4	0.0–0.0	0.5–1.1	0.6–1.4	0.4–1.0	0.1–0.3	0.0–0.0	0.0–0.0	0.1–0.1	0.0–0.0	0.0–0.0	0.3–0.7	3.5–7.9	0.1–0.2	0.0–0.0	0.9–2.0	0.0–0.0
% AFB_1	9.0	0.0	20.1	25.0	16.9	4.1	0.0	0.0	5.4	0.3	0.0	16.0	58.7	5.6	0.0	37.3	0.0
Sweet corn	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.1	0.0	0.0	1.4	0.6	0.0	1.1	0.2	0.1	0.0	0.0	0.3	0.0	0.0
Wheat	0.1–2.6	0.1–2.3	0.0–0.3	0.1–1.9	0.1–1.2	0.1–2.9	0.4–0.5	0.0–0.5	0.0–0.9	0.0–1.5	0.0–1.8	0.1–1.1	0.0–0.5	0.0–0.7	0.2–2.2	0.0–0.2	0.0–0.9
% AFB_1	59.8	58.0	4.4	33.8	20.2	46.3	55.6	54.4	38.4	46.2	80.2	24.1	3.6	23.0	75.7	3.2	35.1

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Tree and groundnuts																	
Almonds	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.1–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	1.0	0.1	0.0	0.5	0.0	0.4	1.2	3.9	0.0	1.8	0.5	0.0	0.0	0.0	0.1	0.0	0.0
Brazil nuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cashew nuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.1	0.1	0.0	0.0	0.3	0.0	0.1	0.3	0.6	1.9	0.2	0.0	0.0	0.0	0.0
Chestnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Hazelnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.1	0.0	0.0	0.0	0.2	0.4	1.3	0.0	0.1	0.8	0.0	0.0	0.0	0.3	0.0	0.0
Peanuts	0.0–0.0	0.1–0.1	0.5–0.6	0.1–0.1	0.4–0.4	0.1–0.1	0.1–0.1	0.1–0.1	0.4–0.4	0.1–0.2	0.1–0.1	0.1–0.1	0.6–0.6	0.0–0.0	0.0–0.0	0.7–0.7	0.0–0.0
% AFB_1	0.8	2.1	9.7	2.2	7.2	2.4	8.9	7.9	16.9	5.2	4.7	1.6	4.6	1.0	1.7	13.2	1.8
Pistachios	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.4	0.1	0.0	0.6	0.0	0.3	3.7	1.6	0.1	0.7	0.8	0.0	0.0	0.0	0.5	0.0	0.0
Walnuts	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.1	0.5	0.0	0.0	0.0	0.3	0.1	0.2	0.1	0.1	0.2	0.0	0.0	0.0	0.3	0.0	0.0
Spices																	
Anise, fennel, coriander	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.3	0.0	0.0	0.0
Cinnamon	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1
Cloves	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ginger	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0

Table 19 (continued)

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
% AFB_1	0.1	0.0	0.1	0.4	0.2	0.0	0.6	0.1	0.4	0.3	0.5	0.2	0.1	0.4	0.0	0.0	0.0
Nutmeg, mace, cardamon	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.7	0.0	0.0	0.4	0.2	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0
Pepper (black, white)	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.3	0.3	0.1	0.0	0.2	0.1	0.0	0.1
Spices	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.2	0.1	0.0	0.6	0.6	0.3	0.6	0.1	0.2	0.2	1.1	0.1	0.0	0.7	0.2	0.0	0.8
Other foods																	
Figs	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.1–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.8	0.3	0.0	0.2	0.0	1.4	0.4	1.1	0.0	0.2	0.1	0.0	0.0	0.0	0.8	0.0	0.0
Soy	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0
% AFB_1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	1.1	4.4	0.0	0.5	0.1	0.0	0.0	0.2	0.0

AFB_1: aflatoxin B_1; % AFB_1: percentage of dietary exposure to AFB_1 that is due to consumption of the associated food commodity, based on the UB estimate of dietary AFB_1 exposure; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. The LB mean estimate was derived by substituting zero for analytical results below the LOD when calculating mean concentration values. The UB estimate was derived by substituting the value of the LOD for analytical results below the LOD or the value of the LOQ for analytical results described as "trace". All exposure estimates are based on a 60 kg body weight.

[b] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

Table 20

Chronic dietary exposure to AFM_1 in commodities in the GEMS/Food clusters

	Estimated LB–UB dietary exposure for GEMS/Food clusters in ng/kg bw per day[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
All foods																	
Mean	0.09–0.27	0.16–0.49	0.01–0.02	0.04–0.13	0.06–0.18	0.05–0.16	0.00–0.48	0.01–0.07	0.01–0.04	0.18–0.56	0.01–0.07	0.03–0.1	0.03–0.1	0.01–0.02	0.16–0.35	0.02–0.06	0.01–0.03
High (90th) percentile[b]	0.17–0.54	0.32–0.98	0.01–0.04	0.08–0.26	0.12–0.36	0.10–0.32	0.00–0.96	0.02–0.13	0.03–0.08	0.35–1.13	0.02–0.14	0.07–0.21	0.06–0.20	0.02–0.05	0.32–0.71	0.04–0.12	0.02–0.06
Butter	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00
% AFM_1	0.6	0.3	0.1	0.9	0.9	1.2	0.9	5.8	0.2	0.3	5.0	0.3	0.2	0.8	0.4	0.0	3.2
Cattle milk	0.08–0.26	0.16–0.47	0.01–0.02	0.04–0.11	0.06–0.18	0.05–0.14	0.00–0.19	0.01–0.03	0.01–0.04	0.17–0.55	0.00–0.05	0.03–0.1	0.03–0.09	0.01–0.02	0.16–0.33	0.02–0.06	0.01–0.03
% AFM_1	94.2	96.5	96.8	81.9	97.7	86.7	38.5	51.8	94.3	97.6	72.0	95.6	87.0	98.4	92.8	99.0	95.2
Cheese	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.00	0.00–0.28	0.00–0.02	0.00–0.00	0.01–0.01	0.01–0.01	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.02	0.00–0.00	0.00–0.00
% AFM_1	0.6	1.1	0.3	6.2	0.7	2.5	57.5	35.8	0.8	2.0	14.9	3.7	1.0	0.4	5.0	0.0	1.3
Fermented milk products	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00
% AFM_1	0.0	0.1	0.2	0.1	0.0	0.0	3.1	6.5	0.0	0.0	8.2	0.4	0.0	0.0	1.1	0.0	0.2
Goat milk	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00
% AFM_1	0.6	0.1	0.9	2.9	0.7	0.8	0.0	0.1	1.4	0.1	0.0	0.0	2.5	0.4	0.1	0.5	0.0
Sheep milk	0.00–0.01	0.00–0.01	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.01	0.00–0.00	0.00–0.00	0.00–0.00	0.00–0.00
% AFM_1	3.8	1.8	1.7	7.9	0.0	8.8	0.0	0.0	3.2	0.0	0.0	0.0	9.3	0.0	0.6	0.3	0.0

AFM_1: aflatoxin M_1; % AFM_1: percentage of dietary exposure to AFM_1 that is due to consumption of the associated food commodity, based on the UB estimate of dietary AFM_1 exposure; bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] The range of dietary exposure estimates refers to LB and UB estimates of mean dietary exposure. The LB mean estimate was derived by substituting zero for analytical results below the LOD when calculating mean concentration values. The UB estimate was derived by substituting the value of the LOD for analytical results below the LOD or the value of the LOQ for analytical results described as "trace". All exposure estimates are based on a 60 kg body weight.

[b] High percentiles are an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

8.4 Potential effect of limits and their enforcement on chronic dietary exposure

The Committee was asked by the CCCF to consider the impact of four potential maximum levels for AFT in ready-to-eat peanuts (4, 8, 10 and 15 µg/kg) on non-compliance rates and resulting impacts on mean AFT and AFB_1 concentrations and dietary exposure to AFT and AFB_1. In order to assess the impact of maximum levels for AFT on AFB_1 concentrations and exposure, it is necessary to have both AFT and AFB_1 concentration data for the same set of samples or to assume a constant relationship between AFT and AFB_1 concentrations in contaminated samples. The GEMS/Food contaminants database contained AFT concentrations for 20 870 samples of peanuts and AFB_1 concentrations for 7907 samples of peanuts. While it is likely that not all of these records relate to ready-to-eat peanuts, there was insufficient information in the database to make a distinction. For the purpose of the current analysis, it was assumed that all records in the GEMS/Food contaminants database for peanuts related to ready-to-eat peanuts. Of these samples, there were 5252 for which both AFT and AFB_1 concentrations were available. In order to use the maximum number of these data, the mean concentrations (lower- and upper-bound) for all samples with an AFT concentration (n = 20 870) and all samples with an AFB_1 concentration (n = 7907) were taken as the concentrations of AFT and AFB_1 in the absence of any maximum level. The proportional decrease in the mean concentration of AFT due to the application of a particular maximum level was assumed to be the same as the proportional decrease in the mean concentration of AFB_1. That is, it was assumed that there was a constant ratio between the concentrations of AFT and AFB_1 in ready-to-eat peanuts.

Table 21 summarizes the impact of different hypothetical maximum levels (4, 8, 10 and 15 µg/kg) for AFT on the statistical distribution of AFB_1 and AFT contents in ready-to-eat peanuts for 2007–2016.

The impacts of different maximum level scenarios for ready-to-eat peanuts (no maximum levels, maximum levels at 4, 8, 10 and 15 µg/kg) on dietary exposure to AFT and AFB_1 are also summarized in Table 22. The corresponding contributions of ready-to-eat peanuts to overall mean dietary AFT exposure (in % AFT) are also presented. The largest impact on mean AFT and AFB_1 concentrations and on estimated dietary exposure is associated with the imposition of any maximum level and the consequent exclusion of samples of ready-to-eat peanuts with very high aflatoxin concentrations. A maximum level of 15 µg/kg reduced the mean AFT concentration for all ready-to-eat peanuts from 9.5–9.8 to 1.3–1.6 µg/kg, a reduction of 84% at the upper bound. Reducing the maximum level from 15 to 4 µg/kg for AFT further reduces the mean AFT

Table 21

Summary of the impact of different proposed maximum levels (4, 8, 10 and 15 µg/kg) for AFT on the mean concentrations of AFB_1 and AFT content (lower–upper bound scenarios) in ready-to-eat peanuts for 2007–2016, including the predicted proportion of rejected samples, globally and by GEMS/Food cluster

		Data per GEMS/Food cluster diet																	
Scenario[a]	Statistic	All	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
No ML																			
AFT	Number of samples	20 870	–	–	–	–	1 423	–	622	1 922	69	15 394	–	–	–	–	553	–	–
	Mean concentration (µg/kg)	9.5–9.8	–	–	–	–	19.4–20.4	–	2.5–2.7	3.2–3.4	2.3–3.2	10.4–10.6	–	–	–	–	2.5–3.5	–	–
AFB_1	Mean concentration (µg/kg)	4.7–4.9	–	–	–	–	16.8–16.8	–	1.9–1.9	2.1–2.2	3.1–3.5	2.0–2.4	–	–	–	–	1.7–2.1	–	–
ML = 15 µg/kg																			
AFT	Number of samples	18 849	–	–	–	–	1 216	–	601	1 853	67	13 694	–	–	–	–	538	–	–
	Mean concentration (µg/kg)	1.3–1.6	–	–	–	–	1.1–2.2	–	0.5–0.7	0.3–0.5	1.7–2.6	1.5–1.8	–	–	–	–	0.2–1.3	–	–
	% of reject samples	9.7	–	–	–	–	14.5	–	3.4	3.6	2.9	11.0	–	–	–	–	2.7	–	–
AFB_1	Mean concentration (µg/kg)	0.6–0.8	–	–	–	–	0.9–1.8	–	0.4–0.5	0.2–0.3	2.4–3	0.3–0.4	–	–	–	–	0.1–0.8	–	–
ML = 10 µg/kg																			
AFT	Number of samples	18 238	–	–	–	–	1 179	–	598	1 837	66	13 145	–	–	–	–	536	–	–
	Mean concentration (µg/kg)	0.9–1.2	–	–	–	–	0.7–1.9	–	0.4–0.6	0.2–0.4	1.6–2.5	1.1–1.3	–	–	–	–	0.1–1.2	–	–
	% of reject samples	12.6	–	–	–	–	17.1	–	3.9	4.4	4.3	14.6	–	–	–	–	3.1	–	–
AFB_1	Mean concentration (µg/kg)	0.4–0.6	–	–	–	–	0.6–1.5	–	0.3–0.5	0.1–0.2	2.2–2.8	0.2–0.3	–	–	–	–	0.1–0.7	–	–
ML = 8 µg/kg																			
AFT	Number of samples	17 952	–	–	–	–	1 160	–	596	1 831	62	12 894	–	–	–	–	536	–	–
	Mean concentration (µg/kg)	0.8–1.1	–	–	–	–	0.6–1.8	–	0.4–0.6	0.2–0.3	1.1–2.1	0.9–1.2	–	–	–	–	0.1–1.2	–	–

Table 21 (continued)

		Data per GEMS/Food cluster diet																	
Scenario[a]	**Statistic**	**All**	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
	% of reject samples	14.0	–	–	–	–	18.5	–	4.2	4.7	10.1	16.2	–	–	–	–	3.1	–	–
AFB_1	Mean concentration (µg/kg)	0.4–0.5	–	–	–	–	0.5–1.4	–	0.3–0.4	0.1–0.2	1.5–2.3	0.2–0.3	–	–	–	–	0.1–0.7	–	–
ML = 4 µg/kg																			
AFT	No. of samples	16 734	–	–	–	–	1 085	–	584	1 805	57	11 814	–	–	–	–	530	–	–
	Mean concentration (µg/kg)	0.4–0.7	–	–	–	–	0.2–1.5	–	0.3–0.5	0.1–0.3	0.7–1.8	0.4–0.7	–	–	–	–	0.1–1.2	–	–
	% of reject samples	19.8	–	–	–	–	23.8	–	6.1	6.1	17.4	23.3	–	–	–	–	4.2	–	–
AFB_1	Mean concentration (µg/kg)	0.2–0.4	–	–	–	–	0.2–1.2	–	0.2–0.4	0.1–0.2	1.0–2.0	0.1–0.2	–	–	–	–	0.1–0.7	–	–

AFB_1: aflatoxin B_1; AFT: total aflatoxin; ML: maximum level

[a] As the MLs are defined in terms of AFT, changes in the mean concentrations of AFB_1 in ready-to-eat peanuts have been estimated by assuming that the proportional decrease in AFB_1 mean concentrations due to application of the maximum level is the same as the proportional decrease in the mean AFT concentrations.

Table 22

Mean estimates of dietary exposure to AFT and AFB_1 from all food sources for the 17 GEMS/Food cluster diets taking into consideration hypothetical maximum level scenarios for AFT in ready-to-eat peanuts and the contribution of ready-to-eat peanuts to total dietary AFT exposure

	Estimated LB–UB mean dietary exposure per GEMS/Food cluster diet																
Scenario	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
No ML																	
AFT (ng/kg bw per day)	3.0–7.3	2.6–6.7	6.5–8.6	5.1–9.1	7.1–10.8	5.8–11.4	1.3–1.7	0.3–1.3	4.4–7.3	1.2–3.9	1.2–3.5	4.4–7.2	31.6–34.8	7.1–10.2	1.5–7.0	4.1–5.6	3.1–5.1
AFB_1 (ng/kg bw per day)	1.2–4.3	1.3–4.0	3.8–5.7	2.3–5.6	2.9–5.8	2.6–6.3	0.7–0.9	0.2–1.0	1.3–2.5	0.7–3.4	0.1–2.2	2.2–4.4	7.0–13.5	1.3–3.2	0.6–3.0	3.2–5.3	1.1–2.4
RTE peanuts (% AFT)	1.0	2.5	13.0	2.7	4.7	2.6	6.2	9.0	4.9	19.9	6.0	2.0	3.6	0.7	1.2	24.6	1.7
ML = 15 µg/kg																	
AFT (ng/kg bw per day)	2.9–7.3	2.5–6.5	5.6–7.6	4.9–8.9	6.7–10.4	5.6–11.1	1.2–1.7	0.2–1.2	4.4–7.2	0.6–3.3	1.1–3.4	4.3–7.1	30.6–33.8	7.0–10.1	1.4–7.0	3.0–4.5	3.0–5.0
AFB_1 (ng/kg bw per day)	1.2–4.3	1.2–3.9	3.4–5.3	2.2–5.5	2.5–5.4	2.5–6.2	0.6–0.8	0.1–0.9	1.0–2.3	0.5–3.2	0.1–2.2	2.1–4.4	6.5–12.9	1.3–3.2	0.6–2.9	2.6–4.7	1.1–2.4
RTE peanuts (% AFT)	0.2	0.4	2.4	0.5	0.5	0.4	1.6	1.4	4.2	4.0	1.0	0.3	0.6	0.1	0.4	5.1	0.3
ML = 10 µg/kg																	
AFT (ng/kg bw per day)	2.9–7.3	2.5–6.5	5.5–7.6	4.9–8.9	6.6–10.4	5.6–11.1	1.2–1.7	0.2–1.2	4.3–7.2	0.5–3.2	1.1–3.4	4.3–7.1	30.5–33.7	7.0–10.1	1.4–7.0	2.9–4.4	3.0–5.0
AFB_1 (ng/kg bw per day)	1.2–4.3	1.2–3.9	3.3–5.2	2.2–5.5	2.5–5.4	2.5–6.2	0.6–0.8	0.1–0.9	1.0–2.3	0.5–3.2	0.1–2.2	2.1–4.4	6.4–12.9	1.3–3.2	0.6–2.9	2.6–4.7	1.1–2.4
RTE peanuts (% AFT)	0.1	0.3	1.9	0.4	0.4	0.3	1.5	1.1	3.9	3.0	0.8	0.3	0.5	0.1	0.4	4.0	0.2
ML = 8 µg/kg																	
AFT (ng/kg bw per day)	2.9–7.3	2.4–6.5	5.5–7.6	4.9–8.9	6.6–10.4	5.6–11.1	1.2–1.6	0.2–1.2	4.3–7.1	0.5–3.2	1.1–3.4	4.3–7.1	30.5–33.7	7.0–10.1	1.4–7.0	2.9–4.4	3.0–5.0

Table 22 (continued)

	Estimated LB–UB mean dietary exposure per GEMS/Food cluster diet																
Scenario	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
AFB_1 (ng/kg bw per day)	1.2–4.3	1.2–3.9	3.3–5.2	2.2–5.5	2.5–5.4	2.4–6.2	0.6–0.8	0.1–0.9	0.9–2.2	0.5–3.2	0.1–2.2	2.1–4.3	6.4–12.9	1.3–3.2	0.6–2.9	2.6–4.6	1.1–2.4
RTE peanuts (% AFT)	0.1	0.3	1.6	0.3	0.4	0.3	1.5	1.0	3.3	2.6	0.7	0.2	0.4	0.1	0.4	3.5	0.2
ML = 4 µg/kg																	
AFT (ng/kg bw per day)	2.9–7.3	2.4–6.5	5.4–7.5	4.8–8.9	6.6–10.4	5.6–11.1	1.2–1.6	0.2–1.2	4.2–7.1	0.5–3.2	1.0–3.3	4.3–7.1	30.4–33.6	7–10.1	1.4–7.0	2.8–4.4	3.0–5.0
AFB_1 (ng/kg bw per day)	1.2–4.3	1.2–3.9	3.3–5.2	2.2–5.5	2.5–5.4	2.4–6.2	0.6–0.8	0.1–0.9	0.9–2.2	0.5–3.2	0.1–2.2	2.1–4.3	6.4–12.9	1.3–3.2	0.6–2.9	2.5–4.6	1.1–2.4
RTE peanuts (% AFT)	0.1	0.2	1.1	0.2	0.4	0.2	1.2	0.8	2.8	1.6	0.5	0.1	0.3	0.0	0.4	2.3	0.1

AFT: total aflatoxins; AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; ML: maximum level; RTE: ready-to-eat; RTE peanuts (% AFT): percentage of dietary exposure to AFT that is due to consumption of ready-to-eat peanuts, based on the UB estimate of dietary AFT exposure; UB: upper bound

concentration for all ready-to-eat peanuts to 0.4–0.7 µg/kg, a further 56% reduction in the mean AFT concentration, at the upper bound.

Applying a maximum level for ready-to-eat peanuts had little impact on dietary exposure to AFT or AFB_1 for most clusters. The exceptions were clusters G03 (mainly African countries), G10 (mainly European and North American countries) and G16 (African countries), for which a maximum level of 15 µg/kg resulted in a decrease in AFT dietary exposure of 12%, 15% and 20%, respectively, at the upper bound. Further reductions in the maximum level for ready-to-eat peanuts had only negligible impacts on dietary aflatoxin exposure.

8.5 Dietary exposures for infants

Aflatoxins may be transferred from mother to child through breastfeeding. A number of factors may affect concentrations of aflatoxins in human breast milk, including maternal place of residence and diet. Occurrence data also show detectable concentrations of aflatoxins in infant formula. While some studies have reported the presence of aflatoxins B and G in human breast milk or infant formula, most studies report only the presence of AFM_1 (Cherkani-Hassani, Mojemmi & Mouane, 2016).

While exposure of infants to aflatoxins from maternal breast milk or infant formula only occurs for a small portion of the individual's total lifetime, infants are potentially at least as sensitive as adults to the toxicity of contaminants. Also, their dependence on maternal breast milk or infant formula as the sole source of nutrition means that they are unable to avoid contamination of their food source. Therefore, infants were included in the evaluation of dietary exposure to aflatoxins where possible. Estimated dietary exposures from both breast milk and infant formula were relevant to the evaluation.

8.5.1 Estimated dietary exposure for breastfed infants

Breast milk has optimal nutrition and immunological benefits for infants, and the advantages of breastfeeding (WHO, 2016) outweigh any possible disadvantages that may be associated with the presence of contaminants such as aflatoxin in breast milk. Concentrations of aflatoxins in human milk are highly variable both between and within countries.

The prevalence of AFM_1 contamination of human breast milk varies considerably from country to country and study to study. Studies in Brazil (Andrade et al., 2013b), France (Wild et al., 1987), the Islamic Republic of Iran (Afshar et al., 2013) and Italy (Turconi et al., 2004) detected AFM_1 in less than 1% of human breast milk samples analysed, whereas studies in the Gambia (Zarba et al., 1992), Jordan (Omar, 2012), Turkey (Gürbay et al., 2010) and the United Republic of Tanzania (Magoha et al., 2016) found AFM_1 in all breast milk samples

examined. Different studies have reported mean AFM_1 concentrations between less than 0.01 µg/L and 44 µg/L (Cherkani-Hassani, Mojemmi & Mouane, 2016), a range of over 3 orders of magnitude.

Assuming consumption of breast milk of 800 mL/day and a body weight of 5 kg, this range of AFM_1 concentrations would equate to dietary AFM_1 exposures from 1.6 to 7000 ng/kg bw per day. It should be noted that this range of AFM_1 exposures is derived from study mean values and individual infants may be exposed to lower or higher levels. The highest breast milk AFM_1 concentrations were reported for African countries (Egypt, Sudan, the United Republic of Tanzania), which is consistent with known greater exposures to dietary aflatoxins in this region. The highest mean breast milk AFM_1 concentration reported for Europe was 0.05 µg/L, for the Middle East and Asia was 0.77 µg/L, and for the Americas was 0.11 µg/L (Cherkani-Hassani, Mojemmi & Mouane, 2016).

An Iranian study estimated dietary AFM_1 exposure in breastfed infants at 1 week of age at 4.6–6.9 ng/day (1.0–1.5 ng/kg bw per day for a 4.5 kg infant; Ghiasian & Maghsood, 2012).

8.5.2 Estimated dietary exposure for fully formula-fed infants

Infant formula may be sold as a ready-to-consume liquid or as a powder that requires addition of water before consumption. Formulas with different compositions are produced for neonates from birth (starter formula) and for the period when the infant diet starts to diversify (follow-on formula). Data have been submitted to the GEMS/Food contaminants database on AFM_1 concentrations in starter and follow-on formulas (n = 1376). While the database contained a small number of additional records related to AFT and AFB_1 in infant formula, unmetabolized aflatoxins would not usually be expected to be present in dairy products, and all the records in the database had results below the LOD. Most records for AFM_1 in infant formula in the database related to liquid infant formula or powdered infant formula, as consumed (prepared by addition of water). For the remaining records for powdered infant formula, results were converted to an "as consumed" basis by multiplying by 0.13. This factor was used because most common brands of infant formula are made up for use at a rate of about 13 g of powder to produce 100 mL of formula. Across all records (starter and follow-on formula) the lower- and upper-bound estimates of the mean AFM_1 concentration were 0.002–0.018 µg/kg.

In order to estimate dietary exposure to AFM_1 for fully formula-fed infants, median infant formula consumption estimates can be derived from estimated energy requirements (EERs) for fully formula-fed infants. Standard body weights and EERs for male and female infants aged 1, 3 and 6 months were taken from daily human energy requirements defined by FAO/WHO/UNU

(2004). It should be noted that the EERs of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. EERs for formula-fed infants have been used here. Dietary exposure estimates for AFM_1 derived using this approach range from 0.32–2.3 ng/kg bw per day for a 6-month-old male to 0.46–3.3 ng/kg bw per day for a 1-month-old male.

A further exposure scenario was considered, using high percentile daily energy intakes (95th percentile) reported for formula-fed infants (Fomon & Bell, 1993). Formula-fed males and females aged 1 month have EERs of 122 and 117 kcal/kg bw per day, respectively (FAO/WHO/UNU, 2004). Fomon & Bell (1993) reported maximum 95th percentile energy intakes for male and female infants at 14–27 days of age of 148.7 and 146.0 kcal/kg bw per day, respectively. These energy intakes equate (using an energy density for prepared infant formula of 67 kcal/100 mL) to dietary exposure estimates for AFM_1 of 0.56–4.0 and 0.55–3.9 ng/kg bw per day, respectively.

Dietary exposure to AFM_1 for fully formula-fed infants is about an order of magnitude greater than for adults (see Table 20). Dietary exposure to AFM_1 for breastfed infants may be similar to that for fully formula-fed infants in developed countries, but has the potential to be substantially greater in some regions, due to maternal exposure to dietary aflatoxins.

9. Dose–response analysis and estimation of toxic/carcinogenic risk

9.1 Identification of key data for dose–response analysis

9.1.1 Pivotal data from human clinical/epidemiological studies

Several studies, both cross-sectional and longitudinal, show a positive correlation between aflatoxin exposure and development of hepatocellular carcinoma with an increased risk in the presence of chronic HBV infection. Although some studies suggest that aflatoxin poses a risk only in the presence of other risk factors such as HBV, since the previous evaluation, there has been work showing the independent risk of aflatoxin exposure. Chen et al. (2013) demonstrated a longitudinal reduction in aflatoxin exposure in a historically hepatocellular carcinoma–endemic population in Qidong, China. AFB_1–alb detection in this population in 1989 showed 100% positivity ($N = 75$); that detection rate declined to 23% in 2009 ($N = 100$) and further to 7% in 2012 ($N = 100$). Although HBV vaccination programmes have been in effect in this region, reductions in hepatocellular carcinoma incidence have occurred in age groups that were not included in the vaccination programme, indicating the independent effect of

aflatoxin reduction on hepatocellular carcinoma incidence. Chen et al. (2013) calculated that 83% of the reduction in primary liver cancer was attributable to decreasing aflatoxin.

A number of factors affect the risk of hepatocellular carcinoma, including tobacco use, alcohol consumption and, most notably, HCV and HBV infection. The potency of aflatoxins in the presence of HBV appears to be significantly enhanced and has been proposed to act in a multiplicative manner (Liu et al., 2012). A lack of background data on hepatocellular carcinoma limits this conclusion and its use in dose–response modelling and risk assessment. The majority of epidemiological evidence for aflatoxin-related hepatocellular carcinoma comes from populations with both high rates of HBV infection and high aflatoxin exposure; however, our understanding of the relationship between these two etiological factors in populations with low aflatoxin exposure and low HBV prevalence remains limited. The Committee has decided to maintain the approach used during the forty-ninth meeting, where aflatoxin potency estimates were calculated contingent upon the dynamics of HBV infection and aflatoxin in a human population. Other risk factors, such as HCV, tobacco use and alcohol consumption, have not been included in the dose–response and potency estimates due to limited evidence for interactions with aflatoxin-induced hepatocellular carcinoma.

9.1.2 Biomarker studies

The Committee determined that while the standard in epidemiology exposure assessment for aflatoxins is to utilize biomarkers of exposure, particularly the AFB_1–alb adduct, and there have been numerous well-conducted studies since the forty-ninth meeting (see Table 23 below), limitations in the use of these biomarkers for dose–response analysis and potency estimates make the use of these studies difficult in the current assessment. Linear relationships between dietary exposure and AFB_1–alb level have been examined only in populations with high exposure, genetic differences in aflatoxin metabolism and risk for hepatocellular carcinoma have been identified, and the consequences of this on the biomarker and its use in risk assessment remain undetermined, and the interaction between this biomarker and other risk factors remains inconclusive.

9.2 General modelling considerations

9.2.1 Selection of data

The Committee determined that despite its limitations, as discussed at the forty-ninth meeting and in the relevant monograph (Annex 1, references *131* and *132*) and in section 2.4.4(c) of the current assessment, the Yeh et al. (1989) study would remain the dataset utilized for the dose–response and potency estimates.

9.2.2 Measure of exposure

In the risk assessments performed for aflatoxin, dose has been expressed as lifetime average exposure to AFB_1 in ng/kg bw per day. If peak exposure or early lifetime exposure impacts the risk other than through an increase in the lifetime average ng/kg bw per day, this exposure measure could bias the risk estimates.

9.2.3 Measure of response

The major toxicological impact of aflatoxins on humans and animals is an increase in primary hepatocellular carcinoma, which is the focus of this risk assessment and all others performed to date.

9.2.4 Selection of mathematical model

To describe the relationship between exposure and disease, mathematical models are routinely used to analyse observed data. For cancer epidemiology, risk is typically modelled multiplicatively,

$$r_M(t,E) = r_O(t) \times f_M(E)$$

or additively,

$$r_A(t,E) = r_O(t) + f_A(E),$$

where $r_M(t,E)$ and $r_A(t,E)$ are functions that describe disease incidence as a function of age (t) and exposure (E). Here, exposure is used generically to define any factor that could affect the incidence rate other than age. For the present discussion, E is represented by HBV status as well as ng/kg bw per day aflatoxin exposure.

In estimating the rate, different authors have used different mathematical models for $f_M(E)$ and $f_A(E)$, which leads to model uncertainty when estimating risk. As these models are not nested, traditional statistical hypothesis testing, which compares models testing individual model parameters, cannot be performed. Instead, models are compared by looking at their Bayesian posterior probability (Kass & Raftery, 1995) in relation to the other models considered. For a given model, the posterior probability is the probability that the model is correct when compared with all the other models considered. Values closer to 1 indicate more support for the model, and values closer to 0 indicate less support given the data. Though the values sum to 1 and represent the correctness of the model given the data, these values should not be thought of as the probability of a given model being true (i.e. as being fully representative of the data-generating mechanism), but rather they should be considered as a relative measure that

quantifies the uncertainty in the model choice given the data. For all analyses, posterior probabilities are computed using the Bayesian information criterion approximation (Hoeting et al., 1999).

9.3 Potency estimates

There are many plausible alternatives as to the form of the mathematical relationship between exposure and response. In the previous analysis, a range of potencies were derived using different models, which were used to provide an indication of the uncertainty in risk. In the following sections, this analysis is reanalysed and a more comprehensive uncertainty analysis conducted. As with the previous analysis, it should be noted that the potential effect of mis-specification of the dose has not been quantitatively addressed, and the use of recent levels of exposure assumes that current exposures are comparable to past exposures. Uncertainty in the cumulative lifetime dose is an additional source of uncertainty in the analysis that is not taken into account. This uncertainty could lower (if the historical exposures were actually higher) or raise (if the historical exposures were lower) the potency estimate.

9.3.1 Potency estimates in humans based on epidemiological data

(a) Potency estimates not accounting for HBV infection

A number of studies have investigated the relative potency of aflatoxin exposure without regard to differences in HBV status. Table 23 shows a list of these studies and the corresponding potencies of aflatoxin exposure. As noted by the forty-ninth JECFA meeting (Annex 1, reference *131*), these values are in line with observed hepatocellular carcinoma rates in the general population (specifically the population rate of 3.4 cancers per 100 000 years in the USA). In addition, these values are comparable to the potencies estimated when HBV status is included.

(b) Potency estimates accounting for HBV infection

The epidemiology study by Yeh et al. (1989) is currently the best cohort available for estimating the potency of aflatoxin. It was the focus of the quantitative risk assessment in the previous JECFA opinion (Annex 1, reference *131*) and has been the focus of several quantitative risk assessments. This prospective cohort study of 7917 men took place in Guangxi Province in southern China.

In the analysis, Yeh et al. (1989) adjusted mortality rates for each region based on the age distribution of the composite study cohort as an internal standard. Wu-Williams, Zeise & Thomas (1992) calculated that the age-adjusted primary liver cancer (primary hepatocellular carcinoma) rate for the total cohort was 121.5 per 100 000 when standardized to the age distribution of the world population, versus 226.3 per 100 000 when standardized to the age distribution

Table 23
Potency estimates for the risk of liver cancer in humans for 1 ng/kg bw per day of AFB_1 exposure

References	Incidence/year per 100 000[a]
Peers & Lensell (1977)	0.11
Stoloff & Friedman (1976)	0
Carlborg (1979)	<0.21
Bruce (1990)	
Based on Stoloff (1983)	0
Based on Van Rensburg et al. (1985), Shank et al. (1972a,b), Peers, Gilman & Linsell (1976), Peers et al. (1987)	0.10
Croy & Crouch (1991)	
Based on Peers, Gilman & Linsell (1976)	0.15 (0.23)
Based on Yeh et al. (1989)	0.14 (0.21)
California Department of Health Services (CDHS, 1990)	
Based on Peers et al. (1976)	0.38 (0.60)
Based on Van Rensburg et al. (1985)	0.14 (0.17)
Based on Peers et al. (1987)	0.17 (0.3)
Based on Yeh et al. (1989)	0.18

HBV: hepatitis B virus
[a] The estimates do not take into account HBV status.
Source: JECFA 49 (Annex 1, reference *131*)

of the study cohort. The ratio of these rates (0.54) was then used to adjust the regional primary liver cancer mortality rates reported by Yeh et al. (1989) to obtain expected incidence rates for a (hypothetical) cohort with age distribution similar to the world population. Adjusted person-years of observation were calculated in each region as the number of deaths due to primary liver cancer observed in that region divided by the adjusted mortality rate. Adjusted person-years of observation were assumed to be distributed among HBsAg+ and HBsAg– carriers according to the regional prevalence of hepatitis B. The data are summarized in Table 24.

The previous JECFA evaluation looked at potency estimates based on the Yeh et al. (1989) study. These studies are described herein with the study-derived potency given. The values given are slightly different from the new analysis described in Table 25 because a different method of estimation was used. The new analysis quantifies the model uncertainty using the Bayesian framework whereas the previous analysis estimates are described using maximum likelihood estimation. As such methods determine the parameter estimates differently, that is, using the mean versus the mode, slight numerical differences, usually in the second or third significant digit, are noticeable.

Table 24
Adjusted person-years of observation[a]

AFB_1 dose (ng/kg bw per day)	No. of PLC cases		Adjusted person-years	
	HBsAg–	HBsAg+	HBsAg–	HBsAg+
12	0	12	9 932	2 727
90	1	7	6 114	2 017
705	4	12	7 733	2 537
2 028	2	23	5 803	1 743
–[b]	7	54	29 582	9 034

AFB_1: aflatoxin B_1; bw: body weight; HBsAg–: hepatitis B surface antigen negative; HBsAg+: hepatitis B surface antigen positive; PLC: primary liver cancer
[a] Epidemiological data originally from Yeh et al. (1989) adjusted by Wu-Williams, Zeise & Thomas (1992).
[b] No data are available for this group.

Table 25
Estimated potency for 1 ng/kg bw per day of AFB_1 exposure from different models applied, fitted to the data of Yeh et al. (1989)[a]

Model	HBsAg status	Potency in 100 000 person-years (95% UB)	Prior weight[b]	Posterior weight[c]
Wu-Williams, Zeise & Thomas (1992)				
Multiplicative linear	–	0.005 (0.009)	0.10	0.09
	+	0.140 (0.326)		
Additive	–	0.029 (0.058)	0.40	0.47
	+	0.410 (0.615)		
Hosenyi (1992)				
Exponential multiplicative	–	0.002 (0.003)	0.10	0.30
	+	0.054 (0.124)		
Bowers et al. (1993)				
Multistage cancer	–	0.018 (0.029)	0.40	0.14
	+	0.350 (0.474)		
Model average				
Expert prior[a]	–	0.017 (0.049)		
	+	0.269 (0.562)		
Uniform prior[b]	–	0.010 (0.040)		
	+	0.163 (0.502)		

bw: body weight; HBsAg: hepatitis B surface antigen; UB: upper bound
[a] Potency values are based upon a reanalysis using Bayesian estimation versus maximum likelihood estimation. As such, they are slightly different from the values reported by the authors, which are given in the text.
[b] Model-average estimates, prior and posterior weights based upon weights chosen by the Committee a priori to the analysis.
[c] Model-average estimates based upon prior weights that were equal across the models considered.

In estimating the potency of AFB_1, Croy & Crouch (1991) separately analysed the HBV-negative and HBV-positive cancer mortality rates in the Yeh et al. (1989) study using additive-linear models. Estimated potencies of 0.036

cancers per 100 000 per year for every ng/kg bw per day exposure for the HBV-negative individuals and 0.50 cancers per 100 000 per year for every ng/kg bw per day exposure for the HBV-positive individuals were reported. Their analysis did not look at the data under a single model but analysed HBV-negative and HBV-positive individuals separately. Consequently, their analysis is not considered further.

Hoseyni (1992) applied the Yeh et al. (1989) data to several different models and compared them based upon goodness-of-fit and likelihood ratio tests. The analysis led to the conclusion that a multiplicative model with linear-exponential effect on AFB_1 exposure and HBV status best fit the data. An interaction term was not explicitly included in any of the models. The potency of the multiplicative model is a function of the background liver cancer rate (in the absence of aflatoxin and HBV). Focusing on risk prediction for the USA population, Hoseyni (1992) chose a background cancer rate of 3.4 per 100 000 in deriving potency estimates. The resulting estimates were 0.0018 cancers per 100 000 per year for every ng/kg bw per day exposure to AFB_1 in HBV-negative individuals and 0.046 cancers per 100 000 per year for every ng/kg bw per day exposure in HBV-positive individuals.

Wu-Williams, Zeise & Thomas (1992) examined the fit of a variety of multiplicative and additive models that incorporated interaction terms. These models were fit to the adjusted person-years data as discussed above. Two models were found to fit the data adequately: an additive-linear model that includes an interaction term and a multiplicative-linear model (similar to that of Hoseyni, 1992) with no interaction term. Under the additive-linear model, the potency estimates were 0.031 and 0.43 for HBV-negative and HBV-positive populations, respectively. Like the Hoseyni (1992) model, potency estimates from the multiplicative-linear model are a function of the background cancer rate. For that analysis, a background cancer risk of 2.8 per 100 000 was used, resulting in potency estimates of 0.0037 and 0.094, respectively.

Bowers et al. (1993) applied an approximation to the two-stage model of carcinogenesis (Kopp-Schneider, Portier & Sherman, 1994) to the Wu-Williams, Zeise & Thomas (1992) data. In this model, the parameters are tied to the biological concepts of induction of mutations and growth of these mutated cells (Thorslund et al., 1987). The model assumed that AFB_1 has a linear effect on the formation of mutations and that HBV has no effect on the rate of mutation. For the growth of mutated cells, Bowers et al. (1993) assumed a linear effect of HBV (presence or absence) and an interaction effect of HBV and AFB_1. The resulting potencies for the HBV-negative and HBV-positive populations were 0.013 and 0.32 cancers per 100 000 per year for every ng/kg bw per day AFB_1 exposure, respectively. Unlike the studies, an upper bound for the potency was not given, but is computed below in the uncertainty analysis.

To investigate the uncertainty of the exposure–response relationship, these studies were reanalysed under a consistent methodological framework that allows for a relative comparison of the model fits as well as a Bayesian model-averaged estimate of the potency (Hoeting et al., 1999). All studies were reanalysed, with the exception of Croy & Crouch (1991), whose model did not take into account HBV status directly. This analysis was done from a Bayesian standpoint assuming two different prior choices. For the first, normal priors having mean zero and high variance (100) were placed over the parameters. Such priors produce analyses that are qualitatively identical to the method of maximum likelihood (the method used in the original analysis) and are used in the final analysis. To compare the resultant posterior model probabilities, a different prior was chosen. Here, parameters were assumed normal with mean and variance identical to the maximum likelihood estimation. As this approach produced a qualitatively similar uncertainty analysis, the results with the diffuse prior are given. The potency estimates for the multiplicative model of Wu-Williams, Zeise & Thomas (1992) and Hoseyni (1992) assumed a different background cancer risk between studies (2.8 cancers per 100 000 per year vs 3.4 cancers per 100 000 per year). These models were analysed under both scenarios. As the difference had minimal impact on the estimate, the value of 3.4 cancers per 100 000 per year is reported. All estimation was done in RSTAN (Carpenter et al., 2017).

The Committee determined prior weights for each model. There is strong biological evidence to conclude that AFB_1 is a low-dose linear genotoxic carcinogen. Consequently, from the low-dose linear argument (i.e. consistency with animal data) and the models' similarity to commonly used toxicological dose–response models, the linear model of Wu-Williams, Zeise & Thomas (1992) and multistage cancer model of Bowers et al. (1993) were given increased weight in comparison with the other models, totalling 80%. The other two models were given a total weight of 20%. Table 25 gives the potency estimates as well as the 95% upper confidence bound on this estimate for all models, including the model average. The Committee decided to use the model-average estimate of 0.017 (0.049, upper bound) for HBsAg– individuals and 0.269 (0.562, upper bound) for HBsAg+ individuals for aflatoxin exposure of 1 ng/kg bw per day. The central estimates are virtually unchanged from the previous Committee's potency estimates of 0.01 for HBsAg– and 0.3 for HBsAg+ for aflatoxin exposure of 1 ng/kg bw per day.

To determine the sensitivity of the analysis to prior specification, the Committee also analysed the data giving each model equal prior weight. For this analysis, the potency estimates are similar, with the upper-bound estimates on uncertainty qualitatively unchanged. Here the potency for HBsAg+ individuals was estimated to be 0.163 with a 95% upper limit of 0.502, and the potency estimate for HBsAg– individuals was estimated at 0.01 with a 95% upper limit of 0.04.

9.3.2 Potency estimates in humans based on biomarkers

The forty-ninth Committee computed potency estimates based upon the studies of Qian et al. (1994) and Wang et al. (1996), which quantified levels of AFB_1 exposure at the individual level. For the Qian et al. (1994) analysis, the potency was estimated to be 0.011 for HBV-negative individuals and 0.11 for HBV-positive individuals; for the Wang et al. (1996) analysis, the potency was estimated to be 0.0082 for HBV-negative individuals and 0.37 for HBV-positive individuals. The potencies from both of these studies are within the values produced in Table 25, although these studies have had some updates.

The Committee, however, had several reservations about this analysis. First, this analysis involved estimating the mean levels of the biomarkers internally as well as the daily AFB_1 intake corresponding to these classifications. As a majority of the data observed biomarker levels below the LOD, an additional complication of the analysis required estimating the mean value to corresponding values that were below this LOD and the strong assumption of the shape of the distribution of these biomarkers in a general population. In addition, the estimates of mean levels corresponding to detectable and non-detectable classifications of AFB_1–N^7-gua or AFB_1–alb are based on very limited data. Furthermore, the conversion factors relating internal exposure (AFB_1–N^7-gua or AFB_1–alb) to dietary AFB_1 intake are based on studies in human populations that may have different genetic characteristics than the study populations to which the conversion factor is applied. For the Wang et al. (1996) study, there is the additional consideration of how the case series was obtained. About half of the identified cases were prevalent cases diagnosed at the onset of the study. Consequently, the determinations of AFB_1 exposure for these cases may reflect alterations in metabolism directly related to the presence of primary liver cancer per se (Annex 1, reference *131*).

9.3.3 Potency estimates in test species

Several investigators have studied the carcinogenic potential of aflatoxins in vivo using laboratory animals (Wieder, Wogan & Shimkin, 1968; Butler, Greenblut & Lijinsky, 1969; Epstein, Bartus & Farber, 1969; Vesselinovitch et al., 1972; Merkow et al., 1973; Newberne & Rogers, 1973; Wogan, Paglialunga & Newberne, 1974; Ward et al., 1975; Reddy & Svoboda, 1976; Sieber et al., 1979; Angsubhakorn et al., 1981a,b; Butler & Hempsall, 1981; Nixon et al., 1981; Moore et al., 1982; Stoner et al., 1986; Cullen et al., 1987). In most of these studies, hepatocarcinogenesis was the main focus although other cancers have been noted, such as colon, kidney, lung and lymphoreticular system. The majority of these studies focused on AFB_1 and one on AFM_1 (Cullen et al., 1987); one study compared aflatoxins B_1, G_1 and B_2 (Butler, Greenblut & Lijinsky, 1969) and another study considered the aflatoxin metabolite aflatoxicol (Nixon et al., 1981). All of these laboratory results

are amenable to quantitative estimation of risks; however, some only contain one experimental dose group, have little indication of dose–response due to 100% response in all dosed animals or include the use of other agents (e.g. vitamin A) in their protocols. Cardis et al. (1997) summarized the calculated potencies from aflatoxin exposure in these test species. With regard to quantitative estimations and prediction of risks for AFB_1, the study by Epstein, Bartus & Farber (1969) has the most experimental dose groups and the most complete data for fitting a model. Using a simple multistage model of carcinogenesis (Cardis et al., 1997), these data predict an added incidence of 0.97 cancers per 100 000 per year for an exposure of 1 ng/kg bw per day of AFB_1 (scaled from the animal data to human risk estimates using body weight raised to the ¾ power). Other potency estimates (extrapolated to humans) ranged from as low as 0.05 per 100 000 per year for the Syrian golden hamster (Moore et al., 1982) to as high as 37 per 100 000 per year for the Fischer 344 rat (Cullen et al., 1987), with median estimate.

The Committee considered the above estimates, but concluded that the potency estimates were based upon studies where the lowest dose level was higher than the highest dose observed in human studies, which may lead to dose–response analyses that estimate potency much higher than the true potency. As an alternative, the Committee computed potency estimates based upon the study of Wogan, Paglialunga & Newberne (1974), which had doses much closer to the Yeh et al. study (1989). In this study, male Fischer rats were exposed to 0, 1, 5, 15 or 100 μg/kg diet of AFB_1 in their feed until a clinical deterioration of animals was observed, at which time all survivors were killed. Dosing, tumour incidence (hepatocellular carcinomas) as well as time-adjusted dosing are shown in Table 26. As the duration of dosing differed for each dose group, all analyses were conducted using the time-adjusted dosing.

To estimate the dose–response relationship, all models available in the USEPA's Benchmark Dose Software (BMDS) version 2.6.1 were fit to the data and a model-averaged benchmark dose estimate dose–response curve, as well as the upper bound on this estimate, was computed. This model-averaged curve was used to estimate the dose that increased the probability of tumorigenesis by 1 in 1000. Though this value is below the value typically used in dose–response analyses (i.e. 1 in 10), it has been shown to accurately reflect the dose–response curve in these regions using model-averaging methods (Wheeler & Bailer, 2007, 2008, 2013). The dose associated with this potency was estimated and used to linearly extrapolate the potency of the dose associated with 1 ng/kg bw per day. Using a body weight to the ¾ power conversion factor between humans and rats, the estimated lifetime potency for AFB_1 in rats is estimated to correspond to 4.7 cases per 100 000 lifetimes in humans (90% confidence interval 1.3–74.9). Using the Yeh et al. (1989) data and assuming a lifetime of 75 years, the potency is

Table 26
Induction of liver tumours (hepatocellular carcinomas) in male Fischer rats after dietary administration of AFB_1

Administered AFB_1 dose (µg/kg bw per day)	Time-adjusted dose (µg/kg bw per day)	Duration of dosing (weeks)	Tumour incidence [a]
0	0	104	0/18 (0)
0.04	0.04	104	2/22 (9%)
0.2	0.2	93	1/22 (5%)
0.6	0.6	96	4/21 (19%)
2.0	2.0	82	20/25 (80%)
4.0	2.0	54	28/28 (100%)

AFB_1: aflatoxin B_1; bw: body weight
[a] Expressed as number of animals with liver tumours / number of animals examined, and the resulting percentage in parentheses.
Source: Wogan, Paglialunga & Newberne (1974)

estimated to be 1.3 cases per 100 000 lifetimes, with an upper-bound estimate of 3.6.

9.3.4 Combining cancer potency estimates with dietary exposure estimates across GEMS/Food cluster diets and prevalence of HBsAg status to estimate the increases in liver cancer incidences worldwide associated with aflatoxin contamination

The combination of updated information regarding aflatoxin dietary exposure by GEMS/Food dietary clusters, HBsAg status and hepatocellular carcinoma incidence and central and upper-bound aflatoxin-related cancer potency estimates permitted an update in the calculation of global aflatoxin-related hepatocellular carcinoma risk. Aflatoxin-related cancer rates were calculated, accounting for different exposure estimates for AFB_1 (Table 27) and prevalence of chronic HBsAg positivity (Schweitzer et al., 2015), by GEMS/Food cluster diets.

Cancer risks were estimated for each of the GEMS/Food cluster diets, based on dietary exposure estimates for AFB_1 (section 8). Aflatoxin-related hepatocellular carcinoma risk for populations was estimated using the following equation:

$$R_i = P_{HBV+} \times (AF\ exposure) \times HBV+ + P_{HBV-} \times (AF\ exposure) \times (1-HBV+)$$

where R_i is the cancer risk for region i, with a population fraction of chronic HBV cases ($HBV+$) and potency estimates P for the HBV+ fraction of the population in region i and the fraction of the general population ($HBV-$) in region i. Estimates of the prevalence of chronic hepatitis B for individual countries were taken from a recent systematic review (Schweitzer et al., 2015). For each GEMS/Food cluster

Table 27

Cancer risk estimates for AFB_1 exposure by GEMS/Food cluster diets

Dietary exposure scenario	Estimated LB–UB dietary exposure of AFB_1 in ng/kg bw per day) and cancer risk (LB–UB; cancers per year per 100 000 population) per GEMS/Food cluster diet																
	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Dietary exposure (LB–UB; ng/kg bw per day)																	
Mean	1.2–4.3	1.3–4.0	3.8–5.7	2.3–5.6	2.9–5.8	2.6–6.3	0.7–0.9	0.2–1.0	1.3–2.5	0.7–3.4	0.1–2.2	2.2–4.4	7.0–13.5	1.3–3.2	0.6–3.0	3.2–5.3	1.1–2.4
High (90th) percentile[b]	2.5–8.6	2.6–7.9	7.6–11.5	4.6–11.3	5.8–11.6	5.2–12.7	1.4–1.7	0.4–1.9	2.3–4.7	1.3–6.7	0.4–4.5	4.4–8.8	13.9–26.9	2.6–6.5	1.2–5.9	6.4–10.5	2.3–4.8
Range of HBV prevalence for GEMS/Food clusters (%)[a]																	
	0.7–16.2	1.5–10.3	4.6–17.6	0.7–4.1	0.2–10.4	1.0–4.0	0.01–0.4	0.3–1.2	0.8–10.8	0.3–4.6	0.4–0.7	4.7–4.7	5.2–19	2.5–22.7	0.3–5.6	6.7–11.5	5.5–5.5
Cancer risk – LB HBV, central estimate of cancer potency (cancers per year per 100 000 population)																	
Mean	0.02–0.08	0.03–0.08	0.11–0.16	0.04–0.11	0.05–0.1	0.05–0.12	0.01–0.01	0–0.02	0.02–0.05	0.01–0.06	0–0.04	0.06–0.13	0.21–0.4	0.03–0.08	0.01–0.05	0.11–0.18	0.03–0.07
High (90th) percentile[b]	0.04–0.16	0.06–0.16	0.22–0.32	0.08–0.22	0.1–0.2	0.1–0.24	0.02–0.02	0–0.04	0.04–0.1	0.02–0.12	0–0.08	0.12–0.26	0.42–0.8	0.06–0.16	0.02–0.1	0.22–0.36	0.06–0.14
Cancer risk – UB HBV, central estimate of cancer potency (cancers per year per 100 000 population)																	
Mean	0.07–0.25	0.06–0.17	0.23–0.35	0.06–0.15	0.13–0.25	0.07–0.17	0.01–0.02	0–0.02	0.05–0.1	0.02–0.1	0–0.04	0.06–0.13	0.45–0.87	0.1–0.24	0.02–0.09	0.15–0.24	0.03–0.07
High (90th) percentile[b]	0.14–0.5	0.12–0.34	0.46–0.7	0.12–0.3	0.26–0.5	0.14–0.34	0.02–0.04	0–0.04	0.1–0.2	0.04–0.2	0–0.08	0.12–0.26	0.9–1.74	0.2–0.48	0.04–0.18	0.3–0.48	0.06–0.14
Cancer risk – LB HBV, UB estimate of cancer potency (cancers per year per 100 000 population)																	
Mean	0.07–0.23	0.07–0.22	0.28–0.42	0.12–0.3	0.15–0.29	0.14–0.34	0.03–0.04	0.01–0.05	0.06–0.13	0.03–0.17	0.01–0.12	0.16–0.32	0.53–1.02	0.08–0.2	0.03–0.15	0.26–0.44	0.09–0.19
High (90th) percentile[b]	0.14–0.46	0.14–0.44	0.56–0.84	0.24–0.6	0.3–0.58	0.28–0.68	0.06–0.08	0.02–0.1	0.12–0.26	0.06–0.34	0.02–0.24	0.32–0.64	1.06–2.04	0.16–0.4	0.06–0.3	0.52–0.88	0.18–0.38
Cancer risk – UB HBV, UB estimate of cancer potency (cancers per year per 100 000 population)																	
Mean	0.16–0.57	0.13–0.4	0.53–0.8	0.16–0.39	0.3–0.59	0.18–0.44	0.04–0.04	0.01–0.05	0.12–0.25	0.05–0.24	0.01–0.12	0.16–0.32	1.02–1.97	0.22–0.54	0.05–0.23	0.34–0.57	0.09–0.19
High (90th) percentile[b]	0.32–1.14	0.26–0.80	1.06–1.6	0.32–0.78	0.6–1.18	0.36–0.88	0.08–0.08	0.02–0.10	0.24–0.5	0.1–0.48	0.02–0.24	0.32–0.64	2.04–3.94	0.44–1.08	0.10–0.46	0.68–1.14	0.18–0.38

Dietary exposure scenario	Estimated LB–UB dietary exposure of AFB1 in ng/kg bw per day) and cancer risk (LB–UB; cancers per year per 100 000 population) per GEMS/Food cluster diet																
	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Age-standardized liver cancer incidence, range by GEMS/Food clusters (per 100 000 population)[c]																	
	1.1–78.1	2.1–8.4	3.0–19.5	1.9–7.0	2.4–16.0	2.5–25.6	1.7–6.7	2.8–5.9	0.9–52.6	2.1–16.2	1.6–3.1	6.3–6.3	1.7–25.8	3.1–15.1	2.5–5.8	2.3–13.3	3.0–3.0

AFB_1: aflatoxin B_1; bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; HBV: hepatitis B virus; LB: lower bound; UB: upper bound

[a] Source: Schweitzer et al. (2015)

[b] High percentiles are based on an approximation of the 90th percentile dietary exposure, calculated as twice the mean dietary exposure (FAO/WHO, 2009).

[c] Source: Globocan http://globocan.iarc.fr/Default.aspx

diet, the lowest and highest reported prevalence figures for individual countries in the cluster were used as lower- and upper-bound estimates of the prevalence of chronic hepatitis B for the GEMS/Food cluster diet. Risk was calculated utilizing both central tendency and upper-bound potency estimates of P_{HBV+} and P_{HBV-} (described in detail in section 9.3.1).

Table 27 summarizes estimates of the cancer risk for each GEMS/Food cluster diet, based on (1) the lower-bound estimate of *HBV*+ and the central tendency estimates of P_{HBV+} and P_{HBV-}; (2) the upper-bound estimate of *HBV*+ and the central tendency estimates of P_{HBV+} and P_{HBV-}; (3) the lower-bound estimate of *HBV*+ and the upper-bound estimates of P_{HBV+} and P_{HBV-}; and (4) the upper-bound estimate of *HBV*+ and the upper-bound estimates of P_{HBV+} and P_{HBV-}. For each of these scenarios, estimates of cancer risk were calculated for the lower- and upper-bound mean dietary exposure estimates and the lower- and upper-bound high percentile (90th) dietary exposure estimates for AFB_1, for each GEMS/Food cluster diet.

The lowest cancer risks, based on AFB_1 exposure levels, were estimated for clusters G07 and G08 (European and other developed countries), with cancer risk estimates between less than 0.01 and 0.10 aflatoxin-induced cancers per year per 100 000 population. For countries within G07 and G08 clusters, HBsAg+ rates were 0.01–1.2%. The highest cancer risks were for cluster G13 (sub-Saharan African countries and Haiti), with cancer risk estimates of 0.21–3.94 aflatoxin-induced cancers per year per 100 000 population. For countries within this cluster, HBsAg+ rates were in the range of 5.2–19%. Other clusters with relatively high cancer risks were G03 (sub-Saharan African countries and Paraguay), G05 (mainly Central and South American countries) and G16 (sub-Saharan African countries).

10. Comments

10.1 Biochemical aspects

The Committee at the forty-ninth meeting of JECFA (Annex 1, reference *131*) considered that the carcinogenicity of aflatoxins was due to metabolic activation to a reactive epoxide and that species differences in metabolism were responsible for different susceptibilities of animals to the toxic effects of exposure to aflatoxins.

A substantial body of additional evidence from subsequent studies that was reviewed by the current Committee adds to the chemical and metabolic determinants for toxicity. The toxicity of aflatoxins stems from the presence of an oxidizable 8,9-double bond in AFB_1 and AFG_1. The action of many hepatic CYP isoforms on AFB_1 produces the highly reactive metabolite AFB_1-8,9-*exo*-

epoxide, which reacts readily with critical biological nucleophiles, such as DNA and proteins, that can initiate toxic sequelae (Eaton et al., 2010). The reaction of AFB_1-8,9-*exo*-epoxide at the N^7-position of guanine residues in DNA produces persistent lesions (Smela et al., 2001). Concomitantly, deactivation of AFB_1-8,9-*exo*-epoxide can occur by other pathways, including hydrolysis, enzyme-mediated reactions with glutathione and conjugation with glucuronic acid and sulfate by uridine diphosphate-glucuronosyltransferases and sulfotransferases, respectively, that enhance excretion (Eaton et al., 2010). Some CYP isoforms directly detoxify AFB_1 through oxidation reactions to produce metabolites, including AFQ_1, AFM_1, AFP_1 and AFB_1-8,9-*endo*-epoxide (Kamdem et al., 2006). Hydrolysis of either the *exo*- or *endo*-epoxide produces AFB-diol, which reacts with lysine residues on serum albumin to form adducts that have proven to be valuable biomarkers of exposure to AFB_1 (Guengerich et al., 1998). AFB_1 and its metabolites, with and without phase II conjugation, are eliminated from the body by excretion in the urine and faeces, and AFM_1 is excreted via lactation (Eaton et al., 2010).

Detoxification of AFB_1-8,9-*exo*-epoxide by the GST isoforms present in the liver appears central to the interspecies differences in susceptibility to AFB_1 toxicity, in which mice are relatively resistant and rats and trout are highly susceptible. A constitutively expressed α-class GST with high activity for the detoxification of AFB_1-8,9-*exo*-epoxide is present in mouse liver; in contrast, rats, trout and humans possess much lower hepatic GST activity towards AFB_1-8,9-*exo*-epoxide, whereas monkeys are intermediate (Eaton et al., 2010).

Measurements of levels of AFB_1 bound to serum albumin, its metabolites in urine and faeces, and its DNA adducts provide a wealth of information related to the balance of activation and detoxification that best correlates AFB_1 exposure with susceptibility to toxic effects. Interindividual variability in human subjects is apparently due to enzyme polymorphisms for the activation and detoxification of AFB_1 catalysed by CYP isoforms and detoxification of AFB_1-8,9-*exo*-epoxide catalysed by GST isoforms (Eaton et al., 2010).

10.2 Toxicological studies

The Committee at its forty-ninth meeting (Annex 1, reference *131*) considered substantial evidence that aflatoxins caused liver damage and hepatocarcinogenicity in laboratory rodents. In particular, the high susceptibility of male F344 rats to the carcinogenic effect of AFB_1 was noted.

A substantial body of additional toxicological evidence from subsequent studies was reviewed by the current Committee to update the risk assessment. The carcinogenic effects of AFB_1 in male F344 rats were quantified through a lifetime dietary study in which concentrations as low as 1 μg/kg produced liver

tumours (Wogan, Paglialunga & Newberne, 1974). Similarly sensitive were rainbow trout, in which dietary administration of AFB_1 at 0.8 µg/kg produced a hepatocarcinogenic effect after 20 months. Large-scale studies designed to test the ED_{001} response (effective dose for a 0.1% increase in tumour incidence) to AFB_1 were conducted in trout using dietary concentrations of 0.05–110 µg/kg over a 4-week exposure period with 1-year termination (Williams, 2012). Trout tumorigenesis data showed no indication of deviation from a log-linear dose–tumorigenic response relationship. A log-linear low-dose relationship was also observed between AFB_1 dose and formation of DNA adducts in the trout (Bailey et al., 1998) and rat liver (Choy, 1993; Cupid et al., 2004; Pottenger et al., 2014). This low-dose log-linearity of tumour responses is presumably a consequence of the very low constitutive hepatic GST activity towards AFB_1 epoxide in these species (Monroe & Eaton, 1987; Valsta, Hendricks & Bailey, 1988). The demonstration of such a relationship that includes doses approaching human exposure levels is rare, but important for a genotoxic carcinogen like AFB_1, as it tends to validate the linear, no-threshold approach to AFB_1 cancer risk assessment. The commonality of critical metabolic processes across mammalian species linked effects and potency in controlled dosing studies in experimental animals with those in humans and provided avenues for molecular epidemiological approaches to study the role of aflatoxin exposure in human liver cancer (Eaton et al., 2010; Kensler et al., 2011). Formation of the AFB_1–N^7-gua DNA adduct leads to the most common AFB_1-associated mutation, the GC → AT transversion (Smela et al., 2001). The predominance of a specific mutational hotspot in human hepatocellular carcinoma was identified, even though animal models do not recapitulate this event (Hussain et al., 2007).

As no new data on AFM_1 carcinogenicity were available, the approximate potency for carcinogenicity of an order of magnitude lower relative to AFB_1 (Bailey et al., 1998), as estimated at the forty-ninth meeting of JECFA, was maintained.

10.3 Observations in domestic animals/veterinary toxicology

The Committee at the forty-ninth meeting of JECFA (Annex 1, reference *131*) did not specifically consider the toxicity of aflatoxins in livestock. The current Committee evaluated information on the effects of aflatoxins on domestic animal health and productivity in cattle, poultry and swine genotypes used in North America and Western Europe. AFB_1 causes a variety of adverse effects in different animal species, especially chickens. In poultry, these effects include liver damage, impaired productivity and reproductive efficiency, decreased egg production, inferior eggshell quality, inferior carcass quality and increased susceptibility to disease. Swine are also highly affected by aflatoxin, with the chronic effects largely attributable to liver damage (Armbrecht, 1978). In cattle, the primary symptoms

are reduced weight gain as well as liver and kidney damage; milk production is also reduced (Pitt et al., 2012). In many developing countries where aflatoxins are a chronic problem, the poorest quality grain may be used for animal feed. An indication of this is the common occurrence of AFM_1 in milk (e.g. Gizachew et al., 2016). In Africa, these impacts are likely to be substantial in poultry and cattle (Atherston et al., 2016).

10.4 Observations in humans

Epidemiological assessment of aflatoxin exposure and its association with human health end-points typically utilizes biomarkers. During the previous JECFA evaluation, the Committee identified the AFB_1–alb biomarker as relating linearly to dietary AFB_1 exposure, but noted that key issues related to its use as an indicator of hepatocellular carcinoma risk were as follows: (1) the linear relationship between dietary exposure and AFB_1–alb level was examined only in populations with high exposure; (2) there is a lack of evidence of a correlation between levels of AFB_1–alb and liver AFB_1–DNA adducts; (3) the relationship between AFB_1–alb level and the genetic consequences of exposure on metabolism remained to be determined; and (4) the interactions between aflatoxins and other major risk factors, such as HBV and HCV infection, were not understood. During the current JECFA meeting, the Committee noted that some of these issues still remain. Differences in metabolism and AFB_1–alb levels due to genetic consequences from continued high exposure and/or HBV and HCV infection remain to be determined. A study in a human cohort (n = 39) demonstrated a linear association between AFB_1–alb levels and levels of liver AFB_1–DNA adducts (Zhang et al., 2006). Since the previous JECFA evaluation of aflatoxins, the analytical methodology for detection of AFB_1–alb biomarkers has been refined to allow the detection of a more specific component of the AFB_1–alb adducts (AFB_1–lys). Although the levels of AFB_1–alb and AFB_1–lys biomarkers have generally been recognized to be correlated, the AFB_1–lys biomarker is more specific, and typically its levels are a factor of 2.6 lower than those for the AFB_1–alb biomarker (Scholl et al., 2006; McCoy et al., 2008). The differences between these two adducts should be considered when comparing studies.

The p53 249^{ser} DNA mutation has been identified as a potential biomarker of effect for aflatoxin-induced hepatocellular carcinoma (Kirk et al., 2005b). Although the presence of a p53 249^{ser} mutation in hepatocellular carcinoma is often associated with aflatoxin exposure, there is still no evidence for causality, and the presence of HBsAg appears to be an important aspect for development of this mutation (Stern et al., 2001). Whether AFB_1 causes these mutations or whether AFB_1 leads to differential promotion of cells that acquire the mutation in human populations remains unclear. The development of chronic aflatoxin

exposure biomarkers and validated effect biomarkers would contribute to a better understanding of the global risk from exposure.

The Committee at the forty-ninth meeting of JECFA (Annex 1, reference *131*) evaluated a large body of epidemiological literature on the incidence of primary liver cancer, especially in the developing world, noting that a major disease determinant was the co-exposure to hepatitis viruses, especially HBV, which can significantly enhance risks from aflatoxin exposures. Aflatoxin effects that had been observed in humans include acute aflatoxicosis, growth stunting, immunotoxicity and development of hepatocellular carcinoma.

Since the previous JECFA evaluation, there have been significant contributions made to the literature in the area of epidemiology and aflatoxin exposure. Historical outbreaks of acute liver failure (jaundice, lethargy, nausea, death), identified as aflatoxicosis, have been observed in human populations since the 1960s. Identification of aflatoxins in primary food staples (e.g. maize and peanuts) that were associated with onset of disease was documented in incidents from India and Kenya (Krishnamachari et al., 1975; Ngindu et al., 1981; CDC, 2004; Azziz-Baumgartner et al., 2005). The Committee noted that there were deaths attributed to aflatoxins in the United Republic of Tanzania during the summer of 2016 (PACA, 2016), but dietary exposure data were not available at the time of the meeting. Reports that evaluated past outbreaks of aflatoxicosis have estimated acutely toxic and potentially lethal AFB_1 doses in humans to be between 20 and 120 μg/kg bw per day when consumed over a period of 1–3 weeks (Wild & Gong, 2010; Groopman et al., 2014); the consumption of staple food containing aflatoxin concentrations of 1 mg/kg or higher has also been suspected to cause acute aflatoxicosis (e.g. Serck-Hanssen, 1970).

Growth suppression has historically been considered an important health end-point for aflatoxins in animal models, with prenatal and postnatal exposure potentially eliciting adverse effects. Growth suppression in humans in cross-sectional and prospective studies in sub-Saharan Africa has been observed, with significant associations between aflatoxin exposure and lower WHO-calculated *z*-scores (primarily height-for-age) (Gong et al., 2002, 2003, 2004; Turner et al., 2003, 2007). However, there were no associations found between aflatoxin exposure and child *z*-scores in populations from Nepal (Mitchell et al., 2016a) and the United Republic of Tanzania (Shirima et al., 2015). Prenatal studies indicated that a decrease in a mother's exposure biomarker (AFB_1–alb) from 110 to 10 pg/mg albumin was associated with an increase of 2 cm in height and a weight increase of 800 g in infants at 52 weeks of age (Turner et al., 2007). A number of mechanisms have been proposed for the effect of aflatoxin on growth, including immune dysfunction leading to increased risk of infections and energy loss, changes in intestinal integrity leading to poor nutrient absorption, disruption of the microbiome and altered expression of the IGF axis (Wild, Miller & Groopman,

2015). Castelino et al. (2015) indicated, in a child cohort from Kenya, an inverse relationship of both IGF1 and IGFBP3 with AFB_1–alb levels. The decreased levels of IGF1 may occur from in utero exposure and DNA hypermethylation of CpG sites for the *IGF1* gene (Hernandez-Vargas et al., 2015).

The data from human studies suggest a negative effect of aflatoxins on child growth; however, causality has yet to be determined. Those populations most affected with child growth faltering and chronic aflatoxin exposure are exposed to a number of other etiological risk factors, such as low socioeconomic status, chronic diarrhoea, infectious disease and malnutrition. There are currently no epidemiological studies that factor all of these potential risk factors into their statistical analysis; thus, many of the studies may be overestimating the impact of aflatoxins on growth. The proposed modes of action would indicate that aflatoxin exposure could be the primary agent, because it could negatively affect the immune system and/or intestinal integrity, which in turn would influence the rates of diarrhoeal and infectious disease as well as nutrient uptake. The association between aflatoxin exposure and either impaired immune system function or intestinal integrity in human populations has yet to be determined. In fact, although a few studies have reported negative associations between aflatoxin exposure and certain measures of immunological function, other studies have failed to detect such negative associations (Turner et al., 2003; Jiang et al., 2005; Hernandez-Vargas et al., 2015).

The Committee at the forty-ninth meeting (Annex 1, reference *131*) identified and described several important aflatoxin and hepatocellular carcinoma–related epidemiological studies, including the 1989 study by Yeh et al. (1989). This prospective study, which was conducted in a large cohort (n = 7917) from China, collected dietary aflatoxin exposure data over a 6-year period and demonstrated a statistically significant, almost perfectly linear relationship between aflatoxin exposure and hepatocellular carcinoma mortality, independent of HBV infection (Yeh et al., 1989). This study does have limitations, which were described by the Committee at the forty-ninth meeting, but was ultimately determined to be the most reliable dataset with which to calculate aflatoxin potency estimates (Annex 1, reference *131*).

The majority of epidemiological studies that have been conducted since the last JECFA evaluation of aflatoxins have demonstrated a positive association between aflatoxin biomarkers and hepatocellular carcinoma; some studies indicate that aflatoxin exposure poses a significant risk only in the presence of other risk factors, such as HBV infection. Dietary changes in a historically hepatocellular carcinoma–endemic population led to decreases in aflatoxin exposure and were associated with a marked decrease in hepatocellular carcinoma incidence, independent of HBV (Chen et al., 2013). Worldwide population-attributable risk for aflatoxin-related hepatocellular carcinoma has been calculated by Liu & Wu

(2010), with aflatoxin alone (no HBV) playing a causative role in 4.6–28.2% of global hepatocellular carcinoma cases. The WHO report entitled *Global Burden of Foodborne Disease* estimated global foodborne aflatoxin disease incidence, mortality and disease burden; aflatoxin was associated with global disease (hepatocellular carcinoma) incidence (8967–56 776 cases per year) following adjustments to account for synergism between HBV and aflatoxin (WHO, 2015). The majority of uncontrolled aflatoxin exposure worldwide remains in those populations most at risk for chronic HBV prevalence.

Reports of case–control and cohort studies from China (including the Province of Taiwan) and Africa have reported relative risk values for aflatoxin-induced hepatocellular carcinoma in the range of 0.3–17.4 for aflatoxin exposure alone. These values are increased in the combined relative risk for HBV and aflatoxins to 1.57–70.0 (Ross et al., 1992; Qian et al., 1994; Wang et al., 1996; Lunn et al., 1997; Kuniholm et al., 2008; Wu HC et al., 2009; Asim et al., 2011; Qi et al., 2015). Meta-analysis of the available data indicated a multiplicative interaction of aflatoxins and HBV infection for the development of hepatocellular carcinoma (Liu et al., 2012).

The majority of epidemiological studies focused on evaluation of aflatoxin exposure and hepatocellular carcinoma incidence utilizing the AFB_1–alb biomarker, which limits their usefulness in development of a dose–response relationship. The serum AFB_1–alb biomarker has a relatively short half-life (~30 days), which creates some uncertainty in risk assessment of a lifetime health end-point, such as liver cancer. Additionally, the metabolism of this biomarker can be influenced by genetic differences (Wojnowski et al., 2004; Kirk et al., 2005b; Long et al., 2013a,b; Yao, Huang & Long, 2014) and probably dietary exposure (Tang et al., 2008; Kensler et al., 2011), leading to high variation within populations. Thus, the association of chronic dietary exposure to aflatoxins with hepatocellular carcinoma is difficult to estimate using biomarkers, because short-term biomarkers are limited in their ability to establish a causal relationship in the etiology of diseases with extended latencies.

10.5 Analytical methods

Aflatoxins are mycotoxins of major importance; therefore, techniques for their detection and analysis have been extensively researched to develop those that are highly specific, useful and practical. Many of the techniques that have been developed are applicable to different and specific situations, so a fit-for-purpose approach needs to be considered in selecting the method for use in a particular instance. The natural or induced fluorescence of aflatoxins aids in their detection, such as in the original TLC method, which is still applicable when combined with scanner instrumentation. However, HPLC, in combination with fluorescence

detection or, more recently, with mass spectrometry, is the most widely applied technique for quantitative analysis. For field (non-laboratory) measurements, the method should be rapid, portable, reproducible and capable of being performed by non-scientific personnel (Turner, Subrahmanyam & Piletsky, 2009). As Shephard (2009) stated, "The need for analytical determination of aflatoxins has resulted in a plethora of methods to meet a range of analytical requirements by various analysts, from regulatory control in official laboratories (such as HPLC-MS) to rapid test kits for factories and grain silos (such as ELISA)". The rapid methods generally involve the use of aflatoxin-specific antibodies, specific for AFB_1 or for AFT (based on cross-reactivity of the antibody for all B and G aflatoxins).

The analytical methods used for aflatoxins (reviewed in Vidal et al., 2013; Wacoo et al., 2014; Shephard, 2016) cover a wide spectrum of analytical science and can be generally divided into (1) quantitative methods (TLC combined with scanner; HPLC, HPLC-MS, LC-MS or LC-MS/MS; capillary electrophoresis); (2) semiquantitative methods (ELISA; lateral flow tests; direct fluorescence; fluorescence polarization immunoassay; biosensors); (3) indirect methods (spectroscopy); and (4) emerging technologies (hyperspectral imaging; electronic nose; aptamer-based biosensors; MIPs).

Numerous methods have been published in the scientific literature for specific purposes, but they may not be validated and therefore may not be applied in practice; these are not further discussed here. Trucksess & Zhang (2016) argued that for analytical methods to be practical, they should meet the basic guideline of reproducibility in different laboratory settings. Several standard development organizations have issued stringent guidelines on accuracy, precision, selectivity, LOD, LOQ, linearity, range, uncertainty and ruggedness as criteria for acceptance of quantitative analytical methods. For screening methods or qualitative methods for mycotoxins, the most commonly applied acceptability criteria include (1) false-negative rates of less than 5% for analytical results at target level; (2) false-positive rates of less than 10–15% at target level; (3) a known threshold (cut-off) level for an intended matrix; and (4) a confirmation method for positive results (against a validated reference method) (Trucksess & Zhang, 2016).

There is a need to develop rapid, low-cost, low-technology, accurate detection methods for aflatoxins to improve surveillance and control in rural areas. Shephard (2016) indicated that sampling remains a problem in many developing countries because subsistence farmers in these countries do not produce enough grain to spare the quantities needed for testing. However, organizations such as the Partnership for Aflatoxin Control in Africa and the World Food Programme are addressing these issues. For example, the World Food Programme has instituted the Purchase for Progress programme to ensure grain quality by creating the Blue Box, which contains test kits for grain quality, including aflatoxins (World Food Programme, 2011, 2014).

10.6 Sampling protocols

The inherent non-homogeneous nature of aflatoxin (and other mycotoxin) contamination in raw agricultural commodities continues to present a major challenge to the obtaining of representative samples. The challenge continues to be addressed by the adoption of sampling protocols. In particular, for peanuts (groundnuts), protocols have been developed by the Codex Alimentarius Commission (FAO/WHO, 2001, 2004) and by the European Commission (European Union, 2006, 2010) and form the basis for the Origin Certificate Program of the USA (Adams & Whitaker, 2004). More recently, the Codex Alimentarius Commission, in setting maximum levels for aflatoxins in peanuts, almonds, Brazil nuts, hazelnuts and pistachios intended for further processing and for ready-to-eat almonds, Brazil nuts, hazelnuts, pistachios and dried figs, has specified sampling protocols for regulatory purposes (FAO/WHO, 2016). As an aid to understanding and implementing appropriate sampling and subsampling protocols for mycotoxins in general, the Joint FAO/International Atomic Energy Agency (IAEA) Programme, Nuclear Techniques in Food and Agriculture, has published a manual (Whitaker et al., 2010), and two training videos have been produced (Brera, Miraglia & Pineiro, 2007; ISS, 2015). In general, associated with each sampling protocol is an operating characteristics curve, which gives the statistical probability of acceptable lots being rejected and contaminated lots being accepted. These curves are specific to each mycotoxin/commodity combination and can vary with the sampling parameters chosen. The statistical research from which these were derived is available in the public domain and consolidated as a "mycotoxin sampling tool" by FAO (2013). Problems of representative sampling are most acute in addressing contamination in rural subsistence villages, where the necessary large samples are generally not available for food security reasons. Additionally, processing of the large sample weights can be challenging in a field setting.

10.7 Effects of processing

In common with other mycotoxins, the milling of cereals does not destroy aflatoxins but merely distributes them among the milling fractions or products. In general, those fractions intended for human food have reduced levels, whereas those intended for animal feed (e.g. the bran fraction) have elevated levels. Prior to milling, processes such as grain cleaning and separation are useful management tools, potentially eliminating contaminated kernels and leading to a reduction of contaminant levels in the final milled product. In this regard, hand sorting is also a viable method in rural farms or in small-scale industrial food processors lacking sophisticated sorting machines. A number of publications have highlighted the reductions in contamination of cereal foods that can be

achieved in rural settings using the common processes of sorting, winnowing, washing, crushing, dehulling and fermentation (either alone or when combined with steeping and cooking) (Adegoke, Otumu & Akanni, 1994; Kpodo, Sorensen & Jakobsen, 1996; Fandohan et al., 2005).

Unit operations such as heating, roasting and baking can reduce the levels of aflatoxins during the processing of foods, but complete elimination does not occur. The degree of elimination is variable and depends on the process and the conditions under which it is applied. For example, the decreases registered during extrusion processes are dependent on the design of extruder, moisture content of food, pressure applied and resulting temperatures.

10.8 Prevention and control

Aflatoxin contamination of crops, both preharvest and postharvest, poses a serious health hazard as well as a significant economic burden from lack of sale of contaminated commodities. A reduction in risk will require an integrated systems approach that includes targeted agronomic cultural practices, biological control methods and enhancement of host plant resistance, coupled with postharvest technologies such as proper drying and storage of affected crop products, with the development of appropriate alternative uses to retain at least some economic value.

Strategies for preharvest mitigation are designed to limit fungal invasion of crops by aflatoxigenic fungi and subsequent aflatoxin production. The strategies to minimize aflatoxin contamination in crops begin prior to planting (best management practices). Decisions must be made with respect to the selection of the cultivar to be planted, planting and harvesting dates, plant density, co-cropping and crop rotation, as well as soil treatments, irrigation and plant protection management (reviewed in Hell, Cardwell & Poehling, 2003; Wagacha & Muthomi, 2008; Waliyar et al., 2008; Abbas et al., 2009; Munkvold, 2014; Torres et al., 2014; Alberts et al., 2017). The contribution of each of these practices may vary by geographical location. However, these practices are considered to have a significant effect on reducing aflatoxin contamination when practised together.

The use of microbes to control aflatoxins in food and feed has been extensively reviewed (Dorner, 2004; Yin et al., 2009; Guan et al., 2011; Bandyopadhyay et al., 2016). Microbes such as bacteria and yeasts have been investigated for their ability to reduce toxin contamination. However, no commercial application of bacteria and yeast biocontrol products has been established. One strategy that has received significant attention for reduction of aflatoxins prior to harvest has been biological control using non-toxigenic (atoxigenic) *A. flavus* isolates. Strains formulated into biological control products may be single isolate or multiple isolates to improve broader adaptability

(Atehnkeng et al., 2014). This approach has been deployed on crops such as cotton, maize, peanuts, figs and pistachios in the USA, maize in Africa (Wild, Miller & Groopman, 2015; Bandyopadhyay et al., 2016) and peanuts in Australia (Pitt & Hocking, 2006), Argentina (Chulze et al., 2015) and China (Yin et al., 2009). This strategy has also been used for maize in Thailand to measure the effectiveness of this treatment preharvest and postharvest; the results were promising, but inconsistent (Pitt et al., 2015). Several factors have been identified that affect efficacy, such as available moisture for spore germination, too much moisture from rainfall, resulting in uneven distribution of the applied material, and time of application of the biocontrol formulation (Bock & Cotty, 1999). The added cost of application also makes this strategy more suitable for areas routinely affected by chronic aflatoxin contamination because, to be effective, the application of the biocontrol formulation has to occur at early stages of crop development. The longer-term implications of the application of biocontrol formulations, such as the very low level of sexual recombination in restoring toxigenicity, adaptability of these applied strains under changing climatic conditions, the effect of such application on the microbiome or population biology of the field, and the potential for unsafe exposure to fungal inoculum (Ehrlich et al., 2015; Horn et al., 2016; Wild, Miller & Groopman, 2015; Alberts et al., 2017), remain to be evaluated.

The most long-term, stable solution to control preharvest aflatoxin contamination is through enhancing the ability of the host crop to prevent fungal infection and/or the production of aflatoxins by the invading fungus. This can be achieved through either plant breeding or genetic engineering of crops of interest. However, these processes are laborious and extremely time consuming. Breeding efforts to obtain germplasm resistant to aflatoxin accumulation is particularly challenging because of strong environmental pressures on infection and aflatoxin production by *A. flavus* (reviewed in Payne, 1998; Brown et al., 2013b; Warburton & Williams, 2014; Bhatnagar-Mathur et al., 2015; Fountain et al., 2015). Additionally, finding resistant lines through traditional breeding is difficult because the phenotypic or agronomic characteristics that the breeder needs to look for are difficult to define. Moreover, it has been established that resistance is not conferred by a single gene and is a quantitative trait needing the combined effect of multiple genes (Kelley et al., 2012). Plant breeding and varietal selection have provided significant maize and peanut genetic material demonstrating resistance to fungal invasion or toxin formation; however, commercial lines have yet to be marketed. Some of these lines are drought resistant and have shown reduced levels of aflatoxins. However, with the advent of new technologies such as genomics, proteomics and transcriptomics, the process of understanding and utilizing host–pathogen interactions has been significantly enhanced. Identification of markers to facilitate the transfer of resistance traits into desirable genetic backgrounds is essential for marker-assisted breeding. Marker

genes or QTLs of interest associated at high frequency with *A. flavus* or aflatoxin resistance due to genetic linkage (i.e. close proximity on the chromosome of both traits – namely, the marker locus and the disease resistance–determining locus) have been identified for maize (Warburton & Williams, 2014). In addition, RAPs have been identified from maize using proteomic studies comparing susceptible and resistant germplasm (Chen et al., 2015). These RAPs have been mapped to the resistance loci (QTL maps). Gene silencing, using genetic engineering (RNAi), of five aflatoxin biosynthetic pathway genes in peanut plants was successful in controlling aflatoxin accumulation following inoculation with *A. flavus* in laboratory studies (Arias, Dang & Sobolev, 2015).

The preharvest contamination of commodities with aflatoxins is generally limited to maize, cottonseed, peanuts and tree nuts. In contrast, postharvest contamination can be found in a variety of other agricultural crops, such as coffee, rice and spices. This contamination during storage can be influenced by factors such as moisture, temperature, mechanical or insect damage to commodities, aeration and the level of fungal inoculum. Therefore, preventive measures against aflatoxin contamination postharvest must address these conditions. Additionally, other measures, such as chemical decontamination or use of enterosorbents, can be used to remove aflatoxins from already-contaminated commodities (Hell et al., 2008; Kolosova & Stroka, 2011; Waliyar et al., 2015).

A number of research groups have attempted to correlate various environmental factors with the potential for *A. flavus* growth, and consequently aflatoxin production, in both preharvest and postharvest situations. "Predictive analytics" is an emerging discipline in which large volumes of climatic and agronomic data are mined for modelling to predict future outbreaks. In contrast to *Fusarium* head blight and deoxynivalenol, for which government and commercial predictive models are widely used by farmers in Canada and the USA, there are no commercially successful models available to predict aflatoxin contamination in any commodity. The reliability and predictive power of the results of successful models depend entirely on the quality and number of data points from farmers' fields coupled with about a decade of field experience to refine the model. A number of models for predicting aflatoxin contamination in both field and storage conditions have been developed (reviewed in Battilani & Leggieri, 2015; Battilani et al., 2016), with relatively high correlation (up to 0.8). Model prediction will never be 100% accurate, especially for predicting aflatoxin contamination, because there are too many factors, other than environmental factors, that significantly influence this contamination at harvest or during storage. The availability of accurate and detailed information on the factors that affect aflatoxin contamination will enable researchers to improve model performance in the future.

10.9 Levels and patterns of contamination in food commodities

The evaluation of the occurrence of aflatoxins was restricted to AFB_1 and AFT and to those human foods most likely to be contaminated (cereals, nuts and spices). The presence of the hydroxylated metabolite AFM_1 in milk, both human and dairy, was also considered. The open literature contains results of a large number of surveys for these contaminants. The methods employed in these papers (mostly HPLC as opposed to traditional TLC or ELISA) were of generally sufficient sensitivity (low LOD/LOQ) to measure low microgram per kilogram levels. A factor in assessing the results of open literature surveys is the problem that not all authors make it clear whether the mean values they report are for positive samples only or for all samples, thus complicating the assessment. Of the studies in cereals, most work was reported for wheat, maize and rice, whereas barley, oats and sorghum received little attention. Generally, it was noted that surveys in developing countries showed higher contaminant levels compared with reports from developed countries. A similar pattern emerged for nuts, particularly peanuts, in which extremely high contamination was reported in markets of developing countries. Of the range of spices investigated, chilli had both the highest contamination prevalence (up to 100%) and the highest contaminant levels. Surveys of dairy milk and its processed products followed the same geographical pattern, with a number of samples reported above the maximum level (0.5 μg/kg) set by the Codex Alimentarius Commission (FAO/WHO, 1995). AFM_1 in human milk is a more complex issue, in that it occurs as a consequence of maternal exposure to AFB_1. This exposure is minimal in developed countries, but can be problematic in rural subsistence farming areas of developing countries.

With respect to the GEMS/Food contaminants database, there was little information on the occurrence of aflatoxins in food from developing countries. In examining entries in the database, it was found that certain data were unreliable, in that some contaminant values had clearly been incorrectly captured (or entered). Further, the wide range of LOD/LOQ values reported made any assessment of per cent positive samples difficult. Also, in cases where the number of samples was relatively small, a few large outliers could heavily influence the calculation of the mean. The nature of the database precludes any conclusions on these contaminated samples and what they represent in terms of sampling for compliance, survey or importation. The level of testing clearly relates to the importance of the commodity in trade and the potential for *Aspergillus* infection and consequent aflatoxin contamination.

The only transfer from feed to food that is a concern for food safety is that of the hydroxylated AFB_1 metabolite, AFM_1, secreted in milk. Although

transfer can also occur in eggs and liver, the relative levels with respect to feed contamination are low (Park & Pohland, 1986).

10.10 Food consumption and dietary exposure assessment

Since the previous evaluation by JECFA, a number of national estimates of dietary exposure have been published. The Committee considered evaluations by Africa (various countries), Argentina, Brazil, China, Egypt, France, Greece, Ireland, Islamic Republic of Iran, Japan, Kenya, Lebanon, Malaysia, Mexico, Morocco, the Netherlands, New Zealand, Pakistan, Portugal, the Republic of Korea, Serbia, Spain, Sri Lanka, Tunisia, Turkey and the United Republic of Tanzania. These reports include dietary exposure assessments for AFT (27 studies), AFB_1 (29 studies), AFB_2 (six studies), AFG_1 (five studies), AFG_2 (five studies) and AFM_1 (19 studies). Studies varied in the range of foods included.

Mean AFT dietary exposures in developed countries are generally less than 1 ng/kg bw per day, even at high exposure percentiles (e.g. 95th). Dietary exposure estimates for AFT for some sub-Saharan African countries exceed 100 ng/kg bw per day. However, it should be noted that these estimates are often based on very minimal data. AFB_1 dietary exposure estimates also indicate differences between developed and developing countries, with dietary exposures in developed countries usually less than 1 ng/kg bw per day, even at high exposure percentiles (e.g. 95th). Mean estimated AFB_1 dietary exposures in developing countries range from less than 0.1 ng/kg bw per day to approximately 49 ng/kg bw per day, with dietary exposure in sub-Saharan African countries reported to be as high as 400 ng/kg bw per day.

Estimates of dietary exposure to AFM_1 rarely exceeded 1 ng/kg bw per day in any country. The exceptions were studies in Serbia and Egypt, which estimated dietary exposures up to 6.5 and 8.8 ng/kg bw per day for young children (1–5 years) and breastfed infants, respectively (El-Tras et al., 2011; Kos et al., 2014).

The Committee prepared additional national estimates of dietary exposure based on food consumption information from CIFOCOss and aflatoxin concentration data from the GEMS/Food contaminants database. Additional national estimates of dietary exposure were determined only for countries for which no national estimates of dietary exposure have been published since the previous assessment by the Committee. All mean estimates of dietary exposure to AFT or AFB_1 were less than 10 ng/kg bw per day at the upper bound, with most less than 5 ng/kg bw per day. Estimates of dietary exposure to AFM_1 were mostly less than 0.5 ng/kg bw per day, except for estimates for Bulgaria (infants, toddlers and other children), with upper-bound dietary exposure estimates up to 2.5 ng/kg bw per day.

The Committee prepared updated international estimates using the food consumption from the GEMS/Food cluster diets. Individual data points on the concentration of the contaminant (AFT, AFB_1 and AFM_1) in foods from each cluster were pooled to derive summary representative concentrations for each cluster for use in the dietary exposure calculations. For each commodity, when concentration data were not available for a cluster, the global total lower-bound and upper-bound means, obtained by pooling the data across all clusters, were used to assess exposure. It should be noted that no data were available from clusters G01–G04, G12, G14 or G16, including mainly African, Middle Eastern and Central Asian countries and island states from the Pacific and Indian oceans and the Caribbean. Cluster G05 (mainly South and Central American countries) provided data only on peanuts, cluster G13 (mainly African countries) provided data only on rice and sorghum, and cluster G06 (mainly Middle Eastern countries) provided very limited data on pistachios and dairy products. A standard body weight of 60 kg was used to assess exposure per kilogram body weight. Exposures estimated are lower- and upper-bound mean exposures expressed in nanograms per kilogram body weight per day and are representative of chronic dietary exposure. Estimates of dietary exposure for a high consumer were derived as twice the mean dietary exposure. It has been suggested that this is a good approximation to the 90th percentile of dietary exposure (FAO/WHO, 2009).

For the upper-bound scenario, the estimated dietary exposure to AFT ranged from 1.3 ng/kg bw per day (cluster G08, including Austria, Germany, Poland and Spain) to 34.8 ng/kg bw per day (cluster G13, including African countries and Haiti). Estimated dietary exposure to AFB_1 ranged from 0.9 ng/kg bw per day (cluster G07, including European countries and Australia, Bermuda and Uruguay) to 13.5 ng/kg bw per day (cluster G13). Estimated dietary exposure to AFM_1 ranged from 0.02 ng/kg bw per day (cluster G03, including African countries and Paraguay, and cluster G14, including island nations in the Pacific and Indian oceans) to 0.56 ng/kg bw per day (cluster G10, including European and North American countries, New Zealand and the Republic of Korea). Similar patterns of exposure were seen under the lower-bound scenario. In the previous evaluation, dietary exposure to AFT and AFB_1 was primarily from consumption of maize and peanuts. However, the inclusion of data from a wider range of cereals in the current assessment has resulted in changes to the main contributing commodities. Rice was the main contributor to upper-bound dietary AFT exposure (range 34.5–80.3%) for clusters G05, G10, G12, G13, G14 and G17 (clusters mainly include countries from Central and South America, island nations, parts of sub-Saharan Africa and a range of developed countries), whereas wheat was the main contributor to upper-bound dietary AFT exposure (range 37.0–76.5%) for clusters G01, G02, G04, G06, G07, G08, G11 and G15 (clusters mainly include countries from North Africa, the Balkans, the Middle East, Central Asia,

Caribbean islands, Europe and various other developed countries). Maize was the main contributor to upper-bound dietary AFT exposure (range 44.7–47.6%) for the remaining three clusters (G03, G09 and G16, clusters mainly include countries from sub-Saharan Africa and East Asian countries). A slightly different pattern was seen for AFB_1, with rice being the main contributor to upper-bound dietary AFB_1 exposure (58.7%) for cluster G14 only (cluster includes mainly Pacific island nations), sorghum (range 37.3–58.7%) for clusters G13 and G16 (clusters include countries in sub-Saharan Africa) and maize (range 29.0–53.7%) for clusters G03, G05, G12 and G17 (clusters include countries from Central and South America, sub-Saharan Africa and some island nations). Wheat was the major contributor to upper-bound AFB_1 dietary exposure (range 33.8–80.2%) for the remaining 10 clusters. Cattle milk was the dominant contributor to upper-bound AFM_1 dietary exposure (range 51.8–99.0%) for all clusters except cluster G07 (cluster includes various developed countries), where a greater contribution came from cheese consumption (57.5% compared with 38.5% for cattle milk). The Committee noted that the international exposure estimates obtained in the present evaluation were higher than those of the previous evaluation, due to the inclusion of a greater range of data on aflatoxins in cereals.

With the exception of very high estimates of dietary exposure to AFT for some African countries (105–850 ng/kg bw per day), all national and international mean estimates of dietary AFT exposure were in the range <0.01–58 ng/kg bw per day, with high consumer (90th or 95th percentile) estimates in the range <0.01–200 ng/kg bw per day. For AFB_1, mean dietary exposure estimates were in the range <0.01–49 ng/kg bw per day, with high percentile estimates in the range <0.01–150 ng/kg bw per day. For AFM_1, mean dietary exposure estimates were in the range <0.001–8.8 ng/kg bw per day, with high percentile exposures in the range <0.001–5.0 ng/kg bw per day. It should be noted that these very wide ranges in estimates of dietary exposure are mainly due to the literature estimates of dietary exposure, with their diverse methodologies. Estimates of dietary exposure derived by the Committee (national and international) encompass a narrower, but still wide, range of estimates. Table 28 provides a summary of the range of exposure estimates derived from each of the three sources outlined above. Exposure estimates have been further separated into those pertaining to children and those pertaining to adults or the general population.

10.11 Impact assessment of implementation of Codex maximum levels in ready-to-eat peanuts

CCCF asked the Committee to consider the impact of establishing maximum levels for AFT in ready-to-eat peanuts. In order to evaluate the potential effect of these maximum levels on chronic dietary exposure, all occurrence data on total AFT

Table 28
Summary of the range of estimates of dietary exposure for AFT, AFB_1 and AFM_1 derived from the literature, CIFOCOss and GEMS/Food cluster diets

Aflatoxin/population group[a]/ estimate type	Range of estimated dietary exposures (ng/kg bw per day)	
	Mean	High percentile[b,c]
AFT		
Children		
National – literature	0.03–25	<0.01–120
National – CIFOCOss	0.5–9.6	1.0–19
Adults		
National – literature		
Sub-Saharan Africa	1.4–850	–
Other countries	<0.01–58	<0.01–200
National – CIFOCOss	0.04–5.0	0.1–11
International	0.3–35	0.7–70
AFB_1		
Children		
National – literature	<0.01–13	<0.01–17
National – CIFOCOss	<0.01–7.9	<0.01–20
Adults		
National – literature		
Sub-Saharan Africa	402	–
Other countries	<0.01–49	0.04–150
National – CIFOCOss	0.03–3.9	0.07–8.1
International	0.2–14	0.4–27
AFM_1		
Children		
National – literature	0.02–8.8	0.13–0.32
National – CIFOCOss	<0.001–2.5	<0.001–5.0
Adults		
National – literature		
Sub-Saharan Africa	0.005–1.4	0.05–0.80
Other countries	<0.001–0.14	<0.001–0.28
National – CIFOCOss	0.001–0.56	0.002–1.1
International	0.2–14	0.4–27

AFT, total aflatoxin; AFB_1: aflatoxin B_1; AFM_1: aflatoxin M_1; bw: body weight; CIFOCOss: Chronic Individual Food Consumption Database – Summary statistics; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme

[a] For the purpose of this summary table, "children" were taken to be any population group described as infants, toddlers or children. "Adults" were taken to be any population group described as adults, adolescents, elderly adults, very elderly adults or the general population.

[b] 90th or 95th percentile.

[c] In some cases, the maximum high-percentile exposure estimate may be less than the maximum mean exposure estimate. This is due to the fact that high percentile dietary exposure estimates were not determined in all studies.

for ready-to-eat peanuts were categorized into the groups for which a maximum level has been proposed (ML = 4, 8, 10 or 15 µg/kg), and resultant lower-bound

and upper-bound mean concentrations were calculated. It was further assumed that the mean concentration of AFB_1 in ready-to-eat peanuts would decrease with lower maximum levels in the same proportions as the decrease in the mean concentration of AFT. An international dietary exposure assessment for AFT and AFB_1 was performed based on these maximum levels. For this, all samples for which the concentration of AFT exceeded its maximum level were excluded from the calculation of the lower-bound and upper-bound mean concentrations. The percentages of rejected samples after implementation of the proposed maximum levels were determined by cluster and overall. A maximum level of 15 µg/kg for ready-to-eat peanuts resulted in 2.7–14.5% of samples being rejected (overall 9.7%), whereas a maximum level of 4 µg/kg resulted in 4.2–23.8% of samples being rejected (overall 19.8%). The highest rejection rates were for cluster G05 (the cluster includes mainly South and Central American countries, whereas the data used for the assessment were solely from Brazil).

The effect of the implementation of the proposed Codex maximum levels on chronic dietary exposure to AFT and AFB_1 was evaluated by means of the GEMS/Food cluster diets. For the upper-bound scenario, imposition of a maximum level of 15 µg/kg for ready-to-eat peanuts reduced chronic dietary exposure to AFT by a maximum of 20% (cluster G16, including sub-Saharan African countries). Imposing the strictest proposed maximum level of 4 µg/kg for ready-to-eat peanuts reduced chronic dietary exposure to AFT by a maximum of 21% compared with dietary exposure without imposition of any maximum level for ready-to-eat peanuts. The additional reduction in dietary exposure to AFT from the reduction in maximum level from 15 to 4 µg/kg was negligible for all clusters. The maximum impact on estimated AFB_1 dietary exposure from imposition of the strictest proposed maximum level (4 µg/kg) was a reduction of 13%.

Table 29 includes a summary of the impact of the various maximum levels considered on estimated AFT dietary exposure and ready-to-eat peanut rejection rates, at a global level.

10.12 Dose–response analysis

The current Committee confirmed liver cancer to be the critical end-point associated with dietary exposure to aflatoxins and used this end-point to characterize the health risk.

The Committee at the forty-ninth meeting of JECFA (Annex 1, reference *131*) evaluated a number of epidemiological studies that associated aflatoxin exposures with risks of liver cancer, from which was determined the potency for HBsAg+ and HBsAg– individuals by selecting the median potency estimate among a suite of competing models. However, the Committee at the forty-ninth

Table 29
Impact of different maximum levels for ready-to-eat peanuts on dietary AFT exposure estimates and ready-to-eat peanut rejection rates, at a global level

Maximum level	Mean AFT dietary exposure for all clusters (LB–UB, ng/kg bw per day)	Proportion of ready-to-eat peanuts rejected (%)
No ML	5.3–8.3	–
ML = 15 µg/kg	5.0–8.0	9.7
ML = 10 µg/kg	5.0–8.0	12.6
ML = 8 µg/kg	5.0–8.0	14.0
ML = 4 µg/kg	4.9–8.0	19.8

AFT: total aflatoxins; bw: body weight; LB: lower bound; ML: maximum level; UB: upper bound

meeting of JECFA did not provide a complete uncertainty analysis of the Yeh at al. (1989) data describing the relative risks between HBsAg+ and HBsAg– populations or upper bounds to the potency estimate. Owing to the availability of advanced statistical methodology, the Committee at the current meeting reanalysed the data of Yeh et al. (1989), which is still considered the critical study, using a consistent methodological framework. As model uncertainty was a significant concern, a Bayesian model-averaged estimate of the potency (Hoeting et al., 1999) was computed.

Model averaging is a Bayesian technique that determines the posterior potency estimate from observed data and prior information. The prior is a probability model that assigns weights to the importance of each model based upon considerations made before modelling the data, and the posterior is also a probability model that assigns weights to the importance of each model given the data.

There is strong biological evidence to conclude that AFB_1 is a low-dose linear genotoxic carcinogen. For the prior probability model, the Committee determined prior weights for each model. From the low-dose linear argument (i.e. consistency with animal data) and the models' similarity to commonly used toxicological dose–response models, the linear model of Wu-Williams, Zeise & Thomas (1992) and multistage cancer model of Bowers et al. (1993) were given increased weight in comparison with the other models, totalling 80%. The other two models were given a total weight of 20%. Table 30 gives the potency estimates as well as the 95% upper bound on these estimates for all models, including the model average. The Committee decided to use the model-average estimate of 0.017 (0.049, UB) for HBsAg– individuals and 0.269 (0.562, UB) for HBsAg+ individuals for aflatoxin exposures of 1 ng/kg bw per day. The central estimates are virtually unchanged from the previous Committee's potency estimates of 0.01 for HBsAg– individuals and 0.3 for HBsAg+ individuals for aflatoxin exposure

Table 30
Estimated potency for 1 ng/kg bw per day (in 100 000 person-years) of aflatoxin exposure from different models applied, fitted to the data of Yeh et al. (1989)

Model	HBsAg status	Potency[a]	Prior weight	Posterior weight
Wu-Williams, Zeise & Thomas (1992)				
Multiplicative linear	–	0.005 (0.009)	0.10	0.09
	+	0.140 (0.326)		
Additive	–	0.029 (0.058)	0.40	0.47
	+	0.410 (0.615)		
Hoseyni (1992)				
Exponential multiplicative	–	0.002 (0.003)	0.10	0.30
	+	0.054 (0.124)		
Bowers et al. (1993)				
Multistage cancer	–	0.018 (0.029)	0.40	0.14
	+	0.350 (0.474)		
Model average	–	0.017 (0.049)		
	+	0.269 (0.562)		

bw: body weight; HBsAg: hepatitis B virus surface antigen; UB: upper bound
[a] 95% UB given in parentheses.

of 1 ng/kg bw per day. However, as that estimate did not include an estimate of the statistical uncertainty of the potency, the current Committee chose the upper-bound values of 0.049 and 0.562 for HBsAg– and HBsAg+ individuals, respectively, which reflect the statistical uncertainty of the estimate.

These human potency estimates were compared with potency estimates computed from a dose–response analysis based upon the animal study of Wogan, Paglialunga & Newberne (1974). For this analysis, the estimate was taken from the model-averaged estimate of the dose–response curve. As this dose–response curve has been found to be less prone to error when extrapolating to potencies as low as 1/1000 (Wheeler & Bailer, 2013), the dose associated with this potency was estimated and used to linearly extrapolate the potency of the dose associated with 1 ng/kg bw per day. Using a body weight to the ¾ power conversion factor between humans and rats, the estimated lifetime potency for AFB_1 in rats is estimated to correspond to 4.7 cases per 100 000 lifetimes in humans, with a 90% confidence interval of 1.3–74.9. Using the data of Yeh et al. (1989) and assuming a lifetime of 75 years, the potency is estimated to be 1.3 cases per 100 000 lifetimes, with an upper-bound estimate of 3.6.

11. Evaluation

The Committee reaffirmed the conclusions of the forty-ninth meeting of JECFA that aflatoxins are among the most potent mutagenic and carcinogenic substances known, based on studies in test species and human epidemiological studies, and that HBV infection is a critical contributor to the potency of aflatoxins in inducing liver cancer. The more recent information about human polymorphisms in metabolizing enzymes (e.g. CYPs, sulfotransferases) has described population variability in the balance between activation and detoxification processes for aflatoxins. This knowledge has been used in conjunction with biomarkers to evaluate the effectiveness of pharmacological and dietary interventions with the aim of reducing cancer risk (Kensler et al., 2011).

Increased reporting and identification of acute aflatoxicosis outbreaks, particularly in areas of Africa, led this Committee to consider the available data on acute exposure. Indeed, loss of lives attributed to aflatoxins was most recently reported in the United Republic of Tanzania during the summer of 2016. Ranges of AFB_1 exposures between 20 and 120 µg/kg bw per day for a period of 1–3 weeks (Wild & Gong, 2010) or consumption of staple food containing concentrations of 1 mg/kg or higher (e.g. Serck-Hanssen, 1970) would be suspected to cause acute aflatoxicosis and possibly death. The Committee did not assess acute dietary exposure, but noted that the estimates of chronic dietary exposure are at least 2–5 orders of magnitude lower than the doses associated with acute effects.

Since the forty-ninth meeting of the Committee, epidemiological data have become available to support the hypothesis that aflatoxin exposure in utero and during early life has negative effects on growth; in particular, decreased height is the most frequently associated anthropometric parameter. The available data did not provide evidence for an exposure level at which there is a significant risk for growth faltering.

The Committee considered that the development of analytical technologies based on aptamers may have relevance in remote areas, because of their inherent stability and ease of production and use.

The Committee noted that there were limited contamination data from developing countries, which hindered a more comprehensive and global evaluation of aflatoxin occurrence and may have resulted in an underestimate of dietary exposure in these countries.

Only five food commodities (maize, peanuts, rice, sorghum and wheat) each contribute more than 10% to international dietary exposure estimates for more than one GEMS/Food cluster diet, for either AFT or AFB_1. The Committee noted that international dietary exposure estimates (AFT and AFB_1) were generally higher than those reported at the sixty-eighth meeting (Annex 1, reference *187*). This was predominantly due to the availability of concentration

data for rice, sorghum and wheat and their inclusion in the international dietary exposure estimates. Although overall concentrations of aflatoxins in rice and wheat are lower than concentrations in maize and groundnuts (a traditional focus for aflatoxin risk management), the high consumption of rice and wheat in some countries means that these cereals may account for up to 80% of dietary aflatoxin exposure for those GEMS/Food cluster diets. Mean AFB_1 concentrations in sorghum from the GEMS/Food contaminants database are higher than those for maize; combined with high consumption levels of sorghum in some GEMS/Food clusters, this cereal contributes 16–59% of dietary exposure in six GEMS/Food clusters. The database on sorghum is considerably more limited than that on maize.

The Committee estimated the cancer potency per 100 000 population for exposure to AFB_1 at 1 ng/kg bw per day. The resulting central estimates are 0.01 additional cancer cases per 100 000 for HBsAg– populations and 0.3 additional cancer cases per 100 000 for HBsAg+ populations. Upper-bound estimates are 0.049 additional cancer cases per 100 000 for HBsAg– populations and 0.562 additional cancer cases per 100 000 for HBsAg+ populations.

The Committee calculated global aflatoxin-related hepatocellular carcinoma risk based on the new central and upper-bound cancer potency estimates from the current dose–response analysis and international dietary exposure estimates described above. Aflatoxin-related cancer rates were calculated, accounting for prevalence of chronic HBsAg positivity (Schweitzer et al., 2015), by GEMS/Food cluster. The low end of the range refers to LB estimates at the mean dietary AFB_1 exposure, minimum HBsAg+ rates for countries in the cluster and the central potency estimate. The high end of the range refers to upper-bound estimates at the 90th percentile of dietary AFB_1 exposure, maximum HBsAg+ rates for countries in the cluster and upper-bound estimates of cancer potency. The lowest cancer risks were estimated for clusters G07 and G08 (European and other developed countries), with cancer risk estimates in the range of <0.01–0.10 aflatoxin-induced cancers per year per 100 000 population, with wheat being the major contributing food commodity. For countries within these clusters, HBsAg+ rates were in the range of 0.01–1.2%. The highest cancer risks were for cluster G13 (sub-Saharan African countries and Haiti), with cancer risk estimates in the range of 0.21–3.94 aflatoxin-induced cancers per year per 100 000 population, with sorghum and maize being the major contributing food commodities. For countries within this cluster, HBsAg+ rates were in the range of 5.2–19%. Other clusters with relatively high cancer risks were G03 (sub-Saharan African countries and Paraguay, with maize and sorghum being the major contributing food commodities), G05 (mainly Central and South American countries, with maize, rice, sorghum and wheat being the major contributing food commodities) and G16 (sub-Saharan African countries, with maize and

sorghum being the major contributing food commodities). The Committee noted that the aflatoxin-related hepatocellular carcinoma risk rates calculated here are within the range of aflatoxin-related foodborne disease (hepatocellular carcinoma) incidences published by WHO.

The Committee noted that a common background cancer rate was used in the cancer potency estimates. A sensitivity analysis showed that changing the background cancer rates has minimal impact on the analysis.

Given the relative cancer potencies and international dietary exposure estimates for AFB_1 and AFM_1, AFM_1 will generally make a negligible (<1%) contribution to aflatoxin-induced cancer risk for the general population.

The Committee concluded that enforcing a maximum limit of 10, 8 or 4 μg/kg for ready-to-eat peanuts would have little further impact on dietary exposure to AFT for the general population, compared with setting a maximum limit of 15 μg/kg. At a maximum limit of 4 μg/kg, the proportion of the world market of ready-to-eat peanuts rejected would be approximately double the proportion rejected at a maximum limit of 15 μg/kg (about 20% versus 10%).

11.1 Recommendations

The Committee recommends that efforts continue to reduce aflatoxin exposure using valid intervention strategies, including the development of effective, sustainable and universally applicable preharvest prevention strategies (e.g. Wild, Miller & Groopman, 2015).

Based on their contribution to dietary aflatoxin exposure in some areas of the world, rice, wheat and sorghum need to be considered in future risk management activities for aflatoxins.

The Committee recommends further research and efforts to alleviate stunting taking aflatoxin exposure into consideration as a possible contributing factor.

The Committee recommends that if additional epidemiological studies are conducted, they should be prospective studies and performed in a high-exposure area (e.g. in Africa).

The Committee advises the development of surveillance programmes for regions for which currently little information on occurrence of aflatoxins exists, carefully considering the impact of these programmes on food security.

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Appendix 1

Table A1-1
Aflatoxin B_1 levels in cereals from FAO scoping review of open literature

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Barley	Croatia	Barley	ELISA	147	6.1	–	–	1.54	–	–	3.22	–	Pleadin et al. (2015)
	Cyprus	Barley	HPLC	3	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Italy, Morocco, Tunisia, Spain	Barley-based products	LC-MS/MS	4	25	–	0.25	–	–	–	24	–	Serrano et al. (2012)
	Malaysia	Barley	ELISA	15	6.7	0.02	–	–	0.58	–	–	–	Reddy & Salleh (2010)
	Republic of Korea	Barley	ELISA	134	0	0.05	0.15	–	–	–	–	–	Ok et al. (2007)
	Spain	Barley	HPLC-FD	105	12.4	0.01	0.035	–	0.1	–	0.61	–	Mateo et al. (2011)
	Spain	Barley	RRLC-FD	123	100	–	–	–	–	–	0.34	0.12	Ibáñez-Vea et al. (2012)
Buckwheat	Japan	Buckwheat flour	HPLC-FD	28	7.1	0.001–0.1	0.005–0.2	–	–	–	0.81	–	Sugita-Konishi et al. (2010)
	Japan	Buckwheat flour	HPLC-FD	28	7.1	0.001–0.1	0.005–0.2	–	–	–	0.99	–	Sugita-Konishi et al. (2010)
Maize	Brazil – Nova Odessa	Corn	TLC-FD	50	2	–	2	0.68	–	–	–	–	Rocha et al. (2009)
	Brazil – Olivera dos Campinhos	Corn	TLC-FD	50	8	–	2	2.05	–	–	–	–	Rocha et al. (2009)
	Brazil – Santa Maria	Corn	TLC-FD	50	14	–	2	50.5	–	–	–	–	Rocha et al. (2009)
	Brazil – Varzea Grande	Corn	TLC-FD	50	18	–	2	27.7	–	–	–	–	Rocha et al. (2009)
	Burkina Faso	Maize	HPLC-MS/MS	26	50	3	–	–	–	–	636	23.6	Warth et al. (2012)
	China	Corn	HPLC-FD	43	100	0.05	–	–	–	–	136	–	Lunn et al. (1977)
	China	Corn	HPLC-FD	34	100	0.05	–	–	–	–	50	–	Lunn et al. (1977)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Maize	China	Corn	HPLC-FD	31	100	0.05	–	–	–	–	2.2	–	Lunn et al. (1977)
	Democratic Republic of the Congo + United Republic of Tanzania	Maize (yellow)	ELISA	3	–	0.001	–	0.46	–	–	10.81	–	Manjula et al. (2009)
	Democratic Republic of the Congo + United Republic of Tanzania	Maize (white)	ELISA	4	–	0.001	–	0.55	–	–	10.81	–	Manjula et al. (2009)
	Democratic Republic of the Congo + United Republic of Tanzania	Maize (mixed)	ELISA	4	–	0.001	–	1.36	–	–	120.1	–	Manjula et al. (2009)
	Democratic Republic of the Congo + United Republic of Tanzania	Maize	ELISA	2	–	0.001	–	1.07	–	–	71.67	–	Manjula et al. (2009)
	Croatia	Maize	ELISA	972	31.4	1	1.5	38.46	–	–	2 072	–	Pleadin et al. (2015)
	Cyprus	Corn	HPLC	18	72.2	0.1	0.33	–	6.6	–	14.9	–	Christofidou et al. (2015)
	Egypt	Snacks (corn-based)	HPLC	24	70.8	–	–	–	4.43	–	15.83	–	El-Sawi & El-Sawi (2012)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	3 001	–	–	–	Akowuah et al. (2015)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	653.9	–	–	–	Akowuah et al. (2015)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	91.3	–	–	–	Akowuah et al. (2015)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	25.35	–	–	–	Akowuah et al. (2015)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	20.26	–	–	–	Akowuah et al. (2015)
	Ghana	Maize	HPLC-FD	1	–	0.8	–	–	18.77	–	–	–	Akowuah et al. (2015)

Table A1-1 (continued)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Maize	Iran (Islamic Republic of)	Maize	HPLC	15	–	0.1	–	–	2.61	–	–	–	Ghiasian, Shephard & Yazdanpanah (2011)
	Iran (Islamic Republic of)	Maize	HPLC	15	–	0.1	–	–	0.31	–	–	–	Ghiasian, Shephard & Yazdanpanah (2011)
	Iran (Islamic Republic of)	Maize	HPLC	15	–	0.1	–	–	–	–	–	–	Ghiasian, Shephard & Yazdanpanah (2011)
	Iran (Islamic Republic of)	Maize	HPLC	15	–	0.1	–	–	–	–	–	–	Ghiasian, Shephard & Yazdanpanah (2011)
	Iraq	Corn	HPLC-FD	15	100	–	–	–	–	–	260	–	Thalij, Hajeej & Mohammed (2015)
	Italy	Conventional corn flour	HPLC-FD	42	21.4	0.15	0.5	–	0.85	–	3.75	0.29	Armorini et al. (2015)
	Italy	Corn flour (conventional + organic)	HPLC-FD	50	26	0.15	0.5	–	0.72	–	3.75	0.29	Armorini et al. (2015)
	Italy	Organic corn flour	HPLC-FD	8	50	0.15	0.5	–	0.42	–	0.64	0.26	Armorini et al. (2015)
	Japan	Corn grits	HPLC-FD	30	6.7	0.001–0.1	0.005–0.2	–	–	–	0.21	–	Sugita-Konishi et al. (2010)
	Japan	Corn grits	HPLC-FD	30	6.7	0.001–0.1	0.005–0.2	–	–	–	0.21	–	Sugita-Konishi et al. (2010)
	Malawi	Maize	ELISA	106	45.3	0.1		–	–	–	16.9	–	Matumba et al. (2009)
	Malaysia	Corn-based foods	ELISA	8	75	0.2–0.5	0.35–0.75	–	3.86	–	8.95	–	Reddy, Farhana & Salleh (2011)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Maize	Mexico	Maize (tortilla)	HPLC	396	9.8	0.5	1.4	12.10	–	–	–	–	Castillo-Urueta et al. (2011)
	Mozambique	Maize	HPLC-MS/MS	13	46.2	3	–	–	–	–	363	69.9	Warth et al. (2012)
	Nigeria	Maize	HPLC-FD	3	100	0.1	0.5	–	0.16	–	0.2	–	Adejumo et al. (2013)
	Nigeria	Maize	HPLC-MS/MS	70	67.1	0.4	–	394.00	–	–	6 738	74	Adetunji et al. (2014)
	Pakistan	Maize	HPTLC	65	27.7	–	1	–	192	–	850	–	Khatoon et al. (2012)
	Republic of Korea	Maize, dry	ELISA	50	4	0.05	0.15	–	24.79	–	48.61	–	Ok et al. (2007)
	Republic of Korea	Sweet corn	ELISA	7	0	0.05	0.15	–	–	–	–	–	Ok et al. (2007)
	Tunisia	Maize	HPLC	17	5.8	0.05	–	–	–	–	0.42	–	Ghali et al. (2010)
	Turkey	Maize flour	HPLC-FD	24	66.7	0.026	0.086	–	0.261	–	1.12	0.11	Kara, Ozbey & Kabak (2015)
	United Republic of Tanzania	Maize	HPLC	120	12	0.06	–	–	–	–	90	38	Kimanya et al. (2008)
Oats	Croatia	Oat	ELISA	136	5.1	–	–	1.18	–	–	1.55	–	Pleadin et al. (2015)
	Ecuador	Oat (flakes)	UHPLC	12	0	1	–	–	–	–	–	–	Ortiz et al. (2013)
	Ecuador	Oat (flakes)	UHPLC	30	3.3	1	–	–	–	–	2.7	–	Ortiz et al. (2013)
	Malaysia	Oat-based foods	ELISA	10	50	0.2–0.5	0.35–0.75	–	1.25	–	2.85	–	Reddy, Farhana & Salleh (2011)
Rice	Austria	Rice	HPLC-FD	81	29.6	0.1	–	–	–	–	9.86	–	Reiter et al. (2010)
	Brazil	Rice (parboiled)	HPLC-FD	32	9	2.6	–	–	46	–	74	–	Dors, Bierhals & Badiale-Furlong (2011)
	Brazil	Rice (husk)	HPLC-FD	27	96.3	0.06	–	6.09	–	–	–	3.79	Almeida et al. (2012)
	Brazil	Rice	HPLC-FD	166	40.4	0.06	–	9.09	–	–	–	2.49	Almeida et al. (2012)

Table A1-1 (continued)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Rice	Brazil	Rice (broken)	HPLC-FD	18	94.4	0.11	–	5.60	–	–	1 707	4.2	Almeida et al. (2012)
	Brazil	Rice (bran)	HPLC-FD	19	89.5	0.11	–	38.65	–	–	180.7	18.27	Almeida et al. (2012)
	Canada	Rice	HPLC-FD	199	49.7	0.002	–	–	0.36	–	–	–	Bansal et al. (2011)
	China	Rice	HPLC-FD	370	63.5	0.009	0.03	–	0.6	–	20	–	Lai et al. (2014)
	China	Rice	HPLC-FD	9	100	0.05	–	–	–	–	1.2	–	Lunn et al. (1977)
	China	Rice	HPLC-FD	10	100	0.05	–	–	–	–	1.4	–	Lunn et al. (1977)
	China	Rice	HPLC-FD	10	100	0.05	–	–	–	–	0.2–0.7	–	Lunn et al. (1977)
	Cyprus	Rice	HPLC	38	34.2	0.1	0.33	–	1.1	–	2.6	–	Christofidou et al. (2015)
	Ecuador	Rice (paddy)	UHPLC	20	15	1	–	20.60	–	–	47.4	–	Ortiz et al. (2013)
	Ecuador	Rice (paddy)	UHPLC	23	0	1	–	–	–	–	–	–	Ortiz et al. (2013)
	Iran (Islamic Republic of)	Rice	HPLC-MS/MS	65	21.5	–	0.3	–	3.9	–	30.83	–	Nazari et al. (2014)
	Iran (Islamic Republic of)	Rice	HPLC-FD	18	50	0.01	0.03	–	4.17	–	30.63	1.17	Yazdanpanah et al. (2013)
	Iraq	Rice	HPLC-FD	15	100	–	–	–	–	–	213	–	Thalij, Hajeej & Mohammed (2015)
	Italy, Morocco, Tunisia, Spain	Rice grain	LC-MS/MS	100	2	–	0.25	–	–	–	33	–	Serrano et al. (2012)
	Japan	Rice (cooked)	HPLC-FD	NA	0	0.1	–	–	–	–	–	–	Sakuma et al. (2013)
	Lebanon	Rice and rice-based products	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Malaysia	Rice-based foods	ELISA	13	69.2	0.2–0.5	0.35–0.75	–	1.75	–	3.79	–	Reddy, Farhana & Salleh (2011)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Rice	Malaysia	Rice (red)	ELISA	50	92	–	–	14.72	–	–	–	–	Samsudin & Abdullah (2013)
	Nigeria	Rice	HPLC-FD	21	90.5	0.1	0.5	–	0.14	–	0.3	–	Adejumo et al. (2013)
	Nigeria	Rice	HPLC-FD	21	100	0.02	–	–	–	–	309	–	Makun et al. (2011)
	Pakistan	Brown rice	TLC UV	262	95.4	1	–	–	3.8	–	24.65	–	Asghar et al. (2014)
	Pakistan	Rice (basmati)	HPLC-MS/MS	1	100	0.1	0.2	–	3.2	–	–	–	Lim et al. (2015)
	Pakistan	Rice (basmati)	HPLC-MS/MS	1	100	0.1	0.2	–	2	–	–	–	Lim et al. (2015)
	Pakistan	Rice (basmati)	HPLC-MS/MS	1	100	0.1	0.2	–	1.7	–	–	–	Lim et al. (2015)
	Pakistan	Rice (parboiled/sella)	HPLC-FD	119	58.8	0.1	–	–	1.25	–	–	–	Firdous et al. (2012)
	Pakistan	Rice (white)	HPLC-FD	200	40	0.1	–	–	1.22	–	–	–	Firdous et al. (2012)
	Pakistan	Rice (basmati)	HPLC-MS/MS	1	100	0.1	0.2	–	1.1	–	–	–	Lim et al. (2015)
	Pakistan	Rice (brown)	HPLC-FD	200	52.5	0.1	–	–	1.08	–	–	–	Firdous et al. (2012)
	Pakistan	Rice (broken)	ELISA	33	39.4	–	–	–	–	–	–	–	Nisa, Zahra & Hina (2014)
	Pakistan	Rice (parboiled)	ELISA	52	26.9	–	–	–	–	–	–	–	Nisa, Zahra & Hina (2014)
	Pakistan	Rice (brown)	ELISA	1 029	34	–	–	–	–	–	–	–	Nisa, Zahra & Hina (2014)
	Pakistan	Rice (white)	ELISA	1 561	21.8	–	–	–	–	–	–	–	Nisa, Zahra & Hina (2014)
	Republic of Korea	Rice	ELISA	134	0	0.05	0.15	–	–	–	–	–	Ok et al. (2007)
	Tunisia	Rice	HPLC	11	0	0.05	–	–	–	–	–	–	Ghali et al. (2010)
	Turkey	Rice	ELISA	100	35	1	–	–	1.12	–	3.02	–	Buyukunal, Kahraman & Ciftcioglu (2010)
	Turkey	Rice flour	HPLC-FD	16	0	0.026	0.086	–	–	–	0.029	0	Kara, Ozbey & Kabak (2015)

Table A1-1 (continued)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Rice	Viet Nam	Rice	HPLC-FD	100	51	0.07	0.22	–	3.31	–	29.9	–	Nguyen et al. (2007)
Sorghum	Italy, Morocco, Tunisia, Spain	Sorghum-based products	LC-MS/MS	4	25	–	0.25	–	–	–	6.4	–	Serrano et al. (2012)
	Tunisia	Sorghum	LC-MS/MS	60	38.3	0.03	0.1	–	1.49	–	8.23	–	Oueslati et al. (2014)
	Tunisia	Sorghum	HPLC	49	73.4	0.05	–	–	–	–	25.1	–	Ghali et al. (2010)
Wheat	China	Wheat flour	HPLC-MS/MS	348	0.28	0.05	–	–	7.3	–	–	–	Liu et al. (2015)
	Croatia	Wheat	ELISA	201	7.5	1.1	1.7	1.65	–	–	5.41	–	Pleadin et al. (2015)
	Cyprus	Wheat	HPLC	30	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Iran (Islamic Republic of)	Bread	HPLC-FD	18	0	0.01	0.03	–	–	–	–	–	Yazdanpanah et al. (2013)
	Iran (Islamic Republic of)	Wheat flour	HPLC-FD	18	0	0.01	0.03	–	–	–	–	–	Yazdanpanah et al. (2013)
	Iraq	Wheat	HPLC-FD	15	100	–	–	–	–	–	254	–	Thalij, Hajeej & Mohammed (2015)
	Italy	Pasta	HPLC-MS/MS	27	0	0.1	–	–	–	–	–	–	Raiola et al. (2012)
	Italy	Wheat flour (conventional + organic)	HPLC-FD	40	0	0.15	0.5	–	–	–	–	–	Armorini et al. (2015)
	Italy	Flour (gluten free)	UPLC-MS/MS	47	0	–	0.88	–	–	–	–	–	Brera et al. (2014)
	Italy	Bread (gluten free)	UPLC-MS/MS	87	0	–	0.88	–	–	–	–	–	Brera et al. (2014)
	Italy	Snack (gluten free)	UPLC-MS/MS	109	0	–	0.88	–	–	–	–	–	Brera et al. (2014)
	Italy	Pasta (gluten free)	UPLC-MS/MS	133	0	–	0.88	–	–	–	–	–	Brera et al. (2014)
	Italy, Morocco, Tunisia, Spain	Wheat-based products	LC-MS/MS	65	15.4	–	0.25	–	–	–	66.7	–	Serrano et al. (2012)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occur-rence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Wheat	Kenya	Wheat	ELISA	23	52	–	–	–	–	–	7	–	Muthomi et al. (2008)
	Kenya	Wheat	ELISA	48	41	–	–	–	–	–	7	–	Muthomi et al. (2008)
	Lebanon	Bread and toast	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Lebanon	Cakes and pastries	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Lebanon	Pizza and pies	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Lebanon	Biscuits and croissants	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Lebanon	Pasta and other cereal products	HPLC-FD	47	–	0.01	0.03	–	–	–	–	–	Raad et al. (2014)
	Malaysia	Wheat-based foods	ELISA	14	64.3	0.2–0.5	0.35–0.75	–	1.6	–	5.07	–	Reddy, Farhana & Salleh (2011)
	Malaysia	Wheat	ELISA	15	20	0.02	–	–	–	–	1.89	–	Reddy & Salleh (2010)
	Nigeria	Wheat meal	HPLC-FD	3	33.3	0.1	0.5	–	0.04	–	0.06	–	Adejumo et al. (2013)
	Pakistan	Wheat (white powder)	HPLC-FD	53	53	0.04	0.12	–	–	–	45.56	–	Iqbal et al. (2014)
	Pakistan	Pasta (spaghetti)	HPLC-FD	25	36	0.04	0.12	–	–	–	39.4	–	Iqbal et al. (2014)
	Pakistan	Pasta (Bucovina)	HPLC-FD	22	36	0.04	0.12	–	–	–	41.5	–	Iqbal et al. (2014)
	Pakistan	Pasta (lasagna)	HPLC-FD	37	24	0.04	0.12	–	–	–	49.2	–	Iqbal et al. (2014)
	Pakistan	Pasta (noodles)	HPLC-FD	34	24	0.04	0.12	–	–	–	47.1	–	Iqbal et al. (2014)
	Pakistan	Pasta (macaroni)	HPLC-FD	29	34	0.04	0.12	–	–	–	33.4	–	Iqbal et al. (2014)
	Republic of Korea	Wheat flour	ELISA	7	0	0.05	0.15	–	–	–	–	–	Ok et al. (2007)
	Republic of Korea	Corn flakes	ELISA	92	0	0.05	0.15	–	–	–	–	–	Ok et al. (2007)
	Tunisia	Wheat	HPLC	46	8.7	0.05	–	–	–	–	25.1	–	Ghali et al. (2010)
	Turkey	Wheat flour	HPLC-FD	60	0	0.026	0.086	–	–	–	0.044	0	Kara, Ozbey & Kabak (2015)

Table A1-1 (continued)

Cereal	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Other	Cyprus	Breakfast cereals	HPLC	3	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Greece	Breakfast cereals	HPLC-FD	55	56.3	0.07	–	–	1.42	–	4.3	0.05	Villa & Markaki (2009)
	Japan	Job's tears	HPLC-FD	17	35.3	0.001–0.1	0.005–0.2	–	–	–	9.71	–	Sugita-Konishi et al. (2010)
	Japan	Job's tears	HPLC-FD	17	35.3	0.001–0.1	0.005–0.2	–	–	–	9	–	Sugita-Konishi et al. (2010)
	Malaysia	Bakery products (with nuts)	HPLC-MS/MS	14	71.4	0.15	0.4	–	12	–	96.2	–	Leong et al. (2011)

ELISA: enzyme-linked immunosorbent assay; FD: fluorescence detection; HPLC: high-performance liquid chromatography; HPTLC: high-performance thin-layer chromatography; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; Med.: median; MS/MS: tandem mass spectrometry; No.: number; RRLC: rapid resolution liquid chromatography; TLC: thin-layer chromatography; UHPLC: ultra-high performance liquid chromatography; UV: ultraviolet detection

[a] Percentage of samples above the LOD/LOQ.

[b] Calculation not clearly defined.

[c] Mean of positive samples.

[d] Mean of all samples. Mean is calculated as lower bound with nondetect values taken as zero.

Table A1-2
Aflatoxin B_1 levels in nuts from FAO scoping review of open literature

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Almonds	Cyprus	Almonds	HPLC	50	10	0.1	0.33	–	32.9	–	144.2	–	Christofidou et al. (2015)
	Iran (Islamic Republic of)	Almonds	–	25	–	–	–	1.7	–	–	3.8	–	Rezaei et al. (2014a)
	Japan	Almonds	HPLC-FD	24	25	0.001–0.1	0.005–0.2	–	–	0	1.06	–	Sugita-Konishi et al. (2010)
	Japan	Almonds	HPLC-FD	24	25	0.001–0.1	0.005–0.2	–	–	0.09	0.89	–	Sugita-Konishi et al. (2010)
	Malaysia	Almonds (roasted)	HPLC-MS/MS	4	25	0.15	0.4	–	0.63	–	0.87	–	Leong et al. (2010)
	Republic of Korea	Almonds	ELISA	15	20	0.05	0.15	–	0.23	–	0.55	–	Ok et al. (2007)
Brazil nuts	Cyprus	Brazil nuts	HPLC	6	16.7	0.1	0.33	–	3	–	3	–	Christofidou et al. (2015)
	Malaysia	Brazil nuts	HPLC-MS/MS	3	66.6	0.15	0.4	–	0.88	–	0.9	–	Leong et al. (2011)
Cashew nuts	Cyprus	Cashew nuts	HPLC	14	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Malaysia	Cashew nut (roasted)	HPLC-MS/MS	5	40	0.15	0.4	–	1.01	–	1.02	–	Reddy, Farhana & Salleh (2011)
Chestnuts	Cyprus	Chestnuts	HPLC	19	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Italy	Chestnuts (fresh)	HPLC-MS/MS	32	15.6	0.04	0.1	0.07	–	–	1.7	–	Bertuzzi, Rastelli & Pietri (2015)
	Italy	Chestnuts (dried)	HPLC-MS/MS	25	40	0.04	0.1	0.25	–	–	2.18	–	Bertuzzi, Rastelli & Pietri (2015)
	Italy	Chestnuts (dried)	HPLC-MS/MS	14	21.4	0.04	0.1	0.47	–	–	5.25	–	Pietri et al. (2012)
	Italy	Chestnuts (flour)	HPLC-MS/MS	25	92	0.04	0.1	4.73	–	–	58.6	0.98	Bertuzzi, Rastelli & Pietri (2015)
	Italy	Chestnuts (flour)	HPLC-MS/MS	37	62.2	0.04	0.1	4.99	–	–	67.88	0.48	Pietri et al. (2012)

Table A1-2 (continued)

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Hazelnuts	Iran (Islamic Republic of)	Hazelnut	–	25	–	–	–	1.6	–	–	3.9	–	Rezaei et al. (2014a)
	Turkey	Hazelnut	HPLC-FD	64	60.9	0.093	0.309	–	12	7.36	55.9	1.45	Golge, Hepsag & Kabak (2016)
Groundnuts / peanuts	Brazil	Peanut candy	HPLC-FD	27	44.4	–	–	5.6	–	–	–	–	Magrine et al. (2011)
	Brazil	Peanut sweet	HPLC-FD	45	53.3	–	–	9.0	–	–	–	–	Magrine et al. (2011)
	Brazil	Peanut (salted)	HPLC-FD	10	50	–	–	2.6	–	–	19.2	–	Magrine et al. (2011)
	Burkina Faso	Groundnuts	HPLC-MS/MS	9	22.2	3	–	–	–	–	15.5	10.5	Warth et al. (2012)
	Cameroon	Groundnut soup	HPLC-MS/MS	15	73	0.14	–	–	15	–	–	–	Abia et al. (2013)
	Cameroon	Groundnut	HPLC-MS/MS	35	97	0.13	–	–	47	–	–	–	Abia et al. (2013)
	China	Peanut	HPLC-FD	7	100	0.05	–	0.10	–	–	0.7	–	Lunn et al. (1977)
	China	Peanut	HPLC-FD	10	100	0.05	–	0.30	–	–	0.5	–	Lunn et al. (1977)
	China	Peanut butter	HPLC-FD	50	–	0.15	–	6.12	–	–	68.51	1.58	Li et al. (2009)
	China	Peanut	HPLC-FD	n.a.	–	0.05	–	–	–	–	–	–	Lunn et al. (1977)
	Cyprus	Peanuts	HPLC	135	38.5	0.1	0.33	–	3	–	49.3	–	Christofidou et al. (2015)
	Democratic Republic of the Congo	Peanuts (dry season)	TLC-FD	30	72	1	–	–	229.1	–	937	–	Kamika & Takoy (2011)
	Democratic Republic of the Congo	Peanuts (rainy season)	TLC-FD	30	90	1	–	–	205.7	–	937	–	Kamika & Takoy (2011)
	Iran (Islamic Republic of)	Groundnut (roast)	ELISA HPLC-FD	30	16.7	0.3	0.6	–	–	–	126	–	Leong et al. (2010)
	Iran (Islamic Republic of)	Peanut	HPLC-FD	18	77.8	0.01	0.03	1.97	–	1.54	9.95	0.95	Yazdanpanah et al. (2013)
	Japan	Peanut butter	HPLC-FD	21	47.6	–	0	1.07	–	–	2.59	–	Kumagai et al. (2008)

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Groundnuts / peanuts	Japan	Peanut butter	HPLC-FD	62	33.9	0.001–0.1	0.005–0.2	–	–	0.30	2.59	–	Sugita-Konishi et al. (2010)
	Japan	Peanut butter	HPLC-FD	62	33.9	0.001–0.1	0.005–0.2	–	–	0.45	3.92	–	Sugita-Konishi et al. (2010)
	Japan	Peanut butter	HPLC-FD	150	0.7	0.001–0.1	0.005–0.2	–	–	0.03	4.88	–	Sugita-Konishi et al. (2010)
	Japan	Peanut butter	HPLC-FD	150	0.7	0.001–0.1	0.005–0.2	–	–	0.19	28	–	Sugita-Konishi et al. (2010)
	Malaysia	Peanut (braised)	HPLC-MS/MS	2	50	0.15	0.4	–	0.68	–	0.96	–	Leong et al. (2011)
	Malaysia	Nuts	ELISA	7	28.6	0.2–0.5	0.35–0.75	–	0.87	–	1.09	–	Reddy, Farhana & Salleh (2011)
	Malaysia	Peanut (roasted, shelled)	HPLC-MS/MS	8	37.5	0.15	0.4	–	0.78	–	1.08	–	Leong et al. (2011)
	Malaysia	Peanut slice	HPLC-MS/MS	3	100	0.15	0.4	–	0.99	–	1.18	–	Leong et al. (2011)
	Malaysia	Peanut (pounded)	HPLC-MS/MS	19	75	0.15	0.4	–	5.2	–	44.1	–	Leong et al. (2011)
	Malaysia	Peanut sauce	HPLC-MS/MS	4	100	0.15	0.4	–	2.77	–	5.98	–	Leong et al. (2011)
	Malaysia	Peanut butter	HPLC-MS/MS	4	47.4	0.15	0.4	–	1.19	–	1.48	–	Leong et al. (2011)
	Malaysia	Peanut soup	HPLC-MS/MS	2	50	0.15	0.4	–	1	–	1.02	–	Leong et al. (2011)
	Malaysia	Peanut (roasted, in shell)	HPLC-MS/MS	8	25	0.15	0.4	–	1.01	–	1.02	–	Leong et al. (2011)
	Malaysia	Raw peanut kernels	HPLC-FD	84	75	0.03	0.1	–	–	9	92.07	2.99	Arzandeh, Selamat & Lioe (2010)
	Malaysia	Peanut (fried)	HPLC-MS/MS	5	60	0.15	0.4	–	58.9	–	222	–	Leong et al. (2011)
	Malaysia	Peanuts	ELISA	13	84.6	0.2–0.5	0.35–0.75	–	4.25	–	15.33	–	Reddy, Farhana & Salleh (2011)
	Malaysia	Peanut cake	HPLC-MS/MS	3	100	0.15	0.4	–	6.03	–	10.2	–	Leong et al. (2011)
	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	–	–	–	–	Afsah-Hejri, Jinap & Radu (2013)

Table A1-2 (continued)

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Groundnuts / peanuts	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	977.7	–	–	–	Afsah-Hejri, Jinap & Radu (2013)
	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	8.26	–	–	–	Afsah-Hejri, Jinap & Radu (2013)
	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	2.14	–	–	–	Afsah-Hejri, Jinap & Radu (2013)
	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	1.63	–	–	–	Afsah-Hejri, Jinap & Radu (2013)
	Malaysia	Peanuts	HPLC	10	–	0.05	0.15	–	1.32	–	–	–	Afsah-Hejri, Jinap & Radu (2013)
	Mozambique	Groundnuts	HPLC-MS/MS	23	13	3	–	–	–	–	297	109	Warth et al. (2012)
	Republic of Korea	Peanuts	ELISA	27	29.6	0.05	0.15	–	4.07	–	18.04	–	Ok et al. (2007)
	Republic of Korea	Peanut butter	ELISA	19	26.3	0.05	0.15	–	3.6	–	6.44	–	Ok et al. (2007)
	Zimbabwe	Peanut butter	HPLC-FD	11	90.9	–	–	51	–	–	191	–	Mupunga et al. (2014)
Pistachios	Cyprus	Pistachio	HPLC	105	33.3	0.1	0.33	–	8.2	–	123.3	–	Christofidou et al. (2015)
	Iran (Islamic Republic of)	Pistachio	–	40	–	–	–	1.5	–	–	5.8	–	Rezaei et al. (2014a)
	Iran (Islamic Republic of)	Pistachio nuts	HPLC-FD	100	95	0.1	0.8	185.9	–	–	–	0.63	Pour et al. (2010)
	Japan	Pistachio	HPLC-FD	5	20	0.001–0.1	0.005–0.2	–	–	0.08	0.38	–	Sugita-Konishi et al. (2010)
	Japan	Pistachio	HPLC-FD	5	20	0.001–0.1	0.005–0.2	–	–	0.08	0.38	–	Sugita-Konishi et al. (2010)
	Malaysia	Pistachio	HPLC-MS/MS	4	0	0.15	0.4	–	–	–	–	–	Leong et al. (2011)
	Republic of Korea	Pistachio	ELISA	15	20	0.05	0.15	–	16.22	–	38.66	–	Ok et al. (2007)
	Spain	Pistachio	HPLC-FD	31	6	–	–	–	0.55	–	0.7	–	Fernane et al. (2010)

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Pistachios	Spain	Pistachio	HPLC-FD	1	100	0.023	–	–	2.21	–	–	–	Fernane et al. (2010)
	Spain	Pistachio	HPLC-FD	1	100	0.023	–	–	1 037	–	–	–	Fernane et al. (2010)
	Spain	Pistachio	HPLC-FD	1	100	0.023	–	–	23.03	–	–	–	Fernane et al. (2010)
	Spain	Pistachio	HPLC-FD	1	100	0.023	–	–	4.82	–	–	–	Fernane et al. (2010)
	Spain	Pistachio	HPLC-FD	1	100	0.023	–	–	2.66	–	–	–	Fernane et al. (2010)
	Spain	Pistachios (bulk)	HPLC-FD	32	50	0.04	–	–	–	0.12	–	–	Ariño et al. (2009)
	Spain	Pistachios (prepacked)	HPLC-FD	32	19	0.04	–	–	–	0.07	–	–	Ariño et al. (2009)
	Spain	Pistachio	HPLC-FD	45	0	0.023	–	–	–	–	–	–	Fernane et al. (2010)
Walnuts	Cyprus	Walnut	HPLC	33	0	0.1	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Iran (Islamic Republic of)	Walnut	–	40	–	–	–	1.8	–	–	3.6	–	Rezaei et al. (2014a)
	Iran (Islamic Republic of)	Walnut	HPLC-FD	35	74.3	0.2	0.7	9	–	–	91.9	3.06	Imani Nejad & Farahani (2012)
	Republic of Korea	Walnut	ELISA	19	0	0.05	0.15	–	0	–	–	–	Ok et al. (2007)
	Turkey	Walnut	HPLC-FD	48	43.8	0.093	0.309	–	2.26	1.01	11.8	0.05	Golge, Hepsag & Kabak (2016)
	Turkey	Walnut (unshelled)	HPLC-FD	50	10	0.02	0.07	0.16	2.65	–	12.2	–	Kabak (2014)
	Turkey	Walnut (shelled)	HPLC-FD	35	8.6	0.02	0.07	0.01	0.16	–	0.2	–	Kabak (2014)
Other	Cameroon	Soybean	HPLC-MS/MS	10	100	0.13	–	–	2.6	–	1–3	–	Abia et al. (2013)
	China	Sesame paste	HPLC-FD	100	–	0.15	–	4.31	–	–	20.45	1.46	Li et al. (2009)
	Iran (Islamic Republic of)	Sunflower	–	40	–	–	–	1.3	–	–	5.2	–	Rezaei et al. (2014a)

Table A1-2 (continued)

Nut	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Other	Iran (Islamic Republic of)	Sesame seeds	TLC	182	18.1	0.45	1.42	–	1.62	–	–	–	Asadi, Beheshti & Feizy (2011)
	Lebanon	Nuts, seeds, olives and dried dates	HPLC-FD	47	–	0.01	0.03	–	–	0.18	–	–	Raad et al. (2014)
	Lebanon	Pulses	HPLC-FD	47	–	0.01	0.03	–	–	0.00	–	–	Raad et al. (2014)
	Malaysia	Sesame	ELISA	8	87.5	0.2–0.5	0.35–0.75	–	0.9	–	1.82	–	Leong et al. (2011)
	Malaysia	Sunflower	ELISA	7	85.7	0.2–0.5	0.35–0.75	–	2.55	–	5.33	–	Leong et al. (2011)
	Nigeria	Beans	HPLC-FD	17	88.2	0.1	0.5	–	0.15	–	0.89	–	Adejumo et al. (2013)
	Republic of Korea	Assorted nuts	ELISA	12	8.3	0.05	0.15	–	6.68	–	6.68	–	Ok et al. (2007)
	Republic of Korea	Pine nut	ELISA	12	0	0.05	0.15	–	0	–	–	–	Ok et al. (2007)
	Republic of Korea	Soybean	ELISA	7	0	0.05	0.15	–	0	–	–	–	Ok et al. (2007)
	Republic of Korea	Pine nut	ELISA	12	0	0.05	0.15	–	0	–	–	–	Ok et al. (2007)
	Republic of Korea	Soybean paste	ELISA	56	3.6	0.05	0.15	–	0.11	–	0.17	–	Ok et al. (2007)

ELISA: enzyme-linked immunosorbent assay; FD: fluorescence detection; HPLC: high-performance liquid chromatography; MS/MS: tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; Med.: median; n.a.: not applicable; No.: number; TLC: thin-layer chromatography

[a] Percentage of samples above the LOD/LOQ.

[b] Calculation not clearly defined.

[c] Mean of positive samples.

[d] Mean of all samples. Mean is calculated as lower bound with nondetect values taken as zero.

Table A1-3

Aflatoxin B_1 levels in foods other than dairy products, cereals and nuts from FAO scoping study of open literature

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Aniseed	China	Star anise	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
	China	Star anise	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
	China	Star anise	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
Cardamom	Pakistan	Cardamom	HPLC-FD	34	34	0.04	0.12	–	–	2.34	9.67	–	Iqbal, Asi & Jinap (2013a)
Chilli	Bahrain	Red chilli powder	HPLC-FD	11	–	–	0.50	27.6	–	–	–	–	Musaiger, Al-Jedah & D'Souza (2008)
	Malaysia	Chillies (dried)	HPLC-FD	80	65	0.02	0.06	–	–	3.37	56.61	1.84	Jalili & Jinap (2012)
	Malaysia	Chilli	ELISA	8	100	0.2–0.5	0.35–0.75	–	2.62	–	3.5	–	Reddy, Farhana & Salleh (2011)
	Spain	Chilli	HPLC	35	–	–	0.13	–	–	–	0.84	–	Santos et al. (2010)
	Sri Lanka	Chilli powder	HPLC-MS/MS	42	83.3	–	–	–	10.4	6.6	31.2	7.4	Yogendrarajah et al. (2014c)
	Sri Lanka	Chilli flakes	HPLC-MS/MS	26	92.3	–	–	–	20.5	16.6	91	16.9	Yogendrarajah et al. (2014c)
	Sri Lanka	Chillies (whole)	HPLC-MS/MS	18	38.9	–	–	–	133.1	44.5	687	22.9	Yogendrarajah et al. (2014c)
	Turkey	Red chilli flake	HPLC-FD	24	79.2	0.05	–	–	2.61	2.07	11.45	0.67	Ozbey & Kabak (2012)
	Turkey	Red chilli powder	HPLC-FD	22	63.6	0.05	–	–	5.10	3.26	35.77	0.51	Ozbey & Kabak (2012)
Cinnamon	Turkey	Cinnamon powder	HPLC-FD	17	0	0.05	–	–	–	–	–	–	Ozbey & Kabak (2012)
Coriander	Bahrain	Coriander powder	HPLC-FD	11	–	–	0.50	0.2	–	–	–	–	Musaiger, Al-Jedah & D'Souza (2008)

Table A1-3 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Cumin	China	Cumin	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
	Malaysia	Cumin	ELISA	3	66.7	0.2–0.5	0.35–0.75	–	3.26	–	4.64	–	Reddy, Farhana & Salleh (2011)
	Turkey	Cumin	HPLC-FD	19	21.1	0.05	–	–	0.58	0.14	0.88	0.03	Ozbey & Kabak (2012)
Curry	Bahrain	Mixed curry powder	HPLC-FD	11	–	–	0.50	1.4	–	–	–	–	Musaiger, Al-Jedah & D'Souza (2008)
	Cyprus	Curry	HPLC	2	100	0.10	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Republic of Korea	Curry powder	ELISA	7	28.6	0.05	0.15	–	3.88	–	4.12	–	Ok et al. (2007)
Fennel	China	Fennel	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
	China	Fennel	HPLC-FD	1	0	0.04	0.15	–	–	–	–	–	Kong et al. (2014)
Liquorice	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	1.21	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.21	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	1.37	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.59	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.13	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.13	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.03	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.07	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.27	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.04	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.08	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.17	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.12	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	–	–	–	–	–	Wei et al. (2013)
	China	Liquorice	HPLC-MS/MS	1	–	0.01	0.02	0.27	–	–	–	–	Wei et al. (2013)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Liquorice	Italy	Liquorice extract (dried)	HPLC-MS/MS	28	17.9	0.25	0.60	–	17.90	1.57[b]	2.4	–	Pietri, Rastelli & Bertuzzi (2010)
	Italy	Liquorice (confectionery)	HPLC-MS/MS	54	14.8	0.25	0.60	–	14.80	2.06[b]	7.7	–	Pietri, Rastelli & Bertuzzi (2010)
Nutmeg	Cyprus	Nutmeg	HPLC	4	100	0.10	0.33	–	–	–	–	–	Christofidou et al. (2015)
Paprika	Cyprus	Paprika	HPLC	9	77.8	0.10	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Spain	Paprika	HPLC	64	–	–	0.13	–	–	–	2.66	–	Santos et al. (2010)
	Spain	Red paprika	HPLC-MS	21	90	0.30	–	–	1.10	–	3.8	–	Hernández-Hierro et al. (2009)
Pepper	Bahrain	Black pepper powder	HPLC-FD	11	–	–	0.50	1.3	–	–	–	–	Musaiger, Al-Jedah & D'Souza (2008)
	Belgium	Black pepper	HPLC-MS/MS	20	5	–	–	–	–	–	–	–	Yogendrarajah et al. (2014a)
	Japan	White pepper	HPLC-FD	5	80	0.001–0.1	0.005–0.2	–	–	0.16	0.3	–	Sugita-Konishi et al. (2010)
	Japan	White pepper	HPLC-FD	5	80	0.001–0.1	0.005–0.2	–	–	0.24	0.5	–	Sugita-Konishi et al. (2010)
	Japan	Red pepper	HPLC-FD	6	16.7	0.001–0.1	0.005–0.2	–	–	0.2	1	–	Sugita-Konishi et al. (2010)
	Japan	Red pepper	HPLC-FD	6	16.7	0.001–0.1	0.005–0.2	–	–	0.2	1	–	Sugita-Konishi et al. (2010)
	Malaysia	Pepper	ELISA	4	100	0.2–0.5	0.35–0.75	–	1.20	–	2.1	–	Reddy, Farhana & Salleh (2011)
	Pakistan	Black pepper	HPLC-MS/MS	82	–	–	–	–	9.00	–	17.3	5.3	Yogendrarajah et al. (2014a)

Table A1-3 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean?[b] (µg/kg)	Mean +ve[c] (µg/kg)	Mean total[d] (µg/kg)	Max.	Med.	Reference
Pepper	Republic of Korea	Hot pepper powder	ELISA	7	57.1	0.05	0.15	–	0.40	–	0.63	–	Ok et al. (2007)
	Republic of Korea	Pepper powder	ELISA	7	14.3	0.05	0.15	–	0.08	–	0.08	–	Ok et al. (2007)
	Turkey	Black pepper powder	HPLC-FD	23	30.4	0.05	–	–	0.20	0.08	0.42	0.03	Ozbey & Kabak (2012)
Spices	Cyprus	Other spices	HPLC	22	0	0.10	0.33	–	–	–	–	–	Christofidou et al. (2015)
	Malaysia	Spices	HPLC	34	85	–	–	–	1.38	–	7.68	–	Ali, Hashim & Shuib (2015)
	Malaysia	Spices	HPLC	24	83	–	–	–	7.31	–	28.43	–	Ali, Hashim & Shuib (2015)
	Tunisia	Spices	HPLC	13	69.2	0.05	–	–	–	–	14.9	–	Ghali et al. (2010)

ELISA: enzyme-linked immunosorbent assay; FD: fluorescence detection; HPLC: high-performance liquid chromatography; MS/MS: tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; max.: maximum; med.: median; no.: number

[a] Percentage of samples above the LOD/LOQ.

[b] Calculation not clearly defined.

[c] Mean of positive samples.

[d] Mean of all samples. Mean is calculated as lower bound with nondetect values taken as zero.

Table A1-4

Aflatoxin M_1 levels in dairy products from FAO scoping study of open literature

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Brazil	Milk (UHT) – Winter	HPLC	48	–	0.052	0.16	ng/L	19.4	–	–	121	21.8	Silva et al. (2015)
	Brazil	Milk (UHT) – Spring	HPLC	41	–	0.052	0.16	ng/L	10.9	–	–	54.7	9.7	Silva et al. (2015)
	Brazil	Milk (UHT) – Summer	HPLC	44	–	0.052	0.16	ng/L	16.8	–	–	45.9	16.6	Silva et al. (2015)
	Brazil	Milk (UHT) – Autumn	HPLC	19	–	0.052	0.16	ng/L	30.4	–	–	72.8	30.3	Silva et al. (2015)
	Brazil	Milk (UHT), all samples	HPLC	152	87.5	0.052	0.16	ng/L	–	–	–	–	–	Silva et al. (2015)
	Brazil	Milk (UHT)	HPLC	17	76	3	–	ng/L	–	–	–	215	–	Iha et al. (2013)
	Brazil	Milk (pasteurized)	HPLC	30	87	3	10	ng/L	–	–	–	437	–	Iha et al. (2013)
	Brazil	Total of milk samples	HPLC-FD	125	95.2	3	–	ng/L	31	–	–	200	–	Shundo et al. (2009)
	Brazil	Milk (powder)	HPLC	12	100	0.052	3	ng/L	–	–	–	760	–	Iha et al. (2013)
	Brazil	Milk (pasteurized)	HPLC-FD	10	70	3	10	ng/L	–	–	–	–	–	Shundo et al. (2009)
	Brazil	Milk (powder)	HPLC-FD	10	100	3	10	ng/L	–	–	–	–	–	Shundo et al. (2009)
	Brazil	Milk (UHT)	HPLC-FD	40	100	3	10	ng/L	–	–	–	–	–	Shundo et al. (2009)
	Brazil	Milk (powder)	HPLC-FD	65	95.4	3	10	ng/L	–	–	–	–	–	Shundo et al. (2009)
	Brazil	Milk (powder)	UHPLC	3	0	1.5	4.5	µg/kg	–	–	–	–	–	Scaglioni et al. (2014)
	Brazil	Milk (UHT)	HPLC-FD	75	30.7	0.15	–	µg/L	–	–	–	–	–	Oliveira et al. (2013)
	Brazil	Milk (goat) – pasteurized	HPLC-FD	12	58.3	0.01	–	µg/L	0.072	–	–	–	–	Oliveira et al. (2013)
	Brazil	Milk (goat) – UHT	HPLC-FD	12	83.3	0.01	–	µg/L	0.058	–	–	–	–	Oliveira & Ferraz (2007)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Brazil	Milk (goat) – powder	HPLC-FD	12	66.7	0.01	–	µg/L	0.056	–	–	–	–	Oliveira & Ferraz (2007)
	Brazil	Milk (raw)	ELISA & HPLC-FD	129	100	0.000 1	0.000 2	µg/kg	0.019 5	0.002 8	–	–	–	Picinin et al. (2013)
	China	Milk (raw)	HPLC-MS/MS	72	59.7	10	–	ng/L	65	–	–	420	31	Xiong et al. (2013)
	China	Milk (pasteurized)	ELISA	26	96.2	0.005	–	µg/kg	–	0.072	–	–	0.07	Zheng et al. (2013)
	China	Milk (UHT)	ELISA	153	54.9	0.005	–	µg/kg	–	0.048	–	–	0.007	Zheng et al. (2013)
	Taiwan, China	Milk	HPLC	144	100	1.4	4.6	µg/kg	0.018–0.015	–	–	–	–	Peng & Chen (2009)
	Croatia	Milk (raw, ewe)	ELISA	18	100	5	–	µg/L	–	–	0.028	0.037	–	Duraković et al. (2012)
	Croatia	Milk (raw)	ELISA	3 736	27.8	23.2	35.8	ng/L	–	118.6	46.6	1 135	–	Bilandžić et al. (2014a)
	Croatia	Milk (raw) – East Croatia	ELISA	194	24.2	23.2	35.8	ng/L	20.6	–	–	162.3	–	Bilandžić et al. (2014b)
	Croatia	Milk (raw) – Rest of Croatia	ELISA	143	8.4	23.2	35.8	ng/L	12.1	–	–	44.9	–	Bilandžić et al. (2014b)
	Croatia	Milk (raw, donkey)	ELISA	14	0	23.2	35.8	ng/L	4.77	–	–	–	–	Bilandžić et al. (2014b)
	Croatia	Milk (raw, goat)	ELISA	32	6.2	23.2	35.8	ng/L	7.61	–	–	40.8	–	Bilandžić et al. (2014b)
	Croatia	Milk (raw, sheep)	ELISA	19	0	23.2	35.8	ng/L	3.69	–	–	–	–	Bilandžić et al. (2014b)
	Croatia	Milk (UHT)	ELISA	706	9.6	23.2	35.8	ng/L	–	69.1	25.7	183.5	–	Bilandžić et al. (2014a)
	Egypt	Milk (powder)	ELISA	30	60	5	–	ng/kg	–	1.81	–	4	–	Ghareeb et al. (2013)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Egypt	Milk (raw)	ELISA	48	97.9	5	–	ng/L	–	62.81	–	110	–	Ghareeb et al. (2013)
	Greece	Milk	ELISA	196	46.5	5	–	ng/L	–	10 (8.6–11.4)	6 (5.2–6.8)	–	–	Tsakiris et al. (2013)
	Iran (Islamic Republic of)	Liquid milk	ELISA	128	100	5	–	µg/L	72.2	–	–	113	–	Oveisi et al. (2007)
	Iran (Islamic Republic of)	Milk	ELISA	23	100	–	–	ng/L	–	–	17.4	55.9	–	Nemati et al. (2010)
	Iran (Islamic Republic of)	Milk	ELISA	23	100	–	–	ng/L	–	–	56.3	85	–	Nemati et al. (2010)
	Iran (Islamic Republic of)	Milk	ELISA	23	100	–	–	ng/L	–	–	22.3	28.9	–	Nemati et al. (2010)
	Iran (Islamic Republic of)	Milk	ELISA	23	100	–	–	ng/L	–	–	52.9	81.9	–	Nemati et al. (2010)
	Iran (Islamic Republic of)	Milk	ELISA	20	100	5	–	ng/L	–	35	–	80	–	Var & Kabak (2009)
	Iran (Islamic Republic of)	Milk	ELISA	186	64	5	–	ng/L	–	43.4	–	740	–	Ghiasian et al. (2007)
	Iran (Islamic Republic of)	Milk	ELISA	60	100	–	–	ng/L	–	16.16	16.16	64	–	Sani & Nikpooyan (2013)
	Iran (Islamic Republic of)	Milk	HPLC-FD	74	85.1	0.005	25.68	µg/kg	–	0.028	–	–	0.021	Garmakhany et al. (2011)
	Iran (Islamic Republic of)	Milk (goat, raw)	EIA	65	43.1	–	–	µg/kg	–	0.018	–	0.055	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Milk (pasteurized)	ELISA	72	100	–	–	ng/kg	–	230	230	253.3	–	Sefidgar et al. (2011)
	Iran (Islamic Republic of)	Milk (pasteurized)	HPLC	196	100	–	–	ng/L	74.91	–	–	126	–	Mohamadi Sani, Nikpooyan & Moshiri (2010)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Iran (Islamic Republic of)	Milk (pasteurized)	TLC	116	71.5	5	–	ng/L	–	73.8	52.8	528.5	–	Fallah (2010)
	Iran (Islamic Republic of)	Milk (pasteurized)	TLC	42	23.8	5	–	ng/L	–	54.39	36.06	62.66	–	Vagef & Mahmoudi (2013)
	Iran (Islamic Republic of)	Milk (pasteurized)	ELISA	24	100	10	–	ng/L	72	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (pasteurized)	ELISA	24	100	10	–	ng/L	49	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (pasteurized)	ELISA	40	–	5	–	ng/L	23.22	–	–	–	–	Mohamadi & Alizadeh (2010)
	Iran (Islamic Republic of)	Milk (pasteurized)	ELISA	80	96.3	–	–	ng/L	–	–	27.8	–	–	Moosavy et al. (2013)
	Iran (Islamic Republic of)	Milk (pasteurized) – Autumn	ELISA	15	26.7	5	–	ng/L	41.3	–	–	80	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Milk (pasteurized) – Spring	ELISA	15	40	5	–	ng/L	21.3	–	–	36	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Milk (pasteurized) – Summer	ELISA	15	33.3	5	–	ng/L	30.2	–	–	41	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Milk (pasteurized) – Winter	ELISA	15	60	5	–	ng/L	43.2	–	–	94	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Milk (raw – industrial farms)	ELISA	48	39.6	5	–	ng/L	–	53.15	34.21	60.71	–	Vagef & Mahmoudi (2013)
	Iran (Islamic Republic of)	Milk (raw – traditional farms)	EIA	54	63	5	–	ng/L	–	56.39	43.98	85.24	–	Vagef & Mahmoudi (2013)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	88	84.1	–	–	µg/kg	–	0.052	–	–	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	2160	100	–	–	ng/L	–	0.052	38.82	148	–	Khosravi et al. (2013)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Iran (Islamic Republic of)	Milk (raw)	ELISA	40	100	–	–	µg/L	–	0.17	–	0.205	–	Rezaei et al. (2014b)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	122	100	–	–	ng/L	–	40.01	–	112.4	38.27	Kamkar, Khaniki & Alavi (2011)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	60	85	10	–	ng/L	62	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	75	78.7	5	–	ng/L	–	60.1	–	–	–	Rahimi et al. (2010)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	80	82.5	10	–	ng/L	72	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (raw)	ELISA	534	34.6	5	–	ng/L	–	13.1	–	–	–	Habibipour et al. (2010)
	Iran (Islamic Republic of)	Milk (raw) – Camel	ELISA	40	12.5	5	–	ng/L	–	19	–	–	–	Rahimi et al. (2010)
	Iran (Islamic Republic of)	Milk (raw) – Goat	ELISA	60	31.7	5	–	ng/L	–	30.1	–	–	–	Rahimi et al. (2010)
	Iran (Islamic Republic of)	Milk (raw) – Sheep	ELISA	51	37.3	5	–	ng/L	–	28.1	–	–	–	Rahimi et al. (2010)
	Iran (Islamic Republic of)	Milk (raw) – Water buffalo	ELISA	75	38.7	5	–	ng/L	–	31.9	–	–	–	Rahimi et al. (2010)
	Iran (Islamic Republic of)	Milk (raw) – Autumn	ELISA	540	100	–	–	ng/L	–	–	0.14	0.28	–	Khosravi et al. (2013)
	Iran (Islamic Republic of)	Milk (raw) – Spring	TLC	540	100	–	–	ng/L	–	–	30.66	65.41	–	Khosravi et al. (2013)
	Iran (Islamic Republic of)	Milk (raw) – Summer	ELISA	540	100	–	–	ng/L	–	–	64.69	148.01	–	Khosravi et al. (2013)
	Iran (Islamic Republic of)	Milk (raw) – Winter	TLC	540	100	–	–	ng/L	–	–	59.78	115.89	–	Khosravi et al. (2013)
	Iran (Islamic Republic of)	Milk (sheep, raw)	EIA	72	59.7	–	–	µg/kg	–	0.027	–	0.102	–	Fallah et al. (2011)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Iran (Islamic Republic of)	Milk (UHT)	TLC	109	62.3	5	–	ng/L	–	74.3	46.4	515.9	–	Fallah (2010)
	Iran (Islamic Republic of)	Milk (UHT)	ELISA	24	100	10	–	ng/L	75	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (UHT)	ELISA	24	100	10	–	ng/L	56	–	–	–	–	Rahimi et al. (2009)
	Iran (Islamic Republic of)	Milk (UHT)	ELISA	40	–	–	–	ng/L	19.53	–	–	–	–	Mohamadi & Alizadeh (2010)
	Iran (Islamic Republic of)	Milk (UHT) – August	ELISA	53	35.8	0.005	–	µg/L	–	0.021	–	0.16	–	Heshmati & Milani (2010)
	Iran (Islamic Republic of)	Milk (UHT) – February	ELISA	53	75.5	0.005	–	µg/L	–	0.087	–	0.249	–	Heshmati & Milani (2010)
	Iran (Islamic Republic of)	Milk (UHT) – May	ELISA	52	50	0.005	–	µg/L	–	0.042	–	0.217	–	Heshmati & Milani (2010)
	Iran (Islamic Republic of)	Milk (UHT) – November	ELISA	52	59.6	0.005	–	µg/L	–	0.057	–	0.194	–	Heshmati & Milani (2010)
	Iran (Islamic Republic of)	Milk (UHT) – Summer	ELISA	22	100	–	–	ng/L	–	65.5	–	84.8	–	Kamkar (2008)
	Iran (Islamic Republic of)	Milk (UHT) – Autumn	HPLC-FD	30	100	–	–	ng/L	–	69.22	–	93.6	–	Kamkar (2008)
	Iraq	Milk powder	ELISA	35	82.8	–	–	µg/kg	–	154.1	–	640	–	Iqbal & Asi (2013)
	Italy	Milk (bulk)	ELISA	10	30	3	4	ng/L	–	–	–	5	–	Santini et al. (2013)
	Italy	Milk (goat)	HPLC-FD	85	34.1	0.003	–	µg/L	–	–	–	48	3	Sacca et al. (2009)
	Italy	Milk (goat)	ELISA	208	17.3	5	–	ng/1	–	14.5 ± 8.4	–	36.1	–	Virdis et al. (2008)
	Italy	Milk (raw)	ELISA	288	100		5	ng/kg	–	–	–	25	–	Schirone et al. (2015)
	Italy	Milk (raw)	ELISA	51	52.9	3	4	ng/L	–	–	–	16	–	Santini et al. (2013)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Italy	Milk (UHT)	ELISA	12	41.7	3	4	ng/L	–	–	–	5	–	Santini et al. (2013)
	Jordan	Milk	ELISA	50	100	–	–	µg/kg	–	–	56.17	–	–	Omar (2012)
	Lebanon	Milk	ELISA	64	40.6	0.005	–	µg/L	–	–	–	–	–	El Khoury, Atoui & Yaghi (2011)
	Lebanon	Milk (goat, pasteurized)	ELISA	4	–	5	–	ng/L	4.55	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk (goat, raw)	ELISA	4	–	5	–	ng/L	6.85	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk (pasteurized)	ELISA	15	–	5	–	ng/L	22.3	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk (raw)	ELISA	4	–	5	–	ng/L	22.05	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk (sheep, pasteurized)	ELISA	4	–	5	–	ng/L	2.1	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk (sheep, raw)	ELISA	4	–	5	–	ng/L	3.33	–	–	–	–	Hassan & Kassaify (2014)
	Lebanon	Milk and milk-based beverages	HPLC-FD	47	–	0.01	–	µg/kg	–	–	0.11	–	–	Raad et al. (2014)
	Lebanon	Milk (pasteurized)	ELISA	25	32	5	–	ng/1	–	–	–	84.4	–	Assem, Mohamad & Oula (2011)
	Lebanon	Milk (powder)	ELISA	14	18	5	–	ng/1	–	–	–	16.5	–	Assem, Mohamad & Oula (2011)
	Lebanon	Milk (raw)	ELISA	38	49	5	–	ng/1	–	–	–	126	–	Assem, Mohamad & Oula (2011)
	Mexico	Milk (UHT)	HPLC-FD	20	–	0.015	–	µg/kg	–	–	–	76.6	16.1	Pere et al. (2008)
	Mexico	Milk (organic)	HPLC-FD	15	–	0.015	–	µg/kg	–	–	–	88.6	23.1	Pere et al. (2008)
	Mexico	Milk (raw)	HPLC-FD	9	–	0.015	–	µg/kg	–	–	–	92.3	16.2	Pere et al. (2008)
	Morocco	Milk (raw)	HPLC-FD	48	27	–	8	µg/kg	–	31	–	100	–	Iqbal et al. (2013)
	Pakistan	Milk (raw)	HPLC-FD	107	71	0.004	–	µg/kg	–	212	150.7	845.4	–	Iqbal et al. (2013)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Pakistan	Milk (UHT) – Summer	HPLC-FD	39	31	0.004	–	µg/kg	–	–	0.021	–	–	Iqbal et al. (2013)
	Pakistan	Milk (UHT) – Winter	HPLC-FD	45	51	0.004	–	µg/kg	–	–	0.06	–	–	Iqbal et al. (2013)
	Pakistan	Milk (raw) – Winter	HPLC-FD	48	40	0.004	–	µg/kg	–	–	0.073	–	–	Iqbal et al. (2013)
	Pakistan	Milk (raw) – Summer	HPLC-FD	56	36	0.004	–	µg/kg	–	–	0.028	–	–	Iqbal et al. (2013)
	Pakistan	Milk (raw)	HPLC	120	52.5	0.004	–	µg/kg	–	0.044	–	–	–	Hussain et al. (2008)
	Pakistan	Milk (raw, buffalo)	HPLC	360	42.5	0.004	–	µg/kg	–	–	–	–	–	Hussain et al. (2008)
	Portugal	Milk (UHT)	ELISA	40	27.5	5	–	ng/L	–	23.4	–	–	–	Duarte et al. (2013)
	Republic of Korea	Milk	HPLC-FD	50	6	125.4	418	ng/kg	–	20.81	14.4	228.6	12.54	Lee & Lee (2015)
	Republic of Korea	Milk	HPLC-FD	100	48	0.002	–	µg/L	–	0.026	–	–	0.023	Lee et al. (2009)
	Republic of Korea	Milk (raw) – 2008	HPLC-FD	100	93.3	0.01	–	µg/L	–	–	–	0.1	–	Kim et al. (2010)
	Republic of Korea	Milk (raw) – 2009	HPLC-FD	100	66	0.01	–	µg/L	–	0.07	–	0.1	–	Kim et al. (2010)
	Republic of Korea	Milk powder	HPLC-FD	50	74	125.4	418	ng/kg	–	270.9	147.6	835.6	171.9	Lee & Lee (2015)
	Serbia	Milk	HPLC	60	90	0.3	1	µg/kg	0.026	–	–	0.104	–	Torovic (2015)
	Serbia	Milk	ELISA	150	98.7	1.5	5	µg/kg		0.21	–	1.2	–	Kos et al. (2014)
	Serbia	Milk	UHPLC-MS/MS	42	94	0.000 2	0.000 7	µg/kg	0.3	–	–	–	–	Škrbić, Živančev & Antić (2013)
	Serbia	Milk powder	ELISA	67	67.2	0.005	0.02	µg/kg	0.847	–	–	–	–	Tomašević et al. (2015)
	Serbia	Milk (raw)	ELISA	79	62	0.005	0.02	µg/kg	0.035	–	–	–	–	Tomašević et al. (2015)
	Serbia	Milk (UHT)	ELISA	105	83.8	0.005	0.02	µg/kg	0.022	–	–	–	–	Tomašević et al. (2015)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Milk	Serbia	Milk (UHT)	ELISA	223	19.3	0.005	0.02	µg/kg	0.071	–	–	–	–	Tomašević et al. (2015)
	Serbia	Milk (raw)	ELISA	599	14.9	0.005	0.02	µg/kg	0.314	–	–	–	–	Tomašević et al. (2015)
	South Africa	Milk (commercial)	HPLC	85	100	0.01	–	µg/kg	0.14	–	–	0.19	–	Mulunda & Mike (2014)
	South Africa	Milk (rural farms)	HPLC	125	78.4	0.01	–	µg/kg	0.15	–	–	0.2	–	Mulunda & Mike (2014)
	Spain	Milk	ELISA	72	94.4	5	25	ng/kg	–	9.69	–	–	–	Calvet et al. (2015)
	Tunisia	Milk (raw)	ELISA	112	59.8	0.01	–	µg/kg	13.62	–	–	–	–	Abbès et al. (2012)
	Turkey	Milk (pasteurized)	ELISA	45	66.7	5	–	ng/L	–	19.1	–	–	–	Kocasar, Tasci & Mor (2012)
	Turkey	Milk (powder)	ELISA	45	93.3	50	–	ng/kg	–	204.3	–	–	–	Kocasar, Tasci & Mor (2012)
	Turkey	Milk (powder)	ELISA	80	67.5	1	–	ng/kg	–	–	–	–	–	Elmali et al. (2008)
	Turkey	Milk (raw)	ELISA	176	30.1	0.021	69.90	µg/kg	–	0.153	–	1.101	–	Golge (2014)
	Turkey	Milk (raw)	ELISA	45	91.1	5	–	ng/L	–	45.3	–	–	–	Kocasar, Tasci & Mor (2012)
	Turkey	Milk (UHT)	ELISA	100	67	10	–	ng/L	67	–	–	630	–	Tekinşen & Eken (2008)
	Turkey	Milk (UHT)	HPLC-FD	41	73.2	2	–	ng/L	17.76	–	–	71.33	–	Sahindokuyucu Kocasari (2014)
	Turkey	Milk (UHT)	HPLC-FD	40	20	0.004	–	µg/kg	–	0.029	–	0.076	–	Kabak & Ozbey (2012)
	Turkey	Milk (UHT, flavoured)	ELISA	90	75.6	–	–	ng/L	–	–	9.2	26.6	–	Kocak et al. (2015)
	Turkey	UHT milk	HPLC	150	60	5		ng/kg	–	36	–	185	–	Atasever & Adigüzel (2010)
	West Bank and Gaza Strip	Milk, raw	ELISA	40	85	5	–	ppt (ng/kg)	–	29.57	–	80	–	Al Zuheir & Omar (2012)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Cheese	Brazil	Cheese	HPLC-FD	58	84	0.052	3	ng/kg	–	–	–	304	–	Iha et al. (2011)
	Brazil	Grated parmesan cheese	HPLC-FD	30	60	0.05	–	µg/kg	–	–	0.16	–	–	Trombete et al. (2014)
	Iran (Islamic Republic of)	Cheese	ELISA	80	86.3	10	–	ng/L	133.2	–	–	572.1	–	Rahimi (2014)
	Iran (Islamic Republic of)	Cheese	TLC	82	47.6	–	–	ng/kg	–	124	–	309	–	Akrami Mohajeri et al. (2013)
	Iran (Islamic Republic of)	Cheese (cream)	ELISA	94	72.3	50	–	ng/kg	–	230.1	166.4	785.4	–	Fallah et al. (2009)
	Iran (Islamic Republic of)	Cheese (creamy)	ELISA	40	–	–	–	ng/L	21.96	–	–	–	–	Mohamadi & Alizadeh (2010)
	Iran (Islamic Republic of)	Cheese (feta)	ELISA	40	–	–	–	ng/L	43.31	–	–	–	–	Mohamadi & Alizadeh (2010)
	Iran (Islamic Republic of)	Cheese (Kashar)	ELISA	20	50	25	–	ng/L	–	119	–	388	–	Var & Kabak (2009)
	Iran (Islamic Republic of)	Cheese (Lighvan)	ELISA	75	65.3	–	–	µg/kg	–	0.085	–	0.313	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Cheese (Lighvan)	TLC	37	27	–	–	ng/kg	–	90.8	–	204	–	Mohajeri et al. (2013)
	Iran (Islamic Republic of)	Cheese (white)	ELISA	50	60	–	–	ng/kg	53.39	–	–	374	–	Tavakoli et al. (2012)
	Iran (Islamic Republic of)	Cheese (white)	ELISA	116	80.1	50	–	ng/kg	–	247.7	198.6	744.5	–	Fallah et al. (2009)
	Iran (Islamic Republic of)	Cheese (white)	HPLC	20	80	25	–	ng/L	–	142	–	263	–	Var & Kabak (2009)
	Iran (Islamic Republic of)	Cheese (white)	TLC	45	64.4	–	–	ng/kg	–	135	–	309	–	Mohajeri et al. (2013)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Cheese	Iran (Islamic Republic of)	Cheese (white) – Autumn	HPLC	15	33.3	5	–	ng/L	138.4	–	–	206	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Cheese (white) – Spring	ELISA	16	75	5	–	ng/L	78.9	–	–	177	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Cheese (white) – Summer	ELISA	15	66.7	5	–	ng/L	70.8	–	–	108	–	Rahimi et al. (2012)
	Iran (Islamic Republic of)	Cheese (white) – Winter	ELISA	15	66.7	5	–	ng/L	148.5	–	–	209	–	Rahimi et al. (2012)
	Italy	Cheese	ELISA	102	83	25	–	ng/kg	–	–	–	281.1	60.4	Anfossi et al. (2012)
	Italy	Cheese	ELISA	17	41.2	3	4	ng/L	–	–	–	18	–	Santini et al. (2013)
	Italy	Cheese (goat)	ELISA	41	9.8	50	–	ng/kg	–	257	–	145	–	Virdis et al. (2008)
	Kuwait	Cheese	ELISA	40	80	50	–	ng/kg	–	87.6	–	452	–	Dashti et al. (2009)
	Lebanon	Cheese	ELISA	111	67.6	50	–	ng/kg	–	–	–	315	–	Elkak et al. (2012)
	Lebanon	Cheese	HPLC-FD	47	–	0.01	–	µg/kg	–	–	0	–	–	Raad et al. (2014)
	Pakistan	Cheese (cream)	HPLC-FD	150	59	0.004	–	µg/kg	–	172.9	102.6	456.3	–	Al-Sawaf, Abdullah & Sheet (2012)
	Pakistan	Cheese (white)	HPLC-FD	119	78	0.004	–	µg/kg	–	189.1	147.8	595.4	–	Omar (2012)
	Portugal	Cheese (hard)	HPLC-FD	128	6.3	0.005	–	µg/kg	–	0.01	–	–	–	Martins et al. (2007)
	Serbia	Hard cheese	ELISA	27	22.2	0.005	0.02	µg/kg	0.379		–	–	–	Tomašević et al. (2015)
	Serbia	White cheese	ELISA	47	17	0.005	0.02	µg/kg	0.146		–	–	–	Tomašević et al. (2015)
	Spain	Cheese	ELISA	72	0	5	25	ng/kg	–		–	–	–	Calvet et al. (2015)
	Turkey	Cheese	ELISA	193	82.4	50	–	ng/kg	–		234.5	860	–	Ardic et al. (2009)
	Turkey	Cheese	ELISA	120	60	50	–	ng/kg	–	221	–	1 043	–	Aygun et al. (2009)
	Turkey	Cheese (cream)	ELISA	100	99	10	–	ng/kg	330	–	–	4 100	–	Tekinşen & Uçar (2008)
	Turkey	Cheese (Kashar)	ELISA	147	98	0.01	–	µg/kg	–	–	–	3.774	0.273	Gul & Dervisoglu (2014)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Cheese	Turkey	Cheese (Kashar)	ELISA	132	82.6	50	–	ng/kg	194	–	–	690	–	Tekinşen & Eken (2008)
	Turkey	Cheese (white)	ELISA	45	88.9	50	–	ng/kg	–	199.4	–	–	–	Kocasar, Tasci & Mor (2012)
	Turkey	Cheese (Helva)	ELISA/ HPLC-FD	50	10	0.625	–	µg/kg	2.63	–	–	–	–	Aydin et al. (2008)
	Turkey	Cheese (Hosmerin)	ELISA/ HPLC-FD	50	6	0.625	–	µg/kg	2.07	–	–	–	–	Aydin et al. (2008)
Yoghurt	Brazil	Yoghurt	HPLC-FD	53	95	0.052	3	ng/kg	–	–	–	529	–	Iha et al. (2011)
	Iran (Islamic Republic of)	Yoghurt	ELISA	50	70	?	–	ng/kg	26.95	–	–	137.6	–	Tavakoli et al. (2013)
	Iran (Islamic Republic of)	Yoghurt	ELISA	60	98.3	–	–	ng/L	–	51.66	–	87	–	Issazadeh et al. (2012)
	Iran (Islamic Republic of)	Yoghurt	EIA	60	80	10	–	ng/L	130.5	–	–	319.4	–	Rahimi (2014)
	Iran (Islamic Republic of)	Yoghurt	ELISA	120	100	–	–	ng/kg	–	–	28.2	78.9	–	Tabari, Tabari & Tabari (2013)
	Iran (Islamic Republic of)	Yoghurt (industrial)	ELISA	61	49.2	–	–	µg/kg	–	0.026	–	0.102	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Yoghurt (traditional)	ELISA	60	23.3	–	–	µg/kg	–	0.007	–	0.036	–	Fallah et al. (2011)
	Lebanon	Yoghurt	ELISA	64	32.8	0.005	–	µg/L	–	–	–	–	–	El Khoury, Atoui & Yaghi (2011)
	Lebanon	Yoghurt and products	HPLC-FD	47	NA	0.01	–	µg/kg	–	–	0	–	–	Raad et al. (2014)
	Pakistan	Yoghurt	HPLC-FD	96	61	0.004	–	µg/kg	–	147.1	90.4	615.8	–	Iqbal & Asi (2013)
	Pakistan	Yoghurt – Summer	HPLC-FD	45	29	0.004	–	µg/kg	–	–	0.019	–	–	Iqbal, Asi & Jinap (2013b)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Yoghurt	Pakistan	Yoghurt – Winter	HPLC-FD	51	37	0.004	–	µg/kg	–	–	0.053	–	–	Iqbal, Asi & Jinap (2013b)
	Republic of Korea	Yoghurt	HPLC-FD	50	14	125.4	418	ng/kg		22.54	16.05	191.3	12.54	Lee & Lee (2015)
	Serbia	Yoghurt	ELISA	56	25	0.005	0.02	µg/kg	0.081	–	–	–	–	Tomašević et al. (2015)
	Spain	Yoghurt (branded label)	ELISA	6	50	25	–	ng/kg	–	21.6	–	–	–	Cano-Sancho et al. (2015)
	Spain	Yoghurt (private label)	ELISA	12	25	25	–	ng/kg	–	16.6	–	–	–	Cano-Sancho et al. (2015)
	Spain	Yoghurt	ELISA	72	2.8	5	25	ng/kg	–	38.34	–	–	–	Calvet et al. (2015)
	Turkey	Yoghurt	ELISA	45	44.4	50		ng/L	–	176.6	–	–	–	Kocasar, Tasci & Mor (2012)
Other	Brazil	Dairy drink	LC-FD	12	95	3	0.052	ng/kg	–	–	–	50		Iha et al. (2011)
	Iran (Islamic Republic of)	Commercial doogh	TLC	110	61.8	–	–	ng/L	46.4	–	–	152	–	Tabari, Tabari & Tabari (2011)
	Iran (Islamic Republic of)	Doogh (industrial)	EIA	71	22.5	–	–	µg/kg	–	0.007	–	0.053	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Doogh (traditional)	HPLC	65	13.8	–	–	µg/kg	–	0.003	–	0.029	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Ice cream	ELISA	60	56.7	10	–	ng/L	65.1	–	–	147.4	–	Rahimi (2014)
	Iran (Islamic Republic of)	Kashk (industrial)	ELISA	64	53.1	–	–	µg/kg	–	0.08	–	0.285	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Kashk (traditional)	ELISA	61	31.2	–	–	µg/kg	–	0.053	–	0.291	–	Fallah et al. (2011)
	Iran (Islamic Republic of)	Doogh (traditional)	TLC	115	71.3	10	–	ng/L	52.3	–	–	211	–	Tabari, Tabari & Tabari (2011)
	Lebanon	Dairy products	ELISA	419	20	5–50	–	ng/L	32.77	–	–	–	–	Hassan & Kassaify (2014)

Table A1-4 (continued)

Food item	Country / Region	Food	Analytical method	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Unit[b]	Mean?[c] (µg/kg)	Mean +ve[d] (µg/kg)	Mean total[e] (µg/kg)	Max.	Med.	Reference
Other	Lebanon	Milk-based ice cream and pudding	HPLC-FD	47	–	0.01	–	µg/kg	–	–	0	–	–	Raad et al. (2014)
	Pakistan	Ice cream – Summer	HPLC-FD	37	24	0.004	–	µg/kg	–	–	0.012	–	–	Iqbal, Asi & Jinap (2013b)
	Pakistan	Ice cream – Winter	HPLC-FD	42	43	0.004	–	µg/kg	–	–	0.021	–	–	Iqbal & Asi (2013)
	Republic of Korea	Ice cream	HPLC-FD	50	36	125.4	418	ng/kg	–	33.16	22.13	121.3	12.54	Lee & Lee (2015)
	Serbia	Ice cream	ELISA	21	33.3	0.005	0.02	µg/kg	0.071		–	–	–	Tomašević et al. (2015)
	Turkey	Ice cream	ELISA	45	75.6	10	–	ng/L	–	32.6	–	–	–	Kocasar, Tasci & Mor (2012)

EIA: enzyme-linked immunoassay; ELISA: enzyme-linked immunosorbent assay; FD: fluorescence detection; HPLC: high-performance liquid chromatography; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; Med.: median; MS/MS: tandem mass spectrometry; No.: number; ppt: parts per trillion; TLC: thin-layer chromatography; UHPLC: ultra-high performance liquid chromatography; UHT: ultra-heat treated

[a] Percentage of samples above the LOD/LOQ.

[b] Note the different units reported.

[c] Calculation not clearly defined.

[d] Mean of positive samples.

[e] Mean of all samples. Mean is calculated as lower bound with nondetect values taken as zero.

Table A1-5

Summary of AFB_1 and AFT occurrence data in cereals, nuts, dairy products and other foods[a] drawn from the GEMS/Food contaminants database for the period 2007 to mid-2016

Food type	Mycotoxin	No. of samples	Occurrence[b] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean[c] (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile[d] (µg/kg)
Cereals									
Barley	AFB_1	577	3.8	0.000 3–0.3	0.001–10	0.056	11	–	–
	AFT	500	6.0	0.000 3–2.6	0.001–8.7	0.079	12	–	–
Buckwheat	AFB_1	210	9.0	0.02–2	0.067–6.67	0.28	20.5	–	–
	AFT	80	16.3	0.023–4	0.077–13.3	0.81	24.86	–	0.2
Maize	AFB_1	2 613	22.0	0.000 3–3.4	0.001–10.2	1.83	476	–	2.1
	AFT	1 860	26.9	0.000 3–4	0.001–13.33	2.13	134.6	–	5.7
Millet	AFB_1	59	10.2	0.02–2	0.067–6.67	0.021	0.22	–	–
	AFT	25	20.0	0.1–2.6	0.33–8.67	0.088	0.8	–	0.2
Oats	AFB_1	378	0	0.000 3–3	0.001–9	0	0	–	–
	AFT	78	0	0.000 3–1.75	0.001–5.83	0	0	–	–
Rice	AFB_1	3 666	22.5	0.002–3.33	0.006 7–10	0.24	23.9	–	0.5
	AFT	2 483	25.7	0.006–3	0.027–10	1.72	347	–	1.0
Rye	AFB_1	206	2.9	0.000 3–3	0.001–9	0.011	0.7	–	–
	AFT	61	4.9	0.000 3–2.6	0.001–8.67	0.019	0.7	–	–
Sorghum	AFB_1	1 614	7.0	0.006–3.75	0.076–10	2.62	359	–	–
	AFT	96	18.8	0.006–3	0.094–4	0.94	12	–	3.9
Sweetcorn on the cob	AFB_1	67	6.0	0.006–2	0.076–6.67	0.035	1.03	–	–
	AFT	51	3.9	0.006–1.2	0.094–4	0.007 8	0.2	–	–
Wheat	AFB_1	2 443	3.6	0.000 3–3	0.001–9	0.026 3	2.36	–	–
	AFT	1 486	11.7	0.000 3–4	0.001–13.33	0.180	4.44	–	1.1
Nuts									
Almonds	AFB_1	3 727	20.0	0.02–3.43	0.067–10.3	1.10	480	–	0.4
	AFT	2 218	23.1	0.01–3	0.033–10	1.14	339	–	0.8

Table A1-5 (continued)

Food type	Mycotoxin	No. of samples	Occurrence[b] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean[c] (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile[d] (µg/kg)
Brazil nuts	AFB_1	395	26.3	0.05–3.37	0.17–10.1	6.48	898	–	1.0
	AFT	84	48.8	0.28–1.75	0.93–5.83	67.3	2 589	–	46
Cashews	AFB_1	933	11.6	0.002–3.3	0.007–10	0.064	11	–	0.2
	AFT	399	22.6	0.008–2.6	0.03–8.7	0.109	3.2	–	0.2
Chestnuts	AFB_1	109	6.4	0.000 5–0.5	0.002–1.67	0.048	2.4	–	–
	AFT	42	7.1	0.1–1	0.33–3.33	0.071	1.6	–	–
Hazelnuts	AFB_1	7 025	30.9	0.000 5–3.3	0.001 7–10	0.58	638	–	1.1
	AFT	4 741	40.5	0.008–10	0.027–33.3	1.50	713	–	3.1
Peanuts	AFB_1	7 907	36.6	0.002–34	0.006 7–102	4.69	754	–	2.3
	AFT	20 870	35.4	0.008–10	0.027–33.3	9.46	3 050	–	14.5
Pistachios	AFB_1	11 612	18.7	0.002–3.33	0.007–10	2.21	711	–	0.8
	AFT	9 949	19.0	0.008–3	0.027–10	2.54	665	–	0.8
Walnuts	AFB_1	940	10.7	0.002–3.3	0.007–10	0.089	11.5	–	0.2
	AFT	411	23.6	0.008–3.5	0.03–11.6	0.20	4.1	–	0.8
Dairy products									
Milk, cattle	AFM_1	3 442	11.4	0.000 8–16	0.002 67–53	0.035 46	50	–	0.007
Milk, goats	AFM_1	287	9.4	0.001–0.05	0.003 3–0.167	0.009 2	0.645	–	–
Milk, sheep	AFM_1	220	4.5	0.002–0.5	0.006 7–1.67	0.000 52	0.034	–	–
Cheese	AFM_1	563	22.6	0.001–1	0.003 3–5	0.014 3	0.64	–	0.04
Fermented milk product	AFM_1	322	7.8	0.001–1	0.003 3–5	0.002 83	0.072	–	–
Other foods									
Aniseed	AFB_1	29	0	0.05–1	0.17–2	–	–	–	–
	AFT	1	0	0.1	0.33	–	–	–	–
Basil	AFB_1	11	9.1	0.12–0.75	0.4–2.5	0.045	0.5	–	–
	AFT	4	25.0	1.3–2	4.5–6.7	0.5	2	–	–
Bay leaves	AFB_1	9	0	0.1–0.25	0.33–0.8	–	–	–	–
	AFT	3	0	0.1–0.28	0.33–0.93	–	–	–	–

Food type	Mycotoxin	No. of samples	Occurrence[b] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean[c] (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile[d] (µg/kg)
Cardamom	AFB_1	25	16.0	0.05–1	0.17–2	0.38	7.7	–	–
	AFT	2	100	–	–	0.36	0.5	–	–
Caraway	AFB_1	37	2.7	0.1–0.5	0.33–3.3	0.062	2.3	–	–
	AFT	10	0	0.1–1.34	0.1–4.5	–	–	–	–
Celery seed	AFB_1	3	0	0.05–0.5	0.17–1.67	–	–	–	–
	AFT	No data							
Chives	AFB_1	4	0	0.2–0.75	0.67–2.5	–	–	–	–
	AFT	2	0	1.3–2	4.4–6.67	–	–	–	–
Cinnamon	AFB_1	63	4.8	0.02–2	0.067–1.67	0.035	1	–	–
	AFT	29	6.9	0.025–8	0.083–26.7	0.28	4	–	–
Cloves	AFB_1	32	21.9	0.05–1	0.17–2	0.70	9.26	–	3.6
	AFT	7	14.3	0.025–2	0.083–6.67	4.14	29	–	–
Coriander	AFB_1	47	6.4	0.05–0.5	0.17–1.67	0.058	1.41	–	–
	AFT	25	8.0	0.05–1	0.17–3.33	0.053	1.17	–	–
Cumin	AFB_1	73	5.5	0.05–1	0.17–2	0.50	35.6	–	–
	AFT	29	3.4	0.025–2	0.083–6.67	0.006 9	0.2	–	–
Dill	AFB_1	3	0	0.12–0.75	0.4–2.5	–	–	–	–
	AFT	1	0	2	6.67	–	–	–	–
Dill seed	AFB_1	7	14.3	0.05–0.5	0.17–1.67	0.16	1.09	–	–
	AFT	No data							
Fennel seed	AFB_1	37	2.7	0.05–1	0.17–3.33	0.005	0.2	–	–
	AFT	1	100	–	–	–	0.8	–	–
Fenugreek	AFB_1	37	10.8	0.05–1	0.17–2	0.038	0.53	–	–
	AFT	1	100	–	–	–	0.86	–	–
Figs	AFB_1	2 858	26.6	0.000 5–3.33	0.001 7–10	1.44	1 139	–	1.5
	AFT	2 135	32.0	0.03–4.1	0.1–13.67	2.47	1 329	–	3.3
Ginger	AFB_1	328	45.1	0.012–2	0.04–6.67	1.00	15	–	3.0
	AFT	271	43.5	0.05–8	0.17–26.7	2.19	41	–	5.8
Herbs	AFB_1	49	12.2	0.05–1.13	0.17–3.77	0.155	2.5	–	0.1

Table A1-5 (continued)

Food type	Mycotoxin	No. of samples	Occurrence[b] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean[c] (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile[d] (µg/kg)
	AFT	32	12.5	0.05–3	0.17–10	0.234	2.5	–	1.4
Hops	AFB_1	1	100	–	–	–	0.23	–	–
	AFT	1	100	–	–	–	0.23	–	–
Juniper berry	AFB_1	1	100	–	–	–	0.6	–	–
	AFT	1	100	–	–	–	1.5	–	–
Mace	AFB_1	28	10.7	0.05–1	0.17–2	0.034	0.5	–	–
	AFT	11	18.2	0.1–0.5	0.34–1.67	0.073	0.5	–	–
Nutmeg	AFB_1	691	70.5	0.02–5	0.067–16.67	2.76	106.5	0.81	6.6
	AFT	528	73.1	0.025–8	0.083–26.7	3.24	87.5	1.02	8.2
Parsley	AFB_1	5	0	0.12–1	0.4–3.33	–	–	–	–
	AFT	1	0	2.66	8.87	–	–	–	–
Pepper	AFB_1	1 022	25.1	0.01–5	0.03–16.67	0.22	20.8	–	0.6
	AFT	633	29.7	0.02–8	0.067–26.7	0.57	30	–	2
Rosemary	AFB_1	1	0	0.25	0.83	–	–	–	–
	AFT	1	0	0.67	2.23	–	–	–	–
Soybean	AFB_1	55	5.5	0.05–3.33	0.167–10	0.011	0.2	–	–
	AFT	24	12.5	0.02–0.4	0.067–1.33	0.075	0.8	–	0.2
Spices	AFB_1	3 797	53.3	0.001 2–5	0.004–16.67	1.21	202	0.2	2.2
	AFT	2 606	48.0	0.01–8	0.033–26.67	1.29	150.9	–	2.6
Thyme	AFB_1	13	7.7	0.01–0.75	0.33–2.33	0.027	0.35	–	–
	AFT	5	0	1.34–2	4.47–6.67	–	–	–	–
Turmeric	AFB_1	30	36.7	0.05–1	0.17–2	1.63	20	–	–
	AFT	No data							

AFB_1: aflatoxin B_1; AFT: total aflatoxins; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LOD; limit of detection; LOQ: limit of quantification; No.: number

[a] The spices category is shown in Table A1-6.

[b] Percentage of samples above the LOD/LOQ.

[c] Mean is calculated as lower bound with nondetect values taken as zero.

[d] This estimate gives an indication of the skewed distribution of results.

Table A1-6

Summary of AFB_1 and AFT occurrence data drawn from the general "Spices" category of the GEMS/Food contaminants database for the period 2007 to mid-2016

Food type	Mycotoxin	No. of samples	Occurrence[a] (%)	LOD (µg/kg)	LOQ (µg/kg)	Mean[b] (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)
Allspice	AFB_1	29	17.2	0.1–1	0.33–2	0.19	4.2	–	–
	AFT	12	33.3	0.1–2.6	0.33–8.7	0.24	2	–	–
Cayenne pepper	AFB_1	53	50.9	0.003–5	0.01–16.7	1.60	22	0.07	4.3
	AFT	34	41.2	0.01–8	0.033–26.7	2.47	65	–	2
Chilli powder	AFB_1	499	54.3	0.003–3	0.01–10	2.04	114	0.26	4.1
	AFT	500	41.8	0.01–7.4	0.033–24.7	1.80	120	–	3.7
Curry	AFB_1	366	55.2	0.012–1	0.04–3.33	1.23	202	0.16	1.8
	AFT	236	53.8	0.03–4	0.1–13.3	0.92	19.2	0.17	2.5
Garam masala	AFB_1	16	50.0	0.1–0.2	0.33–0.67	1.92	10.6	0.25	–
	AFT	16	56.3	0.1–0.2	0.33–0.67	2.01	10.7	0.45	–
Mustard	AFB_1	9	11.1	0.05–1	0.17–3	0.035	0.31	–	–
	AFT	No data							
Paprika	AFB_1	1 670	60.8	0.0012–5	0.004–16.7	1.11	146.3	0.4	2.01
	AFT	1 011	51.0	0.03–8	0.1–26.7	1.19	150.9	0.2	2.4
Spices, mixed	AFB_1	298	44.3	0.02–1	0.067–3.33	1.59	84.2	–	1.8
	AFT	370	49.5	0.03–7.4	0.1–24.7	1.64	106.3	–	2.9
Star anise	AFB_1	18	0	1	2	0	0	–	–
	AFT	No data							
Turmeric	AFB_1	489	45.8	0.012–1	0.04–3.33	0.65	29.1	–	1.6
	AFT	419	44.4	0.05–5	0.17–16.7	0.75	31.7	–	2

AFB_1: aflatoxin B_1; AFT: total aflatoxins; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LOD; limit of detection; LOQ: limit of quantification; No.: number

[a] Percentage of samples above the LOD/LOQ.

[b] Mean is calculated as lower bound with nondetect values taken as zero.

4,15-Diacetoxyscirpenol

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1. Explanation 284
2. Biological data 285
2.1 Biochemical aspects 285
2.1.1 Absorption, distribution and excretion 285
(a) Absorption 285
(b) Distribution 287
(c) Excretion 288
2.1.2 Biotransformation 289
(a) In vivo 289
(b) In vitro 290
2.1.3 Effects on enzymes and other biochemical parameters 293
(a) Enzymes 293
(b) Microflora 293
(c) Biochemical modes of action 294
2.1.4 Transfer from feed to food 297
2.2 Toxicological studies 297
2.2.1 Acute toxicity 297
(a) Oral administration 297
(b) Intraperitoneal administration 299
(c) Intravenous administration 299
(d) Dermal administration 301
2.2.2 Short-term studies of toxicity 303
(a) Dietary administration 303
(b) Combined dietary administration of mycotoxins 309
(c) Intravenous administration 313
2.2.3 Long-term studies of toxicity and carcinogenicity 316
2.2.4 Genotoxicity 317
2.2.5 Reproductive and developmental toxicity 318
(a) Multigeneration reproductive toxicity 318
(b) Developmental toxicity 322

2.2.6 Special studies 322
(a) Gastrointestinal effects 322
(b) Haematological effects 324
(c) Immunological effects 326
(d) Modified forms and metabolites 329
(e) Related contaminants 330
2.3 Observations in domestic animals/veterinary toxicology 336
2.4 Observations in humans 337
2.4.1 Biomarkers of exposure 337
2.4.2 Biomarkers of effects 337
2.4.3 Clinical observations 337
2.4.4 Epidemiological studies 338
3. Analytical methods 339
3.1 Chemistry 339
3.2 Description of analytical methods 340
3.2.1 Introduction 340
3.2.2 Screening tests 341
3.2.3 Quantitative methods 341
3.2.4 Quantitative analysis of biologically modified 4,15-DAS 346
(a) Analysis of conjugates formed by plants or fungi 346
(b) Functionalized phase I metabolites and conjugates formed by animals 346
(c) Quantitative analysis of chemically modified 4,15-DAS 347
4. Sampling protocols 348
5. Effects of processing 350
5.1 Sorting, cleaning and milling 350
5.2 Thermal processing 350
5.3 Alkaline treatment and fermentation 350
6. Prevention and control 351
6.1 Preharvest control 351
6.1.1 Crop management strategies 351
6.1.2 Plant protection products to reduce fungal growth and mycotoxin production 352
6.2 Postharvest control 352
6.3 Decontamination 353
6.3.1 Use of irradiation 353
6.3.2 Use of chemicals 353
6.3.3 Use of microorganisms 353
6.3.4 Use of enzymes 354
6.3.5 Use of adsorbents 354
6.3.6 Emerging technique 354
6.4 Hazard analysis and critical control point (HACCP) and integrated mycotoxin management system 355
7. Levels and patterns of contamination in food commodities 355
7.1 Surveillance data 355
7.1.1 Data from GEMS/Food contaminants database on occurrence of 4,15-DAS 355
(a) African Region 356
(b) Region of the Americas 356

(c) European Region 356
(d) Eastern Mediterranean Region 359
(e) Western Pacific Region 359
7.1.2 Literature search of peer-reviewed publications on occurrence of 4,15-DAS in food 359
(a) African Region 360
(b) Region of the Americas 360
(c) South-East Asia Region 361
(d) European Region 361
(e) Eastern Mediterranean Region 364
(f) Western Pacific Region 365
7.1.3 Co-occurrence of DAS, T-2 toxin and HT-2 toxin 365
(a) Data from GEMS/Food contaminants database on co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin 366
(b) Open literature search on co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin in food 366
7.2 Distribution curves 368
7.3 Data on annual variation in contaminant levels 368
8. Food consumption and dietary exposure estimates 368
8.1 Concentrations in food used in the dietary exposure estimates 368
8.2 Food consumption data used in the dietary exposure estimates 369
8.3 Assessments of dietary exposure 369
8.3.1 National estimates 369
(a) Finland 369
(b) Morocco 369
(c) Spain 370
(d) Tunisia 370
8.3.2 International estimates 371
8.4 Potential effect of limits and their enforcement on chronic dietary exposure 374
9. Dose–response analysis and estimation of toxic/carcinogenic risk 374
9.1 Identification of key data for risk assessment 374
9.1.1 Pivotal data from biochemical and toxicological studies 374
9.1.2 Pivotal data from human clinical/epidemiological studies 375
10. Comments 375
10.1 Biochemical aspects 375
10.2 Toxicological studies 376
10.3 Observations in domestic animals/veterinary toxicology 378
10.4 Observations in humans 378
10.5 Analytical methods 379
10.6 Sampling protocols 380
10.7 Effects of processing 380
10.8 Prevention and control 380
10.9 Levels and patterns of contamination in food commodities 381
10.10 Food consumption and dietary exposure assessment 382
10.11 Dose–response analysis 383
11. Evaluation 384
11.1 Recommendations 385

12. References 386
Appendix 1 405

1. Explanation

4,15-Diacetoxyscirpenol (4,15-DAS; (3α,4β)-3-hydroxy-12,13-epoxytrichothec-9-ene-4,15-diyl diacetate; $C_{19}H_{26}O_7$; 366.4 Da; Chemical Abstracts Service [CAS] No. 2270-40-8) or anguidine is a trichothecene mycotoxin produced mainly by *Fusarium langsethiae*, *F. poae* and *F. sambucinum* (Thrane et al., 2004; Shams et al., 2011; Tamura et al., 2015; Lysøe et al., 2016). All trichothecenes have the same core 12,13-epoxytrichothec-9-ene structure, and trichothecene analogues have different patterns of substitution around this core structure. 4,15-DAS is a type A trichothecene, with similar structure to T-2 toxin and HT-2 toxin. Both T-2 toxin and HT-2 toxin have an ester function at the C-8 position, whereas HT-2 toxin also has a hydroxyl group at the C-4 position (Fig. 1).

The main food groups reported to be contaminated with 4,15-DAS are cereals and cereal-based products, which include wheat, oat, barley, rice, rye, maize and sorghum (Schollenberger et al., 2006; Serrano et al., 2012; WHO, 2013; Tamura et al., 2015). In addition to cereals, 4,15-DAS has been found in coffee beans (Garcia-Moraleja et al., 2015a).

4,15-DAS has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The structurally related type A trichothecenes T-2 toxin and HT-2 toxin were evaluated by JECFA at the fifty-sixth meeting (Annex 1, reference *152*). The Committee evaluated 4,15-DAS at the present meeting in response to a request from the Codex Committee on Contaminants in Foods (CCCF).

At the present meeting, the Committee reviewed published studies relevant to the human health risk assessment of 4,15-DAS obtained through a comprehensive search of peer-reviewed literature using PubMed, Embase and Global Health. The literature search on the occurrence of and dietary exposure to 4,15-DAS was run using three databases (Scopus, PubMed and Ovid) and a cut-off date of 2000.

Fig. 1
Chemical structures of 4,15-DAS and the type A trichothecenes

4,15-DAS

T-2 (R_1 = OAc) and HT-2 (R_1 = OH) toxins

2. Biological data

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

Oral administration

Female pigs (n = 4; 20 ± 1 kg) were administered 2 mg/kg body weight (bw) of 4,15-DAS (purity not specified) in a soluble gelatine capsule by intubation to determine the pharmacokinetic profile of 4,15-DAS and two unconjugated metabolites, monoacetoxyscirpenol (MAS) and scirpentriol (SCP) in the serum. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 12, 24, 48, 72 and 96 hours after dosing.

4,15-DAS was rapidly absorbed (peak within 30–60 minutes). The mean values of the data for the four pigs demonstrate increasing amounts of the two metabolites and decreasing levels of 4,15-DAS during the first hour, after which

the levels of all three trichothecenes decreased to non-detectable amounts within 48 hours (Bauer et al., 1985).

Intravenous administration

4,15-DAS (98% purity) was administered intravenously to pigs (n = 7) at doses of 0.1 (n = 1), 0.5 (n = 4) or 1 mg/kg bw (n = 3), 48 hours after halothane anaesthesia. Plasma was collected before treatment and at intervals up to 8 hours after treatment. The plasma half-life of 4,15-DAS doses of 0.1, 0.5 and 1 mg/kg bw were reported to be 4, 7.5–8.7 and 7.5–20.4 minutes, respectively, with a total body clearance of 71.55–191.7 mg/min per kg. One pig, with reported subclinical liver disease characterized as chronic mild multifocal hepatocellular necrosis, appeared to have altered the pharmacokinetics of 4,15-DAS (plasma half-life of 150.7 minutes and total body clearance of 27.25 mg/min per kg). In an analysis of the plasma 8 hours after intravenous administration of 4,15-DAS, only SCP was detected while 4,15-DAS and 15-monoacetoxyscirpenol (15-MAS) were not (Coppock et al., 1987).

Dermal administration

To determine the absorption, distribution and excretion of 4,15-DAS, male Fischer rats (90–110 g) and CD-1 mice (25–30 g) were administered a single topical application of [^{3}H]DAS (purity not specified; in dimethyl sulfoxide [DMSO]) proportional to body weight, namely, 0.98 mg to 1.44 cm^2 of rat skin and 0.28 mg to 0.42 cm^2 of mouse skin. Animals were shaved around the body circumference, and 4,15-DAS was evenly spread over the application site. A skin patch was then attached. Four animals were killed at each time point, namely, 90 minutes, 24 hours and 7 days after dosing.

Total radioactivity recovered, expressed as a percentage of the administered dose, was 90.9–97.2% for the rat and 94.2–96.1% for the mouse over the course of the experiment. Rats and mice exhibited different patterns of absorption, excretion and tissue distribution. In rats, absorption and excretion increased over the 90-minute to 7-day time periods, while retention in tissues decreased. In mice, absorption from the application site occurred only during the first 24 hours (which was also reflected in the amount excreted); at 7 days post-treatment, mice retained more in tissues than rats. Over the 7-day period, 57.5% of the dose was absorbed in rats compared with 13.1% in mice (Wang, Busby & Wogan, 1996).

(b) Distribution

Oral administration

Male Fischer rats (90–110 g) and CD-1 mice (25–30 g) were administered a single intragastric dose of [^{3}H]DAS equal to 0.55 mg/kg bw for rats and 0.66 mg/kg bw for mice in DMSO (<10% of oral median lethal dose [LD_{50}] for rats) to determine the time-course tissue distribution and excretion of 4,15-DAS (purity not specified). Four animals were killed at 90 minutes, 24 hours and 7 days after dosing. The authors reported that no visible signs of toxicity or tissue damage were observed.

When expressed as a percentage of dose, the tissue distribution in the rats and mice was quantitatively similar, with most of the radiolabel detected in the carcass, skin, small intestine (duodenum), stomach, liver and kidney (each contained approximately >1% of the initial dose). The rank order was generally stable over the course of the experiment.

When expressed as specific radioactivity (disintegrations per minute per g of tissue), the stomach, small intestine, spleen, liver and kidney ranked higher than the carcass and skin. After 7 days, the rank order shifted to include tissues associated with toxicity such as the lympho-haematopoietic system (spleen, thymus and femur bone marrow), heart and testis (in mice), as well as caecum and large intestine. The decrease in radioactivity with time in the spleen, thymus, femur, heart and testis was less than for liver, kidney, skin and carcass. Radioactivity in the brain, although low (>0.1% of dose), diminished relatively slowly.

The very few interspecies differences were observed in the target tissues (with higher levels generally present in the mouse) and in the small intestine (higher levels in the rat) and kidney and liver (higher levels in the mouse). The authors suggested that these latter findings indicate that the mouse is somewhat more efficient than the rat in metabolizing and excreting 4,15-DAS (Wang, Busby & Wogan, 1990).

Intravenous administration

4,15-DAS (98% purity) was administered by intravenous injection in doses of 0, 0.1, 0.5 or 1 mg/kg bw in pigs (n = 19; 32.7 ± 7.8 kg bw). Animals were killed 8 hours after administration and the liver, kidney, skeletal muscle, mesenteric lymph node and spleen were analysed for 4,15-DAS. The limit of detection (LOD) was 10 ng/g tissue. Residues (≤ 10 ng/g of tissue) were found in all tissues examined without a dose relationship. The highest concentrations of 4,15-DAS were found in the spleen and mesenteric lymph nodes (Coppock et al., 1988).

Dermal administration

Following a single topical application of [^{3}H]DAS (in DMSO) proportional to body weight, 0.98 mg to 1.44 cm^2 of rat skin and 0.28 mg to 0.42 cm^2 of mouse skin (previously described in section 2.1.1(a)), the absorbed radiolabel was rapidly and widely distributed in the tissues, especially carcass, skin (not the application site), liver, kidney, gastrointestinal tract, spleen, bladder, testes and femur. In rats, there was a shift with time of localization towards target tissues such as immune and haematopoietic tissues, gastrointestinal tract and testes; in mice, this shift was from the target tissues (spleen, thymus) and excretory organs (kidney and bladder) to carcass and skin. In both species, despite rapid absorption and excretion, the level of DAS or its metabolites (residues not identified) remained relatively stable in the tissues (Wang, Busby & Wogan, 1996).

(c) **Excretion**

Oral administration

Following a single intragastric dose of [^{3}H]DAS (in DMSO) equal to 0.55 mg/kg bw for rats and 0.66 mg/kg bw for mice, urinary and faecal excretions were rapid and essentially complete in both rats and mice within the first 24 hours, with 93.7% and 90.3% of the dose excreted, respectively. The ratio of urinary to faecal excretion was approximately 4.5:1 for both species. The excretion within the first 24 hours was paralleled by a decline of radiolabel in the gastrointestinal tract contents, organs and carcass. The remaining radioactivity plateaued over the following 6 days (1.4–2.8% in rats and 2.8–3.4% in mice). These residues were not identified.

Urine and faecal analysis following administration of a soluble gelatine capsule of 2.0 mg/kg bw of 4,15-DAS (purity not specified) to pigs by intubation showed that most of the administered 4,15-DAS was excreted in the faeces as metabolites and only low amounts of 4,15-DAS or the metabolites were detected in the urine. The main metabolites analysed in the faeces were determined to be SCP and de-epoxy SCP. In addition, up to 26% of the orally intubated dose was eliminated by vomit, either as unchanged 4,15-DAS or as 15-MAS (approximately 2.3% of dose) (Bauer, Gareis & Gedek, 1989).

Intravenous administration

Coppock et al. (1987) reported that urine collected 15 minutes after 4,15-DAS was intravenously administered to pigs at a dose of 0.5 mg/kg bw was found to contain less than 1% of the parent compound. The relative concentrations of 4,15-DAS, 15-MAS and SCP in urine 15 minutes after dosing were SCP >> 15-MAS > 4,15-DAS. Urine from additional time points was not analysed.

Dermal administration

Following a single topical application of [^{3}H]DAS (in DMSO) proportional to body weight, 0.98 mg to 1.44 cm^2 of rat skin and 0.28 mg to 0.42 cm^2 of mouse skin, the total excretion of the radiolabel by rats was approximately 6-fold higher than by mice (56% versus 9%), with an excretion ratio for urine to faeces of 2:1 for rats and 3.5:1 for mice. More than 90% of total excretion occurred within 3 days of treatment, with 70–80% of total excretion within the first 24 hours (Wang, Busby & Wogan, 1996).

2.1.2 Biotransformation

(a) In vivo

Male Wistar rats were orally administered DAS (details not provided) at 2.8 mg/kg bw, 3 times at 7-day intervals. Urine and faeces were collected daily for 21 days. DAS was not detected in the urine or faeces, but MAS, SCP, de-epoxy MAS and de-epoxy SCP were detected in urine. However, only the two de-epoxy metabolites were detected in the faeces. The authors concluded that these results indicate that the liver microsomal enzymes are involved in the hydrolysis of DAS and that the gastrointestinal microorganisms participate in the de-epoxidation reaction (Sakamoto et al., 1986).

Wistar rats (n = 3/sex; 200–250 g) and chickens (n = 3/sex; 1.0–1.2 kg) were fasted for 12 hours before receiving a single dose of 4,15-DAS (>98% pure) of 3 mg/kg bw in ethanol/water (30:70, volume per volume) by gavage. Urine and faecal samples were collected prior to dosing and during the 0–12 hours and 12–24 hours after dosing. The urine and faecal samples were analysed by ultra-high performance liquid chromatography–quadrupole time of flight–mass spectrometry (UHPLC-Q/TOFMS) (Yang et al., 2015).

For the 0–12 hours after administration, 4,15-DAS, 4-MAS, 15-MAS, neosolaniol (NEO) and 7-OH-DAS were detected in rat urine and 15-MAS and 7-OH-DAS in rat faeces. In the 12–24 hours after administration, no metabolites were detected. Similar results were found in the chickens: during the 0–12 hours after administration, 15-MAS and 7-OH-DAS were detected in the faeces.

These results confirm that 4,15-DAS is rapidly absorbed from the gastrointestinal tract and extensively metabolized and excreted in rats and chickens.

Consistent with the in vitro results, 15-MAS and 7-OH-DAS were the major metabolites in rats and chickens. Of the two hydrolysed metabolites, a large amount of 15-MAS and trace amounts of 4-MAS were detected in the urine of rats, which further confirms that the deacetylation of 4,15-DAS occurs first at the C-4 and then at the C-15 positions. A large amount of 15-MAS was also detected in the chicken faeces. SCP, which was identified in vitro, was not detected in vivo.

The two hydroxylated metabolites, NEO and 7-OH-DAS, were both detected in rat urine whereas only 7-OH-DAS was detected in chicken urine. One epimer of 7-OH-DAS (M7) was produced in large amounts in both rats and chickens; this is consistent with in vitro results, indicating that hydroxylation at C-7 was a major metabolic pathway. With respect to phase II metabolites, no glucuronide conjugates were detected in rats and chickens in vivo. In vitro, glucuronidation at C-3 was the weakest in rats and chickens compared with other species (Yang et al., 2015).

As previously described (section 2.1.1(a)), following oral administration of 4,15-DAS (2 mg/kg bw) to female pigs, 4,15-DAS and its two metabolites, 15-MAS and SCP, were detected in the serum (Bauer et al., 1985; Bauer, Gareis & Gedek, 1989). The authors suggested that 4,15-DAS is deacetylated in a stepwise manner, first at C-4 and then at C-15. In addition, the majority of the administered 4,15-DAS was excreted in the faeces as 15-MAS, SCP, de-epoxy-15-MAS or de-epoxy SCP. The authors suggested that the presence of 15-MAS in the vomit of the pigs within 1 hour of administration indicates a presystemic hydrolysis of DAS in the stomach, which may be due to the activity of gastric enzymes or acid.

The proposed phase I and II metabolic pathway of 4,15-DAS is shown in Fig. 2.

(b) **In vitro**

4,15-DAS (purity not specified) was extensively biotransformed when incubated with bovine rumen fluid from a fistulated dairy cow for 12, 24 and 48 hours. Under anaerobic conditions, 4,15-DAS was biotransformed to MAS, SCP and their corresponding de-epoxides. These results indicate pre-systemic metabolism and partial detoxification of 4,15-DAS. Direct de-epoxidation of 4,15-DAS (resulting in de-epoxy DAS) was not detected, suggesting that C-4 deacetylation occurred prior to de-epoxidation (Swanson et al., 1987).

Following the anaerobic incubation of 4,15-DAS (purity not specified) with the faecal microflora of rats, cattle and pigs, 4,15-DAS was completely biotransformed, primarily to de-epoxy MAS and de-epoxy SCP. In contrast, faecal microflora from chickens, horses and dogs failed to reduce the epoxide group in 4,15-DAS and yielded only MAS in addition to smaller amounts of SCP and unmetabolized 4,15-DAS. Intestinal (caecal) microflora from rats completely biotransformed 4,15-DAS to de-epoxy MAS and de-epoxy SCP, with traces of SCP. No de-epoxy metabolites were formed upon incubation of 4,15-DAS with rat faecal and intestinal microflora under aerobic conditions (Swanson et al., 1988).

Fig. 2

Proposed phase I and II metabolic pathways of 4,15-DAS: (A) hydrolysation, (B) hydroxylation and (C) glucuronidation

Source: Yang et al. (2015)

The results of the Young et al. (2007) study, examining the degradation of 4,15-DAS (purity not specified) by chicken intestinal microbes, were consistent with that reported by Swanson et al. (1988). There was no evidence of de-epoxy products, and mono-deacetylation (~66%) at the C-4 position was favoured over the sterically protected C-15 acetyl (di-deacetylation ~20%).

After DAS (details not provided) was incubated with rat and rabbit liver microsomes, 15-MAS was identified by gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. The microsomal nonspecific carboxyesterase from the rat and rabbit liver was attributed with hydrolysing DAS to 15-MAS (Ohta et al., 1978). Similarly, carboxylesterase isoenzymes isolated from CD-1 mouse liver microsomes hydrolysed 4,15-DAS only at the C-4 acetyl group and not the C-15 to form 15-MAS (Wu & Marletta, 1988).

In bile obtained from isolated rat liver perfused with DAS (details not provided), the glucuronide conjugates of MAS (the major component) and SCP were detected. No unconjugated metabolites were detected (Gareis et al., 1986).

Three metabolites were found when 4,15-DAS was incubated with uridine 5′-diphospho-glucuronic acid (12 mmol/L), β-naphthoflavone-induced hepatic microsomes from male Long–Evans rats, $MgCl_2$ and K_2HPO_4 for 1.5 and 3.3 hours. The metabolites were identified as glucuronide-DAS, 15-MAS and 4-MAS (Roush et al., 1985).

Phase I and II metabolism of 4,15-DAS (>98% pure) was investigated using rat, chicken, pig, goat, cow or human liver microsomes by UHPLC-Q/TOF. A total of seven phase I and three phase II 4,15-DAS metabolites were detected. The major metabolic pathways of 4,15-DAS in vitro were hydrolysation at the C-4 (15-MAS [M2]) and C-15 (4-MAS [M1]) positions with further hydrolysation to form SCP (M3), hydroxylation at the C-7 (7-OH-DAS [M6 or M7 epimers] and C-8 (8β-OH-DAS [M4] and NEO or 8α-OH-DAS [M5]) positions and conjugation (DAS-3-glucoside [M8], 15-MAS-3-glucoside [M9] and 15-MAS-4-glucoside [M10]). Qualitative differences in phase I and II metabolic profiles of 4,15-DAS between the five animal species and humans were observed. After incubation for 2 hours with liver microsomes, the majority of the 4,15-DAS was converted into its various metabolites. Only a small amount of 4,15-DAS remained after incubation with human or goat microsomes, whereas a larger amount of 4,15-DAS was found with cow liver microsomes. After a comparison of the residual amounts of 4,15-DAS in the liver microsomes of the different species, the ability of human liver microsomes to metabolize 4,15-DAS was found to be the greatest, followed by that of goat, rat, chicken, pig and cow.

M2 (15-MAS) was the most common metabolite in liver microsomes, especially human, goat and chicken, indicating that the hydrolysis of the ester bond at C-4 varied in different species. M1 (4-MAS) hydrolysis at C-15 was only detected in rat liver microsomes in trace amounts. Small amounts of M3 (SCP) were detected in the rat, pig and goat and none in the other species. Hydroxylated metabolites, including M4 (8β-OH-DAS) and M5 (NEO) hydroxylated at the C-8 position, were detected in the rat, pig, cow and human but not the chicken. The amount of M5 in the rat, pig, goat and cow liver microsomes was greater than M4, while in humans, the amount of M5 was roughly equal to M4. Of the other hydroxylated metabolites, M6 and M7 (7-OH-DAS), a pair of epimers, and M7 were detected in large amounts in the chicken, rat and human liver microsomes.

A total of three glucuronide conjugates of 4,15-DAS were detected in the liver microsomes of all species except chicken. M8 was the main phase II metabolite of 4,15-DAS in pig, goat, cow and human liver microsomes, whereas in rat microsomes this was M9. Glucuronidation ability at C-3 was reported to be highest in the cow followed by the goat, pig, human, rat and chicken, respectively.

On incubating pig, goat, cow and human liver microsomes, trace amounts of M10 were found with larger amounts of M8 and M9.

Overall, the available biological data suggest that 4,15-DAS is metabolized by mammalian gut microflora to several metabolites including de-epoxides. Metabolism continues in the liver biphasically: phase I involves deacetylation (first at C-4 and then at C-15), and phase II involves conjugation with glucuronic acid (Yang et al., 2015).

2.1.3 Effects on enzymes and other biochemical parameters

(a) Enzymes

Male Sprague Dawley rats (n = 8; 140–160 g) were administered either 4,15-DAS (purity not specified) or T-2 toxin at a dose of 1 mg/kg bw daily in corn oil for 1, 4 or 8 days. Animals were killed 24 hours after the last treatment, and liver, lung and kidney tissue were examined. In plasma, significant decreases in the total proteins were seen in all groups of rats treated with either 4,15-DAS or T-2 toxin (20% and 39%, respectively). Increases in the aspartate aminotransferase activity were observed in 4- or 8-day 4,15-DAS and T-2 toxin treatment groups. The decline in total proteins paralleled the decrease in microsomal (17% and 31%, respectively) and cytosolic proteins (approximately 19%) in the liver. Reduced levels of hepatic microsomal cytochrome P450 were observed in animals following 8 days of treatment with 4,15-DAS (50%) or T-2 toxin (22%). Of the conjugating enzymes, *p*-nitrophenol glucuronyltransferase activity increased in all groups of animals, whereas there was no change in conjugation to glutathione or acetate. There was no change in any renal enzymes, but there was a significant increase in pulmonary monooxygenase in the 4- and 8-day T-2 toxin treatment groups (Galtier et al., 1989)

(b) Microflora

In order to examine the effect on the microflora of the gut and on plasma glucocorticoid levels, female HUNGAHYB synthetic-line piglets (40–50 days old; 10–12 kg bw) were fed 4,15-DAS (purity not specified) ad libitum at a concentration of 5 mg/kg feed for 7 days followed by 10 mg/kg feed for another 7 days. Similarly, Wistar rats (100–150 g bw) were fed 4,15-DAS at 10 mg/kg for 20 days. Half of the animals were killed after 5 days on experimental feed and the other half at the end of the experimental period. After 5 days, a substantial increase in aerobic bacteria count in the intestine was observed in both pigs and rats. Increasing the 4,15-DAS dose failed to elicit a response. The plasma cortisol and corticosterone levels were 2- to 3-fold higher than the controls in both pigs and rats (Tenk, Fodor & Szathmary, 1982).

(c) Biochemical modes of action

In whole animals, the trichothecene mycotoxins are known to target tissues with high proliferative or turnover rates such as epithelium, thymus, spleen, bone marrow, ovary, testis and lymph nodes. Cyto-morphological surveys have demonstrated karyorrhexis,[1] in a manner similar to radiometric injury (Saito & Ohtsubo, 1974). Trichothecenes are very cytotoxic to eukaryotic cells, and this biological activity is closely related to their lethal toxicity to whole animals. Early evidence for the mechanism of the cytotoxicity included a breakdown of the polyribosomal profile at a cellular level. Based on the different modes of action on polyribosomal behaviour, the trichothecene mycotoxins were grouped into two types: type one mycotoxins inhibit the initial step of protein synthesis (I-type; e.g. DAS, T-2 toxin, HT-2 toxin, nivalenol [NIV]); and type two inhibit the elongation–termination step (ET-type; e.g. deoxynivalenol [DON]). The target organelle is the 60S subunit of eukaryotic ribosomes with inhibition of peptidyl transferase and subsequent inhibition of peptide bond formation. It has been shown that the protein inhibition activity correlates well with ribosome affinity (Ueno, 1983).

Trichothecene mycotoxins are also potent inhibitors of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) syntheses in whole cells. Since the inhibition of DNA and RNA synthesis requires higher toxin concentrations and the degree of inhibition was less when compared to that of protein synthesis, it was thought that the DNA and RNA synthesis inhibition was secondary to the impairment of protein synthesis. However, the inhibition of nucleic acid synthesis is not simply a secondary effect caused by the impairment of protein synthesis. Damage in cell organization may have an important effect on nucleic acid synthesis. It is assumed that damage to membrane structure and cytoskeleton components affects macromolecule synthesis (Ueno, 1983; RIVM, 2002).

Cytotoxicity and protein synthesis inhibition

Tscherne & Pestka (1975) examined 4,15-DAS (purity not specified) for the effects of protein inhibition on HeLa cell polysomes under three conditions (directly; during hypertonicity; and during recovery from hypertonicity) to localize the site of action in the intact cell. Treatment with 4,15-DAS (1 μmol/L or 0.366 μg/mL) resulted in either few or no polysomes under all three test conditions, which was consistent with inhibition of initiation in intact HeLa cells.

Mizuno (1975) studied the mechanism of inhibition of initiation using DAS (details not provided) treatments (1–2 μmol/L or 0.366–0.732 μg/mL) on

[1] Fragmentation of the nucleus whereby its chromatin is distributed irregularly throughout the cytoplasm; a stage of necrosis usually followed by destruction of the nucleus.

rabbit reticulocyte lysates and found that the inhibition occurs after the formation of the first peptide bond.

The reversibility of protein synthesis inhibition was studied by Liao, Grollman & Horwitz (1976) using HeLa cells and rabbit reticulocyte lysates. 4,15-DAS (purity not specified) and T-2 toxin were irreversible inhibitors. After the addition of 1 μmol/L (0.366 μg/mL) of 4,15-DAS to rabbit reticulocyte lysates, polyribosomes were broken down to monosomes.

Cundliffe & Davies (1977) also found that DAS (details not provided) caused a complete breakdown of polyribosomes, behaving as an I-type inhibitor. However, polyribosomes were extensively preserved when H-HeLa cells were exposed to high concentrations of DAS (100 μg/mL), indicating that DAS has a secondary effect upon polypeptide chain elongation at very high concentrations. It was concluded that DAS acts as an intermediate between an I-type and an ET-type inhibitor of protein synthesis.

Hernandez & Cannon (1982) reported similar results in yeast cells and spheroplasts, whereby 4,15-DAS (purity not specified) inhibited recently initiated ribosomes and at a high concentration efficiently blocked elongation. This inhibition was reversible when 4,15-DAS was removed from the target site by washing, in contrast to the findings of Liao, Grollman & Horwitz (1976).

Some examples of the cytotoxicity and inhibition of protein synthesis of 4,15-DAS and other type A trichothecenes are shown in Table 1. The most potent type A trichothecenes are those with an acetyl group at R_3. Removal of this acetyl group results in a pronounced decrease in potency, while removal of the acetyl group at R_2 and the side-chain at C-8 results in a smaller loss of activity (Thompson & Wannemacher, 1986).

The potency ranking, in vitro and in vivo, was relatively consistent; however, for 4,15-DAS and 15-MAS, the in vitro cell system demonstrated a reduced potency, which is the expected response to the removal of acetyl groups from the basic ring structure, but in vivo, 4,15-DAS was significantly less toxic than 15-MAS. Thompson & Wannemacher (1986) suggested that this discrepancy indicates that either secondary effects or more potent metabolites are involved or a mechanism of action other than protein synthesis inhibition accounts for the toxicity.

DNA synthesis inhibition

4,15-DAS (purity not specified) has also been shown to inhibit DNA synthesis (total inhibition by 0.008 μg/mL; 50% inhibition by 0.003 μg/mL) in mitogen-stimulated human peripheral blood lymphocytes. When metabolic activation (by isolated rat liver cells) was present, the inhibitory effect of 4,15-DAS was

Table 1
Cytotoxicity and protein synthesis inhibition of 4,15-DAS, 4,15-DAS metabolites and other type A trichothecenes in cultured human[a] and animal cells

					IC_{50} (µg/mL)			IC_{50} (nmol/L) / relative potency[b]			i.p. LD_{50} (mg/kg bw)
Metabolite	R_1	R_2	R_3	C-8	HeLa	HEK	HL	Rabbit reticulocytes whole cells	Monkey kidney Vero cells	Rat spleen lymphocytes	Mouse
T-2 toxin	OH	OAc	OAc	$OCOCH_2CH(CH_3)_2$	0.01	0.02	0.003	0.03	14 / 100	6 / 100	9.1
HT-2 toxin	OH	OH	OAc	$OCOCH_2CH(CH_3)_2$	0.01	0.1	0.01	0.03	65 / 22	10 / 63	10.1
4,15-DAS	OH	OAc	OAc	H	0.01	0.01	0.001	0.03	27 / 53	12 / 53	15.3
NEO	OH	OAc	OAc	OH	0.1	0.06	0.05	0.25	273 / 5.2	127 / 4.8	14.8
15-MAS	OH	OH	OAc	H	0.1	0.1	0.3	–	73 / 20	30 / 21	4.5
SCP	OH	OH	OH	H	–	–	–	–	861 / 1.7	737 / 0.83	–

bw: body weight; DAS: diacetoxyscirpenol; IC_{50}: median inhibitory concentration; i.p.: intraperitoneal; LD_{50}: median lethal dose; MAS: monoacetoxyscirpenol; NEO: neosolaniol; SCP: scirpentriol
[a] Uterine carcinoma (HeLa), embryonic kidney (HEK), lymphocytes (HL) cells.
[b] Compared to T-2 toxin, which was set as standard and as having 100% potency.
Sources: Ueno (1977, 1983); Thompson & Wannemacher (1986)

somewhat reduced (Cooray, 1984), which suggests that the observed effect was due to 4,15-DAS rather than one or more of the metabolites.

Induction of apoptosis

Lee, Park & Kim (2006) demonstrated that human Jurkat T-cells exposed to 4,15-DAS, 15-MAS, 4-MAS and acetyldiacetoxyscirpenol (TAS; 0.05–0.1 µg/mL; purity not specified) exhibited typical cascades of apoptotic cell death as well as phosphatidyserine externalization, the loss of mitochondrial-membrane potential, the acceleration of poly (ADP-ribose) polymerase cleavage and the fragmentation of genomic DNA in the absence of necrosis. Acetoxylation at the C-15 appeared to be essential for the manifestation of apoptotic activity in Jurkat T-cells. The order of the DNA fragmentation activity in the Jurkat T-cells was 4,15-DAS > 15-MAS > TAS > 4-MAS.

Nasri et al. (2006) compared the potency of 4,15-DAS and T-2 toxin (type A trichothecenes) and DON and NIV (type B trichothecenes) (purity not specified) to reduce mitochondrial activity and to induce apoptosis of Jurkat T-cells. 4,15-DAS and T-2 toxin were found to be more cytotoxic at low concentrations than type B trichothecenes (median inhibitory concentration [IC_{50}]: 0.003–0.005 versus 0.4–0.8 µmol/L, type A and type B, respectively). In addition, the mechanism by which the type B trichothecenes induced cytotoxicity was mainly

via apoptosis, whereas type A trichothecenes reduced mitochondrial activity at concentrations approximately 1000-fold lower than the type B trichothecenes, resulting in necrosis (0.01 µmol/L type A versus 10 µmol/L type B).

Jun et al. (2007) demonstrated that Jurkat T-cell toxicity of 4,15-DAS (purity not specified; 0.01–0.15 µmol/L or 0.004–0.06 µg/mL) was due in part to apoptosis initiated by caspase-8 activation and subsequent mitochondrion-dependent or -independent activation of caspase cascades and also in part to the interruption of cell cycle progression initiated by downregulation of cyclin-dependent kinase 4 and cyclin B_1 proteins.

2.1.4 Transfer from feed to food

No evidence was found on the transfer of 4,15-DAS from feed to food of animal origin.

2.2 Toxicological studies

2.2.1 Acute toxicity

The acute lethal doses of 4,15-DAS for various routes of exposure are summarized in Table 2, and the systemic effects of acute exposure to 4,15-DAS are described in the following text.

(a) Oral administration

In an acute lethality test conducted by Conner et al. (1986), multiple groups of 4–6 male CD-1 mice (21–34 g) were administered 4,15-DAS via oral gavage at doses ranging from 2 to 22 mg/kg bw. Based on the mortality rates observed within 96 hours of dosing, the LD_{50} of 15.5 mg/kg bw was calculated using graphical methods. Prior to death (within 36 hours), animals showed signs of lethargy, trembling, diarrhoea and cyanosis. Characteristic morphological changes in animals that died included extensive necrosis of bone marrow, thymic cortex, epithelium of the large and small intestines, and splenic red pulp. In some animals, degeneration of germinal epithelium of seminiferous tubules of the testis with formation of syncytia was found. Liver, heart, brain, stomach, lungs and kidneys showed no morphological evidence of damage. Animals that survived to 96 hours had similar but less severe lesions.

Craddock, Hill & Henderson (1988) exposed female Wistar rats (8/dose; 195–205 g) to single doses of 0.0125, 0.06, 0.125, 0.5 or 2.0 mg/kg bw of 4,15-DAS (purity not specified) by oral gavage. Animals exposed to 2.0 mg/kg bw showed an increase in cell replication in the oesophagus and stomach (squamous and glandular) 1 day after dosing. Within 4 days of dosing, the oesophagus and glandular stomach of affected animals returned to normal. At lower doses, the

Table 2
Acute toxicity of 4,15-DAS

Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
Mouse	M	Oral	15.5	Conner et al. (1986)
Mouse	M	i.p.	20.0	Conner et al. (1986)
Mouse	M	i.p.	15.3	Thompson & Wannemacher (1986)
Mouse	M	s.c.	19.5	Thompson & Wannemacher (1986)
Mouse	NS	i.v.	12	Ueno (1983)[a]
Mouse (newborn)	NS	s.c.	0.17	Ueno (1983)
Rat	NS	oral	7.3	Ueno (1983)
Rat	NS	i.p.	0.75	Ueno (1983)
Rat	NS	i.v.	1.3	Ueno (1983)
Rabbit	NS	i.v.	1.0	Ueno (1983)
Dog	M & F	i.v.	~1.0	IRDC (1973); Ueno (1983)
Pig	NS	i.v.	0.376	Weaver et al. (1978a)
Chicks	NS	Oral	2.0	Richardson & Hamilton (1990)[b]
Chicks (1 day old)	NS	Oral	3.82	Chi et al. (1978)
Chicks (7 days old)	M	Oral	5.0	Hoerr, Carlton & Yagen (1981a)

bw: body weight; DAS: diacetoxyscirpenol; F: female; i.p: intraperitoneal; i.v.: intravenous; LD_{50}: median lethal dose; M: male; NS: not stated; s.c.: subcutaneous
[a] Details not provided.
[b] Chick LD_{50} for 3,4-DAS >30 mg/kg bw; for 3,15-DAS = 10.1 mg/kg bw.

effects were limited to the squamous and glandular stomach. According to the authors, patchy hyperplasia was still apparent at a dose of 0.06 mg/kg bw.

Hoerr, Carlton & Yagen (1981a) exposed male broiler chicks (7 days old) to 4,15-DAS via a single oral gavage dose of 2.7 mg/kg bw. Clinical signs of toxicity following dosing included reduced spontaneous activity and slightly fluid faecal droppings within the first 24 hours. Gross necropsy revealed clear fluid in the small intestine; diffusely reddened skeletal muscle; disseminated red or white 1 mm diameter foci in the liver; and distended and inflamed gall bladders. Histopathology revealed necrosis of the epithelium in the small and large intestine and in the proventriculus and gizzard; hyperplasia of bile ductules; necrosis of gall bladder epithelium; necrosis of lymphoid tissue and bone marrow; and necrosis of the feather epidermis and of the follicular epidermis at the neck of the feather follicle. Most lesions had recovered within 168 hours of treatment.

Following the exposure of four female pigs (about 20 kg) to 2 mg/kg bw of 4,15-DAS via intubation with a stomach-soluble gelatine capsule (section 2.1.1(a)), three of the four pigs showed strong salivation 10 minutes after dosing. Within approximately 1 hour of dosing, all the animals showed emesis that lasted for 30–60 minutes. Up to approximately 26% of the oral dose was eliminated via the vomit. Subsequently, apathy, anorexia and posterior paresis were observed

for approximately 12 hours. However, 24 hours after dosing, no clinical signs of intoxication were apparent. All animals survived the 96-hour observation period (Bauer et al., 1985; Bauer, Gareis & Gedek, 1989).

(b) Intraperitoneal administration

In an acute lethality test conducted by Conner et al. (1986), multiple groups of 4–6 male CD-1 mice (21–34 g) were administered 4,15-DAS via intraperitoneal injection at doses ranging from 14 to 24 mg/kg bw. Based on the mortality rates observed within 96 hours of dosing the LD_{50} of 20.0 mg/kg bw was calculated using graphical methods. Prior to death (within 36 hours) animals showed similar signs as were observed after gavage dosing including lethargy, trembling, diarrhoea and cyanosis. In a follow-up study, intraperitoneal exposure to as little as 5 mg/kg bw caused cell depletion in the bone marrow associated with leukocytosis and nucleated erythrocytes in the peripheral blood shortly after exposure to higher doses. Following intraperitoneal exposure to 15 mg/kg bw, 4,15-DAS caused anaemia with rapid onset (1 day) and relatively rapid recovery (7 days). Terminal necropsy showed extensive necrosis in the bone marrow, thymic cortex, epithelium of the large and small intestines and splenic red pulp. Necrosis in the intestine of most of the moribund animals was described as transmural. Degeneration of the germinal epithelium of the seminiferous tubules in the testes was also commonly found. The liver, stomach, lungs and kidneys appeared unaffected.

(c) Intravenous administration

Pigs appear to be the most sensitive to the acute lethal effects of intravenous 4,15-DAS (see Table 2 for a comparison of LD_{50}s). Weaver et al. (1978a) administered purified 4,15-DAS via intravenous injection to 12 young feeder pigs (9–23 kg bw) and one sow (113 kg bw) at doses from 0.3 to 0.5 mg/kg bw. Within 20 minutes of the intravenous injection, signs of toxicity (i.e. frequent defecation) were observed and continued to worsen. Within 3 hours of treatment, animals showed vomiting, lethargy and diarrhoea. Death occurred in 7/12 feeder pigs within 18 hours. An intravenous LD_{50} of 0.376 ± 0.043 mg/kg bw was calculated using the Probit method. The animals that survived past 18 hours recovered within 8 days. Most of the treatment pigs did not show gross intestinal lesions. However, two of the feeder pigs that died showed 4,15-DAS–induced haemorrhagic bowel lesions whereas the sow showed severe mucosal congestion of the jejunum and ileum. In addition, acute necrosis of the germinal centres of the mesenteric lymph nodes and spleen were consistently observed in the feeder pigs that died.

Coppock et al. (1985) exposed male and female pigs (~35 kg bw) to 0, 0.5 or 1 mg/kg bw doses of 4,15-DAS (purity not specified) via intravenous injection

in order to define the distribution and extent of pathological changes produced by 4,15-DAS. The pigs (4/group) were dosed, observed for 8 hours, killed and examined for gross and histopathological lesions. Shortly after dosing (e.g. <10 minutes), treatment pigs showed involuntary or habitual grinding of the teeth and vomiting. Within 1–3 hours, treatment pigs developed diarrhoea. Ataxia, flushing of the skin, muscular weakness, depression and coma were sequentially observed. One male pig exposed to 0.5 mg/kg bw died within 4 hours and one male pig exposed to 1 mg/kg bw died within 7 hours. Upon gross necropsy, dose-responsive lesions were observed in "mitotically and metabolically active tissues" of treatment pigs, that is, gastrointestinal tract, all lymphoid tissue and adrenal glands. The gastric lesions were limited to the glandular stomach and intestines and were characterized as necrotizing, haemorrhagic gastritis. Congestion and haemorrhage occurred more frequently around the Peyer patches. Severity of lesions in the intestine were in the following order: caecum > colon > ileum > jejunum and duodenum. Lymph tissues showed lympholysis in the germinal centres. The adrenal cortex showed oedema, congestion and haemorrhage. Other organs/tissues affected by 4,15-DAS treatment included the pancreas (e.g. pyknosis, karyorrhexis and necrosis), brain (e.g. multifocal capillary endothelial necrosis and haemorrhage), kidney (e.g. necrosis and detachment of the epithelial cells in the proximal tubules), gall bladder (e.g. oedema and haemorrhage), bone marrow (e.g. pyknosis, karyorrhexis and cell debris) and salivary gland (e.g. necrosis of the ductal secretory cells of the parotid salivary gland).

Beagle dogs (n = 1/sex per treatment) were administered 4,15-DAS (purity not specified) as a single intravenous dose of 0, 0.031, 0.063, 0.125, 0.25, 0.5, 1.0 (n = 4 dogs) or 2.0 mg/kg bw. One female dog at 0.5 mg/kg bw died 36 hours after treatment and one at 2.0 mg/kg bw died 22 hours after treatment. Clinical observations included emesis and erythema in dogs at 0.063 mg/kg bw and higher doses; diarrhoea and polydipsia at 0.125 mg/kg bw and higher doses; hypoactivity at 0.25 mg/kg bw and higher doses; hypothermia at 0.5 mg/kg bw and higher doses; and weakness and ataxia at 1.0 mg/kg bw and higher doses. Necropsy of the moribund animals revealed congestion of the heart, spleen, lung, stomach, kidney and thymus and haemorrhage and/or necrosis of the small intestine, Peyer patches and bone marrow. Necropsy of all the dogs that survived the study period (8 or 45 days) was reported to be unremarkable (IRDC, 1973).

In terms of interspecies differences, Coppock et al. (1989) showed that pigs are more sensitive to acute sublethal effects (i.e. haematotoxicity) of 4,15-DAS (>98% pure) than dogs and cattle (female calves) following intravenous administration. Pigs (~35 kg), dogs (~14 kg) and cattle (~137 kg) (4 animals/dose group) were exposed to 0 or 0.5 mg/kg bw 4,15-DAS via intravenous injection and killed after 8 hours (before death ensued). An additional group of pigs were exposed to 1 mg/kg bw 4,15-DAS via intravenous injection. Blood samples were

taken at half-hour intervals and the acute haematological aspects of 4,15-DAS intoxication were investigated. The following sequential clinical signs of toxicity were observed in all treatment animals: ptyalism, diarrhoea, ataxia, muscular weakness and depression. Pathological investigation revealed lesions in the bone marrow of treatment animals consisting of cellular necrosis in the haematopoietic cords. The order of intensity of the lesions was pigs ~ dogs >> cattle. In terms of effects of 4,15-DAS on blood cell numbers and morphology (i.e. a marked increase in the number of immature neutrophils and replacement of lymphocytes with immature cells), the following order in species sensitivity was observed: pigs > dogs >> cattle.

(d) Dermal administration

After the discovery of the dermal toxicity of metabolites of *F. sporotrichioides* and related fungi associated with the occurrence of alimentary toxic aleukia, a dermal toxicity test was introduced to screen trichothecene mycotoxins and the fungi that produce them. DAS, T-2 toxin and HT-2 toxin were isolated from a culture of *F. tricinctum*, and the relative dermal toxicity was compared in mice, rats and guinea-pigs using topical, intradermal and subcutaneous routes of exposure. Sequential and gross histological investigations of cutaneous lesions produced by low concentrations (not quantified) of DAS and T-2 toxin revealed a nonspecific acute dermal inflammation reaction, characterized by hyperaemia, oedema and neutrophil exudation, with varying degrees of necrosis of the epidermis. The degree of dermal toxicity of trichothecenes depended on the chemical structure, with the highest toxicity observed with macrocyclic (type D), followed by type A and type B in decreasing order. This relative toxicity is comparable to the cytotoxicity observed in cultured cells (Ueno, 1983).

Ueno et al. (1970) described cutaneous lesions produced in guinea-pigs. DAS (details not provided; 0.2 μg) resulted in necrosis of the epidermis with cellular infiltration of the epidermis and 1 μg caused necrosis and cellular infiltration of the entire width of epidermis accompanied by necrosis of hair follicles.

Hayes & Schiefer (1979) examined the cutaneous responses to topical irritation by 4,15-DAS (purity not specified) and T-2 toxin in rats and rabbits. Sequential gross and histopathological observations on cutaneous lesions were described for a topically applied concentration (80 μg/mL) of T-2 toxin to rats. Twelve hours after administration, flat hyperaemic plaques were observed that subsequently increased in redness to a maximum at 48 hours after administration. Plaques became swollen and centrally pale by 18 hours and were covered with moist exudation by 24–30 hours. After 48 hours, the redness subsided and the plaques became covered with a dry friable exudate. Scaly superficial flakes were

frequently present over the reaction site at 72 hours but broke away by 6 days leaving a smooth pink hairless spot that gradually diminished over the following week to be virtually unnoticeable by day 14.

Neutrophils were sequestered in dermal capillaries and at the luminal margins of venules by 3 hours after application. Between 6 and 12 hours after application, the nuclei of fibroblasts in the interfollicular dermis were pyknotic and karyorrhectic. Neutrophils had infiltrated throughout the dermis by 12 hours and into the outer layers of the epidermis by 24 hours, concentrated in and below the epidermis at 48 hours. Few neutrophils were observed at 72 hours or later. Changes in the epidermis and hair follicles were variable during the first 24 hours. Isolated pyknotic nuclei were found infrequently in the basal layer of the epidermis. Epidermal spongiosis and subepidermal oedema were evident at some sites. By 24 hours, focal areas of coagulation necrosis of the epidermis were found in the more severe reactions but in milder reactions, the epidermis overlying the dermal inflammatory response was intact. Hyperplasia of the epidermis began after 24 hours, became prominent by 48 hours and persisted throughout the 14-day observation period. Subepidermal fibroplasia appeared by 6 days and subsequently increased.

Reactions to low doses (10–30 µg/mL) of T-2 toxin and 4,15-DAS were often greater at 24 hours than at 48 hours, by which time some reaction had disappeared. At slightly higher concentrations (30–100 µg/mL), reactions generally increased to a peak intensity at 48 hours and were more clearly graded according to dose. These differences in reactions were attributed to differences in concentration of the irritant applied and to the reactions intensifying over 48 hours because of secondary dermal and epidermal damage associated with more severe purulent inflammation (Hayes & Schiefer, 1979).

Overall, 4,15-DAS induces similar acute systemic responses in all of the test species regardless of the route of exposure. According to the lower LD_{50} values reported by Ueno et al. (1983), rats appear more sensitive than mice to the acute toxicity of DAS (details not reported) via intraperitoneal, intravenous and oral routes. The lowest oral LD_{50} was observed in chickens. This expected result, based on the biotransformation information, indicates that de-epoxidation by the gut microflora and conjugations in the liver did not take place in chickens. Quantitative differences observed in intraspecies toxic responses to 4,15-DAS may be related to the differences in the respective developmental stages of the animals during testing. For example, a comparison of a subcutaneous LD_{50} in newborn mice (0.17 mg/kg bw) with that of adult mice (19.5 mg/kg bw) indicates that newborn mice are much more sensitive than the adults (Thompson & Wannemacher, 1986).

2.2.2 Short-term studies of toxicity

No short-term studies of toxicity conducted with mice or rats were available.

(a) Dietary administration

Duck

Muscovy ducklings (1 day old; 484–503 g bw) were fed, ad libitum, diets containing either 4,15-DAS or T-2 toxin (purity not specified) at 0 (n = 50), 0.25 (n = 20), 0.5 (n = 30) or 1 (n = 40) mg/kg feed per day for 7 days (equivalent[2] to 0, 0.06, 0.10 and 0.18 mg/kg bw per day). Basal diet was analysed for the absence of mycotoxins (not specified) prior to study initiation. The only parameters assessed were feed consumption, body weight, feed conversion and occurrence of oral lesions. All concentrations of both mycotoxins caused oral lesions; these appeared within the first day for 4,15-DAS at 0.5 and 1 mg/kg feed and for T-2 toxin at all concentrations. Oral lesions from the 0.25 mg/kg feed concentration of 4,15-DAS were not observed until day 3. Birds fed 1 mg/kg feed of 4,15-DAS had more severe lesions than those given the same concentration of T-2 toxin. No differences in severity were noted at the lower concentrations. Feed consumption was slightly decreased in birds fed 4,15-DAS at 1 mg/kg feed; however, there were no significant differences in body weight or feed conversion at any concentration for both 4,15-DAS and T-2 toxin (Shlosberg, Klinger & Malkinson, 1986).

Chicken

Male broiler chicks (n = 10/treatment; 7 days old) were given T-2 toxin by crop gavage at doses of 0, 1.5, 2, 2.5 or 3 mg/kg bw or 4,15-DAS (purity not specified) at doses of 2.5, 3 or 3.5 mg/kg bw per day or in combination for 14 days. Only clinical signs of toxicity were assessed and these were compared with clinical signs observed after single gavage dose administration (Hoerr, Carlton & Yagen, 1981a; see section 2.2.1(a)). Clinical signs were similar to those described after single doses of the toxins, including weight loss and liquid faecal droppings. Birds that died succumbed by the seventh day of dosing. The oral LD_{50} following repeated dosing with T-2 toxin was 2.9 mg/kg bw and of 4,15-DAS was 4.15 mg/kg bw. When given in combination, T-2 toxin and 4,15-DAS had additive lethal toxicity (Hoerr, Carlton & Yagen, 1981b).

Male broiler chicks (n = 10/treatment; 1 day old) were fed, ad libitum, diets containing SCP or TAS at 0, 1, 2, 4 or 8 mg/kg feed (equivalent[3] to 0, 0.53/0.53,

[2] Dietary intake of DAS was calculated using the reported cumulative feed consumption divided by 7 days to estimate a daily feed consumption and the reported initial and terminal body weights; therefore, the presented dietary intake on a body weight basis was calculated using the average of the dietary intakes calculated with the initial and terminal body weights.

[3] Dietary intakes were calculated using a reference average daily feed consumption for 1–3-week-old male

1.05/1.06, 2.10/2.12 and 4.23/4.31 mg/kg bw per day for TAS/SCP, respectively) or MAS or 4,15-DAS (purity not specified) at 0, 0.5, 1, 2 or 4 mg/kg feed (equivalent[3] to 0, 0.27/0.27, 0.53/0.53, 1.07/1.06 and 1.89/1.89 mg/kg bw per day for MAS/4,15-DAS, respectively) for 21 days. The absence of SCP, MAS, 4,15-DAS, TAS and T-2 toxin in the basal diet was confirmed prior to study initiation. The incidence of oral lesions and growth inhibition were the only parameters assessed. Feed consumption or efficiency was not reported. The lowest-observed-effect level (LOEL) for growth inhibition (based on body-weight decreases compared with controls) was reported to be 2 mg/kg feed for SCP (−7%), MAS (−14%) and 4,15-DAS (−9%) and 8 mg/kg feed for TAS (−8%). Without information about feed consumption or feed efficiency of these animals, the toxicological significance of the reported body-weight reductions is uncertain. Oral lesions were dose-related in all treatment groups. The LOELs were 4, 2, 1 and 0.5 mg/kg feed for, respectively, TAS, SCP, 4,15-DAS and MAS, irrespective of whether the number of affected birds or the number of affected mouth parts (angles, upper beak, lower beak and tongue) was the measured response. Oral lesions were clearly visible with each toxin after feeding for 1 week and the number of affected mouth parts almost tripled after 2 weeks of exposure. During week 3 of exposure, only MAS caused a significant increase ($P < 0.05$) in total lesions (Ademoyero & Hamilton, 1991a).

Broiler breeder hens (n = 20/treatment) were fed, ad libitum, diets containing 4,15-DAS (purity not specified; prepared from cultures of *F. sambucinum* NRRL 13495) at 0, 5, 10 or 20 mg/kg feed (equal to 0, 0.33, 0.68 and 1.08 mg/kg bw per day) for 14 days beginning at week 24 of age, followed by a 7-week recovery period. Basal diets were analysed to confirm the absence of background DAS and aflatoxin. The only parameters assessed were body weights, feed consumption and oral lesions. Feed efficiency was not reported. There were dose-related decreases in feed consumption (−18%, −26% and −47% at 5, 10 and 20 mg/kg feed, respectively, compared with controls) during the first 2 weeks of the experimental period. Feed consumption increased rapidly and was comparable to controls 1 week after 4,15-DAS was removed from the diet. Body weights were also decreased in a dose-related manner (approximately −4%, −13% and −18% at 5, 10 and 20 mg/kg feed, respectively, compared with controls) by week 2 of treatment. Despite consumption of equivalent amounts of feed for 7 weeks after exposure to 4,15-DAS, body weights decreased compared with controls. Oral lesions were observed in all treated birds. The palatine area,

broiler chicks of 0.043 kg feed/day (NRC, 1994). The presented dietary intakes on a body weight basis were calculated using the average of the dietary intakes calculated with reference to the initial body weight for 1-day-old broiler chicks of 43.8 g (Neto et al., 2013) and terminal body weight (provided in the study report). Calculated dietary intakes of DAS and other mycotoxins are likely an overestimate as insufficient information was provided to account for reduced feed consumption over the experimental period.

sublingual area, internal angles of the mouth and tongue were affected. There was a dose-related increase in the average size of lesions on the palatine, angles of the mouth and tongue. At 20 mg/kg feed of 4,15-DAS, the necrotic lesions culminated in the loss of the tongue tip (Brake, Hamilton & Kittrell, 2000).

In a second experiment, male and female broiler breeders (25–27 weeks of age) were fed diets containing 4,15-DAS at 0, 5, 10 or 20 mg/kg feed (equal to 0, 0.19/0.22, 0.34/0.40 and 0.61/0.65 mg/kg bw per day for males and females, respectively) under a restricted diet of 125 and 150 g/day for females and males, respectively, for 21 days. The only parameters assessed were body weights, feed consumption and oral lesions. Feed efficiency was not reported. A dose-related decrease in body weight and feed consumption was observed in all treated females. By the third week of treatment, body weights had decreased by 5%, 7% and 14% and feed consumption had decreased by 15%, 27% and 44%, compared with controls, at 5, 10 and 20 mg/kg feed, respectively. In males, a decrease in feed consumption was seen at 10 and 20 mg/kg feed (−33% and −46%, respectively, at week 2), with no significant differences in body weight compared with controls. Oral lesions were observed in all treated animals in a dose-related manner (Brake, Hamilton & Kittrell, 2000).

Male broiler chicks ($n = 20$; 1 day old) were fed, ad libitum, diets containing 4,15-DAS (95% purity; prepared from cultures of *F. sambucinum* NRRL 13495) at 1 or 2 mg/kg feed (equal[4] to 0.36 and 0.69 mg/kg bw per day) with or without the addition of a feed additive (Mycofix; a bacterium capable of biotransforming the epoxide group of trichothecenes into a diene) at 0.75 or 1.5 mg/kg feed for 21 days. The basal diet was analysed for aflatoxin, ochratoxin A (OTA), T-2 toxin, 4,15-DAS, HT-2 toxin and NEO; no detectable levels were found. Parameters analysed included body weight, feed consumption, oral lesions and organ weights (liver, heart, spleen, proventriculus and gizzard). When no feed additive was included, both concentrations of 4,15-DAS significantly decreased body weight (−11% and −15%, respectively, at 21 weeks) and feed consumption (−6% and −9%, respectively, of cumulative feed consumption) and caused oral lesions, with the effect at 2 mg/kg feed being more severe. Feed efficiency was not significantly different from control. Both concentrations of Mycofix protected against the effects of 4,15-DAS at 1 mg/kg feed on feed consumption and body weight but not the effects on oral lesions. When 4,15-DAS was present at 2 mg/kg feed, Mycofix only provided partial protection against decreased feed consumption and body weight (Diaz, 2002).

[4] Dietary intake of DAS was calculated using body weights reported on a weekly basis; therefore, the presented dietary intake on a body weight basis was calculated using the average of the dietary intake calculated for each week. Cumulative feed consumption was divided by 21 days for daily consumption.

Male broiler chicks (n = 10; 1 day old) were fed, ad libitum, diets containing 4,15-DAS (purity not specified; prepared from cultures of *F. sambucinum* NRRL 13495, using the method of Richardson & Hamilton, 1987) at 0, 1, 2, 4 or 8 mg/kg feed (equivalent[3] to 0, 0.5, 1.1, 2.2 and 4.4 mg/kg bw per day) in diets containing 6% or 12% fat for 21 days. The only parameters assessed were body weight, feed consumption and faecal lipid content. Body weight was significantly decreased at 4,15-DAS concentrations of 4 mg/kg feed (–20% and –33%, respectively), with the fat content of the diet having no significant effect. However, at the high concentration of 4,15-DAS, a greater decrease in body weight was observed in the high-fat diet than in the low-fat diet. Feed consumption was not reported, but neither feed conversion nor percentage of fat in faecal material was reported to be affected significantly by 4,15-DAS. Ademoyero & Hamilton (1991b) concluded that high-fat diets may promote lipid micellar absorption of DAS.

Turkey

Male turkey poults (n = 12/treatment; 1 day old) were fed, ad libitum, diets containing 4,15-DAS (purity not specified) at 0, 0.223, 0.429 or 0.860 mg/kg feed (equivalent[5] to 0, 0.048, 0.085 and 0.192 mg/kg bw per day) or T-2 toxin at 0, 0.241, 0.485 or 0.982 mg/kg feed (equal to 0, 0.036, 0.075 and 0.158 mg/kg bw per day) for 32 or 33 days, respectively. The authors did not mention whether the basal diet was analysed for other mycotoxins prior to study initiation. Parameters analysed included body weights, feed consumption, feed efficiency, oral lesions, macroscopic and microscopic examination (small intestine, liver, pancreas, kidney, spleen and bursa) and antibody production. Some slight diarrhoea was observed in some birds fed more than 0.4 mg/kg feed of 4,15-DAS or T-2 toxin. There was no significant effect on body weight, feed consumption or feed efficiency in any of the treatment groups. Oral lesions were observed in all treatment groups after 7 days. The lesions, which plateaued after 7–15 days, were more severe in the birds exposed to T-2 toxin. At the end of the treatment period, some of the birds fed more than 0.4 mg/kg feed of 4,15-DAS or T-2 toxin had mild diffuse intestinal changes; however, no other gross lesions were observed and no microscopic abnormalities were observed in the proventriculus, liver, pancreas, kidney, spleen or bursa tissue. Intestinal morphology was further examined in control and high-dose birds; both mycotoxins affected the jejunum where villi were shorter and thinner. In addition, both mycotoxins enhanced the proportion of proliferating cells both in the crypts and along the villi. Migration rates were reduced in the jejunum of birds fed T-2 toxin but did not change in

[5] Dietary intake of DAS was calculated using body weights reported on a weekly basis; therefore the presented dietary intake on a body weight basis was calculated using the average of the dietary intake calculated for each week. Cumulative feed consumption was divided by 32 or 33 days for daily consumption.

the duodenum or in birds fed 4,15-DAS. Antibodies to parenterally administered Newcastle disease virus and to bovine serum albumin solution administered per os were determined in all birds at 12 and 32 days of treatment. No significant effects of T-2 toxin or 4,15-DAS on antibody production were observed (Sklan et al., 2003).

Pigs

Crossbred weanling pigs (7.1–9.1 kg bw) were fed, ad libitum, diets containing 4,15-DAS (purity 94–96%) at 0, 2, 4, 8 or 10 mg/kg feed (equivalent[6] to 0, 0.23, 0.42, 0.38 and 0.31 mg/kg bw per day) for 9 weeks. Two pigs were assigned to each treatment group except for the control and the 10 mg/kg feed groups, which only had one pig. After 4 weeks of treatment, one pig from the 2, 4 and 8 mg/kg feed groups was killed. The authors did not mention whether the basal diet was analysed for the presence of other mycotoxins prior to study initiation. Pigs were weighed weekly and observed daily for clinical signs of illness. Blood was sampled prior to treatment and at weekly intervals for haematological (packed cell volume, haemoglobin concentration, total red blood cell [RBC] and total white blood cell [WBC] counts, and a 300-cell differential stain from bone marrow samples) and biochemical (alanine transaminase [ALT], aspartate transaminase [AST], lactate dehydrogenase) analysis. At necropsy, brain, gingiva, tongue, oesophagus, stomach, duodenum, jejunum, ileum, caecum, spiral colon, rectum, liver, pancreas, lung, myocardium, adrenal gland, kidney, mesenteric lymph node and spleen tissues were examined microscopically.

Total feed refusal was observed by the pig in the 10 mg/kg feed group. This animal was given a control diet for 96 hours and then a 9 mg 4,15-DAS/kg feed diet for the remaining 8 weeks of the study. No abnormal behaviour or clinical signs of illness were reported in any of the pigs. Decreased feed consumption was reported in all treatment groups. Consumption of diets containing 4,15-DAS at 2 mg/kg feed differed significantly from control ($P < 0.05$), but feed consumption did not differ significantly between the 2 and 4 mg/kg feed groups (−19%, −25% and −66%, compared with controls, at 2, 4 and 8 mg/kg feed, respectively). Decreased weight gain was also reported for all treatment groups; however, the data were not provided. Feed efficiency was reported as not being significantly affected (data not provided). There was no effect of treatment with 4,15-DAS reported on any of the blood parameters or on the terminal bone marrow smears (data not provided). After 2 weeks at 4 mg/kg feed, a mucosal ulceration was observed on the tongue. With time, the number, size or severity of oral lesions increased and more pigs

[6] Dietary intakes of DAS were calculated using the reported feed consumption and the average of the initial body weights of 7.1–9.1 kg, that is, 8.1 kg. The dietary intakes calculated on a body weight basis are likely an overestimate as insufficient information was reported to account for the body-weight gains during the experimental period.

developed similar oral lesions (lingual, gingival and buccal mucosal ulcerations). All levels of 4,15-DAS caused oral lesions. Microscopic examination revealed lingual lesions that were described as proliferative with suppurative exudation over the superficial surface. Haemorrhage and neutrophilic infiltration as well as fibrin were deposited in the area. The ulcer base had fibroplasia and neutrophils present. Gingival lesions were observed to have an ulcerated surface with loss of epithelium and a suppurative base to the area. In the 8 mg/kg feed group, the small intestine had both glandular and mucosal epithelial cell hyperplasia. No other gross or microscopic lesions were reported in the tissues examined at other 4,15-DAS concentrations.

In a follow-up study, five crossbred pigs were fed diets containing 4,15-DAS at 4 mg/kg feed (equal to 0.23 mg/kg bw per day) for 4 weeks. The oral lesions previously observed were reproduced. No other effects on feed consumption, weight gain, feed efficiency, clinical observations or gross lesions were reported (Weaver et al., 1981).

Crossbred barrows (n = 9/treatment; 9 weeks old; 25.2 kg bw) were fed, ad libitum, diets containing 4,15-DAS (purity >98%) at 0 or 2 mg/kg feed (equivalent[7] to 0.1 mg/kg bw per day) or aflatoxin at 2.5 mg/kg feed (equivalent[7] to 0.13 mg/kg bw per day) or a combination of the mycotoxins (total 4.5 mg/kg feed; equivalent[7] to 0.24 mg/kg bw per day) for 4 weeks. Basal diets were analysed for aflatoxin, 4,15-DAS, T-2 toxin, zearalenone, DON, patulin, penicillic acid, OTA and cyclopiazonic acid; no detectable concentrations (<10 µg/kg diet) were found. Barrows were observed twice daily and weighed once each week. At the end of the treatment period, blood was collected for haematological analyses (RBC count, total WBC count, packed cell volume, haemoglobin concentration, mean cell volume, mean cell haemoglobin concentration, prothrombin time and activated partial thromboplastin time); immunological analysis (lymphocyte stimulation indices); serum biochemical analyses (activities of AST, alkaline phosphatase [ALP], cholinesterase, creatine kinase, lactate dehydrogenase and γ-glutamyltransferase, and concentrations of albumin, cholesterol, creatinine, glucose, inorganic phosphorus, total iron, total protein and urea nitrogen); and analyses of unsaturated iron–binding capacity and total iron–binding capacity (TIBC). At necropsy, liver, left kidney, spleen and heart were weighed and histological examinations were conducted that included gastrointestinal tract tissues.

[7] Dietary intake was calculated using a reference average daily feed consumption for this age and production stage of pigs of 1.582 kg feed/pig (NRC, 2012). Dietary intake on a body weight basis was calculated using the average of the dietary intake calculated from the initial and terminal body weights (provided in the study report). Calculated dietary intakes are likely an overestimate as insufficient information was provided to account for reduced feed consumption over the experimental period.

Treatment with 4,15-DAS significantly decreased body-weight gain (−14% compared with controls; $P < 0.05$). Although final body weights were decreased (−8% compared with controls), this difference was not statistically significant. Feed consumption was not reported but there were no significant differences in the feed-to-gain ratios between treated groups and controls, suggesting that the observed reduced body-weight gain is associated with decreased feed consumption. Barrows fed both toxins had a larger decrease in weight gain (−56%) and a statistically significant lower final body weight (−27.5%) compared with controls. Barrows in the treatment groups were reported to be noticeably smaller, were observed to be eating less and were less active than the control group. Treatment with 4,15-DAS was associated with a statistically significant reduction in serum iron–binding capacity (−14% unsaturated iron–binding capacity; −13% TIBC). Although not statistically significant, total iron decreased by 9% while total RBC count and haemoglobin concentrations increased slightly compared with controls. The slight reduction in serum iron–binding capacity was not considered biologically relevant. All other haematological, immunological and clinical chemistry parameters were comparable with controls. There were no significant differences in organ weights compared with controls. No oral, cutaneous or small intestinal lesions were reported in the 4,15-DAS-treated barrows. The only lesions observed in the 4,15-DAS-fed group were reported as consisting of very mild diffuse hepatocellular cytoplasmic vacuolar changes of a very early degenerative nature characterized by foamy cytoplasm. Barrows fed both toxins had significantly greater liver and spleen weights compared with controls. In the combined toxin group, hepatic lesions consisted of diffuse hepatocellular cytoplasmic vacuolar changes accompanied by early portal fibrosis and bile duct hyperplasia. The kidney and heart were unaffected by treatments. The authors suggest that the low incidence of toxic effects associated with 4,15-DAS in the present study may be attributed to the low dose and short exposure. The effects of the combined treatment of 4,15-DAS and aflatoxin were considered to be additive or less-than-additive and primarily reflected the toxicity of aflatoxin (Harvey et al., 1991).

(b) **Combined dietary administration of mycotoxins**

Chicken

Male broiler chicks (n = 7/treatment; 1 day old) were fed, ad libitum, diets containing 6 mg/kg feed of 4,15-DAS (99% purity; equivalent[3] to 3.23 mg/kg bw per day) or 2 mg/kg feed of OTA (95% purity; equivalent[3] to 1.06 mg/kg bw per day) or in combination (total 8 mg/kg feed; equivalent[3] to 4.31 mg/kg bw per day) for 19 days. The basal diet was analysed for aflatoxin, DON, zearalenone and cyclopiazonic acid and all were below the detection limits.

The analysed parameters included body weight, feed consumption, mortality, oral lesions, serum biochemistry and haematology and organ weights (liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius). 4,15-DAS and OTA significantly decreased body weights when fed alone (−28% and −12%, respectively). The body weights of chicks fed the toxin combination were similar to the body weights of chicks fed 4,15-DAS alone, characterized as a less-than-additive effect. Feed consumption was not reported. The efficiency of feed utilization was reduced by 4,15-DAS alone and the toxin combination with OTA, characterized as additive toxicity. Over 90% of chicks fed diets containing 4,15-DAS with or without OTA had oral lesions. Relative liver and kidney weights were increased in the OTA and combined toxin groups; however, no changes in relative organ weights were observed in the 4,15-DAS group. When compared with controls, mean corpuscular volumes were significantly decreased in chicks fed diets of 4,15-DAS, OTA and the combination, characterized as additive toxicity. Mean corpuscular haemoglobin values were decreased in chicks receiving 4,15-DAS alone or 4,15-DAS in combination with OTA. Haemoglobin, erythrocyte count, haematocrit and mean corpuscular haemoglobin concentrations did not differ from controls in any of the treatment groups (Kubena et al., 1994).

Male broiler chicks (n = 10; 1 day old) were fed, ad libitum, diets containing either 5 mg/kg feed of 4,15-DAS (99% purity; equivalent[3] to 2.6 mg/kg bw per day), 3.5 mg/kg feed of aflatoxin (equivalent[3] to 1.9 mg/kg bw per day) or a combination of the mycotoxins (total 8.5 mg/kg feed; equivalent[3] to 4.39 mg/kg bw per day) for 21 days. The authors did not mention whether the basal diet was analysed for the presence of other mycotoxins prior to study initiation. Parameters analysed included body weight, feed consumption, mortality, oral lesions, serum biochemistry and haematology and organ weights (liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius). Compared with controls, body-weight gains were significantly decreased by 4,15-DAS (−11%) and aflatoxin (−16%), and a synergistic interaction was also reported by the study authors (−36%). Feed consumption was not reported but feed efficiency was only reduced in the combined toxin group. Feeding aflatoxin alone resulted in significant increases in the relative weights of the liver, kidney, heart, gizzard, spleen and pancreas. Treatment with 4,15-DAS alone did not alter organ weights. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were significantly reduced in chicks fed aflatoxin alone. 4,15-DAS alone resulted in a decrease in the mean corpuscular volume only, whereas the combination resulted in a decrease in both mean corpuscular volume and haemoglobin. Various other alterations in serum biochemical values, as well as serum enzyme activities, were observed for chicks fed aflatoxin alone or in combination; however, these effects were not observed in the 4,15-DAS group. Addition of hydrated sodium calcium aluminosilicate

(0.5%) into the diets provided almost total protection against the effects of aflatoxin, but only limited protection against the combination and no protection against the effects of 4,15-DAS alone (Kubena et al., 1993).

Single comb White Leghorn hens (n = 10/treatment; 33 weeks old) were fed, ad libitum, diets containing either 2 mg/kg feed of T-2 toxin (equivalent[8] to 0.12 mg/kg bw per day), 2 mg/kg feed of 4,15-DAS (purity not specified; equivalent[8] to 0.12 mg/kg bw per day) or a combination of the mycotoxins (4 mg/kg feed total; equivalent[8] to 0.22 mg/kg bw per day) for 24 days. The basal diet was analysed for the presence of various toxins and only a small amount of DON (0.3 mg/kg feed) was detected. Body weight, feed consumption, oral lesions, egg production, serum biochemistry, liver malondialdehyde concentrations and histopathological examination (oesophagus, crop, proventriculus, duodenum, jejunum, ileum, liver and kidney) were assessed. No effect on body weight was reported (data not provided). Feed consumption was decreased in the T-2 toxin and 4,15-DAS groups compared with controls for the first 12 days. Overall feed consumption was also lower in the treated groups (−8%, −6% and −12% for T-2 toxin, 4,15-DAS and the combined treatment groups, respectively). T-2 toxin and 4,15-DAS induced similar oral lesions in up to half of the hens by day 24. Lesions were observed on day 1 following treatment initiation, and the number of affected hens increased with time. Total egg production over the experimental period was significantly lower in the treated groups and was lowest in hens receiving 4,15-DAS alone or in combination with T-2 toxin. During the first 12 days, egg production in hens receiving 4,15-DAS alone was significantly decreased (−12%); egg production in the T-2 toxin or the combination groups was similar to controls. However, during days 19–24, egg production in hens receiving the combination toxin diet was significantly reduced (−15.3%), while that of hens receiving T-2 toxin or 4,15-DAS alone was comparable to controls. The effect of the combination of T-2 toxin and 4,15-DAS was additive for reduced feed consumption and incidence of oral lesions. The study authors concluded that the effect on reduced egg production was synergistic, but due to the variation in egg production observed over the experimental period, it is not clear if the reduction in the combined toxin group is more than additive. Plasma enzyme activities (AST, ALT, glutamate dehydrogenase, lactate dehydrogenase and creatinine kinase) were not significantly different from controls prior to treatment and at day 24. No change in liver malondialdehyde content was detected. Macroscopic and microscopic evaluations found no treatment-related lesions beyond the oral lesions observed mainly on the tongue, palate and margins of the beak (Diaz et al., 1994).

[8] Dietary intake was calculated using the cumulative feed consumption (provided in the study report) divided by 24 days and a reference average body weight for 33–37-week-old hens of 1.72 kg (http://www.isapoultry.com/en/products/isa/isa-white).

Turkey

Female turkey poults (n = 6; 1 day old) were fed, ad libitum, diets containing 4,15-DAS (purity >99%) at concentrations of 4 mg/kg feed (equivalent[9] to 0.6 mg/kg bw per day) or fumonisin B_1 at 300 mg/kg feed (equivalent[9] to 43 mg/kg bw per day) or in combination (total 304 mg/kg feed; equivalent[9] to 41 mg/kg bw per day) for 21 days. The basal diet was analysed for aflatoxin, DON, zearalenone and cyclopiazonic acid and all were below the LOD. The analysed parameters included body weight, feed consumption, mortality, oral lesions, serum biochemistry and haematology and organ weights (liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius). Overall body-weight gains were significantly decreased in all treatment groups (28% for 4,15-DAS, 36% for fumonisin B_1 and 51% for the combination) during the treatment period. Overall feed consumption (13% for 4,15-DAS, 21% for fumonisin B_1 and 37% for the combination) and efficiency of feed utilization were reduced in all treatment groups compared with controls. Oral lesions were observed in most of the birds fed 4,15-DAS; however, the severity scores were lower in birds fed the combination diet than in those fed 4,15-DAS alone. Relative weights of the liver and gizzard were increased and the relative weight of the heart was decreased with the fumonisin B_1 diet with or without 4,15-DAS. Relative spleen weights were decreased in all treatment groups. The authors concluded that there was an additive or less-than-additive toxicity of the combination of 4,15-DAS and fumonisin B_1 in the diet (Kubena et al., 1997a).

Lambs

Rambouillet × Suffolk crossbred ewe lambs (n = 6/treatment group; 38 kg bw) were fed, ad libitum, diets containing aflatoxin at concentrations of either 0 or 2.5 mg/kg feed (equivalent[10] to 0.08 mg/kg bw per day), 4,15-DAS at 5.0 mg/kg feed (purity 98%; equivalent[10] to 0.17 mg/kg bw per day) or a combination of the mycotoxins (total 7.5 mg/kg feed; equivalent[10] to 0.24 mg/kg bw per day) for 34 days. Basal diets were analysed for aflatoxin, 4,15-DAS, T-2 toxin, zearalenone, DON, OTA and cyclopiazonic acid; no detectable concentrations were found. Lambs were observed twice daily and weighed weekly. At the end of the treatment

[9] Dietary intake was calculated using reported feed consumption, and an average body weight over the experimental period was estimated using a reference initial body weight for 1-day-old turkey poults of 60 g (http://www.turkey.mb.ca/turkey-production-stages) and the reported body-weight gains over the experimental period.

[10] Dietary intake was calculated using feed consumption data, initial body weight and body-weight gain provided in the study report. Feed intake data were provided per pen without a clear indication of the number of lambs per pen, when compared to reference feed consumption for small ruminants (NRC, 2007). The values provided in the study report were divided by 2 to represent feed intake per lamb per day.

period, blood samples were collected for haematological, serum biochemical and immunological measurements. The liver, left kidney, spleen and heart were weighed and the liver was microscopically examined. The diet containing aflatoxin at 2.5 mg/kg feed resulted in hepatocellular alterations (pale, mild lipidosis accompanied by early periportal and interlobular fibrosis) and serum biochemical alterations (increased cholesterol and TIBC). The diet containing 5.0 mg/kg feed of 4,15-DAS resulted in diarrhoea for the first week of the study, decreased weight gain resulting in a lower final body weight compared with controls (9%), decreased feed consumption (9.5%) and altered serum biochemical values (decreased cholinesterase activity and urea nitrogen). No oral lesions were observed in the 4,15-DAS-treated animals. The combination of the mycotoxins in the diet resulted in a significant synergistic reduction in weight gain (−2.7 ± 0.3 kg) and increased serum activities of γ-glutamyltransferase. Altered serum urea nitrogen, cholesterol and TIBC concentrations and cholinesterase activities observed in the combined mycotoxin treatment group were described as additive or less-than-additive. Lymphocyte blastogenic responses were not significantly altered (Harvey et al., 1995).

(c) Intravenous administration

Dogs

Beagle dogs (n = 1/sex per treatment) were administered a daily intravenous dose of 4,15-DAS (purity not specified) at 0, 0.016, 0.031, 0.063, 0.125 or 0.25 mg/kg bw for 5 consecutive days. Control animals were intravenously administered sterile water on the same regimen as the treated dogs. All animals were observed daily for clinical signs of toxicity. Body weights were recorded on each day of dosing and twice weekly during the observation periods. Ophthalmoscopic examinations were conducted prior to treatment, on day 8 following treatment and weekly thereafter. Water and feed consumption were measured daily. Prior to treatment, haematological, biochemical and urine analysis parameters were analysed. Haematological and biochemical parameters were re-analysed on day 2, 4, 6 (urine analysis) or 8 of the study and weekly thereafter. One dog from each dose group was killed on day 12 and the remaining dogs were killed on days 50–52. The organs were weighed and examined macroscopically and microscopically.

One male dog at 0.25 mg/kg bw died at day 7 of the study. Necropsy revealed moderate perivascular haemorrhage and moderate myocardial congestion of the heart and slight congestion of the mucosa of the colon and stomach. Clinical observations included emesis and erythema in dogs at 0.063 mg/kg bw and higher doses; hypersalivation, tremors and diarrhoea at 0.125 mg/kg bw and higher doses; and polydipsia, hypoactivity, ataxia and dehydration at 0.25 mg/kg bw. Body weights and feed consumption were comparable to controls.

Slight changes in haematological parameters (increased nucleated RBCs, anaemia and neutrophilia) were observed at 0.031 mg/kg bw and higher doses. Increased ALT, AST and alkaline phosphatase (ALP) activities and blood urine nitrogen (BUN) and reticulocyte counts as well as leukopenia and neutrophilia were observed at 0.063 mg/kg bw and higher doses. The surviving female dog at 0.25 mg/kg bw showed severe leukopenia with neutropenia and relative lymphocytosis 2 weeks after treatment. It was noted that control animals also had diarrhoea and neutrophilia. The ophthalmological and urine analysis findings at all dose levels were reported to be unremarkable. Necropsy was reported to be unremarkable for all the dogs that survived the study.

The no-observed-adverse-effect level (NOAEL) for 4,15-DAS was 0.016 mg/kg bw based on anaemia and increased nucleated RBCs following 5 consecutive days of intravenous administration at 0.031 mg/kg bw (IRDC, 1973).

Following a single intravenous dose of 4,15-DAS to beagle dogs (described in section 2.2.1(c)), increased nucleated RBCs were observed at a dose of 0.125 mg/kg bw as well as in control animals; by day 8 following treatment, the nucleated RBC counts had reverted to pretreatment levels. The lowest-observed-adverse-effect level (LOAEL) was 0.063 mg/kg bw based on clinical observations of emesis and erythema (IRDC, 1973), indicating that increased toxicity was observed following consecutive repeated exposures to 4,15-DAS.

Beagle dogs (n = 1/sex per treatment) were administered 4,15-DAS (purity not specified) as a daily intravenous dose of 0.031 or 0.125 mg/kg bw for 5 consecutive days followed by a 9-day rest period, and repeated for three treatments for a total of 15 doses over a 42-day period. All animals were observed daily for clinical signs of toxicity. Body weights were recorded on each day of dosing and twice weekly during observation periods. Ophthalmoscopic examinations were conducted prior to treatment, on day 8 after commencement of treatment and weekly thereafter. Water and feed consumption were measured daily. Haematological and biochemical parameters were analysed prior to treatment and on days 2, 4, 9, 11, 15, 16, 18, 23, 25, 29, 30, 32, 37, 39, 47, 54, 61, 68 and 75 of the study. Urine analysis was conducted prior to treatment and on days 6, 20 and 34 of the study. One dog from each dose group was killed on day 40 and the remaining dogs were killed on day 78 of the study. Select organs were weighed and examined macroscopically and microscopically.

There were no deaths during this study. At 0.031 mg/kg bw hypothermia was occasionally observed after dosing and at 0.125 mg/kg bw emesis, soft stools and occasional tremors were observed after dosing. In both dose groups, slight increases in AST and ALT activities were observed during the latter part of the treatment periods and the early part of the post-treatment periods. Slight neutrophilia, monocytosis, eosinophilia and increased nucleated RBCs were occasionally noted in male dogs at 0.031 mg/kg bw, whereas slight

anaemia, increased nucleated RBCs, leukopenia, neutropenia, lymphocytosis, thrombocytopenia and polychromasia of RBCs were noted in male dogs at 0.125 mg/kg bw. The ophthalmological and urine analysis findings at all dose levels were unremarkable. Necropsy was reported to be unremarkable for all dogs. When compared with the results for dogs administered 4,15-DAS over 5 consecutive days only, this repeat regimen of dosing did not significantly increase the toxicity (IRDC, 1973).

Beagle dogs (n = 1/sex per treatment) were administered 4,15-DAS (purity not specified) as a single intravenous dose of 0.031, 0.063, 0.125 or 0.25 mg/kg bw once per week for 6 consecutive weeks. All animals were observed daily for clinical signs of toxicity. Body weights were recorded on each day of dosing and twice weekly during observation periods. Ophthalmoscopic examinations were conducted prior to treatment, on day 8 after initiation of treatment and weekly thereafter. Water and feed consumption were measured daily. Haematological and biochemical parameters were analysed prior to treatment and on days 2, 4, 6, 8, 9, 11, 13, 15, 16, 18, 20, 22, 23, 25, 27, 30, 32, 43, 36, 37, 39, 41, 43, 51, 57, 65, 71 or 72/79 of the study. Urine analysis was performed prior to treatment and on days 6, 13, 20, 27, 34 and 41 of study. One dog from each dose was killed on day 43 and the remaining dogs were killed on day 81. Select organs were weighed and examined macroscopically and microscopically.

There were no deaths during this study. Clinical observations included emesis and erythema in dogs at 0.063 mg/kg bw and higher doses and diarrhoea at 0.125 mg/kg bw and higher doses. Slight changes in clinical chemistry and haematological parameters, which included increases in AST and ALT activities, decreased haemoglobin and slight anaemia, were observed at 0.031 mg/kg bw and higher doses. At 0.125 mg/kg bw and higher doses, increases in BUN and nucleated RBCs were also observed, but were reversible by the end of the recovery periods with the exception of persistent slight anaemia. The ophthalmological and urine analysis findings at all dose levels were reported to be unremarkable. Necropsy was reported to be unremarkable for all dogs. When compared with the results for dogs administered single intravenous doses of 4,15-DAS, repeated weekly injections in dogs at toxic dose levels did not significantly alter the severity of toxicity (IRDC, 1973).

Rhesus monkeys

Rhesus monkeys (n = 1/sex per treatment) were administered 4,15-DAS (purity not specified) as a daily intravenous dose of 0, 0.125, 0.25, 0.5 or 1.0 mg/kg bw for 5 consecutive days. Control animals were intravenously administered sterile water on the same regimen. All animals were observed daily for clinical signs of toxicity. Body weights were recorded on each day of dosing and twice weekly

during observation periods. Ophthalmoscopic examinations were conducted prior to treatment, on day 8 after initiation of treatment and weekly thereafter. Water and feed consumption were measured daily. Haematological, biochemical and urine analysis parameters were analysed prior to treatment and on days 2, 4, 6 (urine analysis) or 8 of the study and weekly thereafter. One monkey from each dose group was killed on day 12 and the remaining monkeys were killed on day 50. Select organs were weighed and examined macroscopically and microscopically.

Both male and female monkeys at 0.5 and 1.0 mg/kg bw died on days 4 and 3 of the study, respectively. Body weights in these monkeys were decreased by approximately 10% compared with controls. Necropsy revealed a consistent moderate to severe necrosis of the germinal centre of Peyer patches, spleen and lymph nodes and a consistent severe to very severe perivascular haemorrhage with moderate to severe neutrophil infiltrate in the skin (injection site). Variable congestion (very slight to severe) of the lung, small and large intestine, spinal cord, liver, adrenal, brain, uterus, heart, pancreas, skeletal muscle, ovary, salivary gland, pituitary gland, eye, kidney, spleen and lymph nodes was observed. Clinical observations included hypoactivity (1–2 days), emesis, anorexia and soft stool/diarrhoea at doses of 0.25 mg/kg bw and higher. Body weights and feed consumption were comparable to controls in animals that survived the study period. Slight increases in AST and ALT during the treatment period and increased reticulocytes and slight anaemia post-treatment (reversible by 4 weeks) were observed at 0.125 mg/kg bw and higher doses. At 0.25 mg/kg bw and higher doses, marked neutrophilia and lymphopenia and slightly increased BUN were also observed during the treatment period. Nucleated RBCs were observed post-treatment (reversible by 4 weeks in the 0.25 mg/kg bw group). It is noted that control animals were also observed to have reversible increases in AST, reticulocytes, decreased haematocrit, slight anaemia, decreased BUN, neutrophilia and lymphopenia. The ophthalmological and urine analysis findings at all dose levels were reported to be unremarkable. Necropsy of monkeys at 0.25 mg/kg bw revealed consistent severe perivascular haemorrhage with moderate to severe leukocytic infiltrate and moderate focal fibrosis in skin at the injection site. No other treatment-related lesions were observed. The clinical chemistry and haematological parameter changes observed at 0.125 mg/kg bw were also observed in control animals.

The NOAEL for 4,15-DAS was 0.125 mg/kg bw based on clinical observations including emesis and increased nucleated RBCs following 5 consecutive days of intravenous administration of 0.25 mg/kg bw (IRDC, 1973).

2.2.3 Long-term studies of toxicity and carcinogenicity

No long-term studies of toxicity or carcinogenicity were available.

Table 3
Genotoxicity of DAS in vitro and in vivo

End-point	Test system	Concentration/dose	Results	Reference
In vitro				
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA1538	DAS at 0, 0.1, 1, 10, 100 µg/plate ±S9	Negative[a]	Kuczuk et al. (1978)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	DAS at 0, 0.25, 2.5, 25, 250 µg/plate ±S9	Negative[b]	Wehner, Marasas & Thiel (1978)
Reverse mutation	*S. typhimurium* TA100	DAS at 0, 0.01, 0.1, 2.5, 5.0, 10, 15 µmol/L ±S9 (equivalent to 3.66, 36.6, 366, 916, 1 382, 3 664, 5 496 µg/L)[c]	Negative[d]	Sinsheimer et al. (1989)
Mutation (wing spot assay)	*Drosophila melanogaster*	DAS at 0, 5, 10, 20, 40 µmol/L (equivalent to concentrations of 1.83, 3.66, 7.33, 14.65 µg/L)[c]	Negative[e]	Gürbüzel, Uysal & Kizilet (2015)
Mutation	*Saccharomyces cerevisiae*	DAS at 1 000 µg/mL (−S9) and 500 µg/mL (+S9)[f]	Negative[g]	Kuczuk et al. (1978)
Mutation (SCE)	Peripheral blood lymphocytes	DAS at 1.5, 3.0, 6.0, 12.0 ng/mL with metabolic activation	Negative[h]	Cooray (1984)
Mutation (SOS chromotest)	*Escherichia coli* PQ37 and PQ35	DAS at 0.001 to 60 µg/mL ±S9	Negative[i]	Krivobok et al. (1987)
In vivo				
Somatic/germ cell mutation	Mouse bone marrow and sperm	1 i.p. dose of DAS at 500, 750 or 1 000 µg/kg bw or i.p. doses of DAS at 500, 750, 1 000 µg/kg bw on days 1, 10 and 20	Positive[c]	Hassanane et al. (2000)

bw: body weight; DAS: diacetoxyscirpenol; i.p.: intraperitoneal; S9: 9000 × *g* supernatant fraction (metabolic activation); SCE: sister chromatid exchange

[a] The experiment was performed in duplicate according to the plate incorporation method as described by Ames et al. (1975). Strains were assessed in the presence and absence of S9 prepared from livers of male Sprague Dawley rats exposed to sodium hexobarbital. DAS did not result in an increased number of revertants at any dose in the presence or absence of rat liver S9, relative to the respective controls.

[b] The main experiment was performed according to the plate incorporation method as described by Ames et al. (1973). S9 was prepared from the livers of male Wistar rats exposed to Aroclor 1254. DAS did not result in an increased number of revertants at any dose in the presence or absence of rat liver S9, relative to controls.

[c] Equivalent doses were calculated based on a molar mass of 366.41 g/mol.

[d] The experiment was performed in triplicate according to the plate incorporation method as described by Maron & Ames (1983). *S. typhimurium* tester strain TA100 was assessed for presence of mutations with and without S9 metabolic activation. No increase in the number of revertants was observed relative to controls.

[e] Transheterozygous (*mwh/flr*^{3}) third instar larvae were exposed to the test substance in *Drosophila* instant medium until emergence from the pupal stage. Wings were removed and dorsal and ventral sides were examined for presence of mutant clones. No increase in the number of spots was observed at 5 and 10 µmol/L. Increases at 20 and 40 µmol/L were considered inconclusive.

[f] The relevant Organisation for Economic Co-operation and Development (OECD) test guideline for this assay was deleted in 2014.

[g] Diploid D-3 yeast cells were incubated for 3 days with the test substance in the presence and absence of S9 prepared from livers of male Sprague Dawley rats exposed to sodium hexobarbital and observed for induction of red colonies/sectors. Assays were conducted a minimum of 2 times. Survival was 92% and 69% that of control, with and without S9 activation, respectively. No increase in the frequency of mitotic crossing over was reported with or without metabolic activation.

[h] The experiment was performed using human peripheral blood lymphocytes with or without co-culture with rat liver cells obtained from female Sprague Dawley rats. Incubation with DAS did not result in an increase in sister chromatid exchanges, with or without co-culture with rat liver cells.

[i] The experiment was performed at doses ranging from 0.001 to 60 µg/mL in triplicate in *E. coli* PQ37 and PQ35 cells incubated in medium with or without S9 activation mixture. Incubation with DAS did not result in genotoxicity activity based on a lack of induction of the SOS promotor and associated β-galactosidase activity.

2.2.4 Genotoxicity

In vitro and in vivo studies of DAS are shown in Table 3.

There is no evidence of genotoxicity or mutagenicity in bacterial or eukaryotic in vitro systems, with or without S9 metabolic activation. Slight increases in the incidence of mutant clones in the *Drosophila* wing spot assay were

reported. However, Gürbüzel, Uysal & Kizilet (2015) were unable to conclusively diagnose a positive result at the two highest concentrations. Positive results for genotoxicity were limited to a single in vivo assay in the mouse.

Adult male Swiss albino mice (n = 20/treatment; 20–25 g) were administered 4,15-DAS at 0, 500, 750 or 1000 µg/kg bw per day as either a single intraperitoneal dose or a repeated dose on days 1, 10 and 20. Dosing was performed in a cumulative manner with five animals per group receiving a dose on day 1, days 1 and 10, or days 1, 10 and 20. An additional group (n = 5) was maintained for a 10-day recovery period after day 20. Mice were killed 2.5 hours after the intraperitoneal injection and 50 metaphases were examined for bone marrow chromosomes. No positive control was used; propylene glycol was used as a negative control. Sperm from the mice administered 500 µg/kg bw per day in a single intraperitoneal injection were also used to assess morphology with 50 metaphases examined for the spermatocytes. In this phase of the study, cyclophosphamide was used as a positive control. Furthermore, the health of the animals was not reported on in this study.

In animals receiving a single injection, a reduced mitotic index was noted in all dose groups. Statistically significant increases in breaks were observed at 500 µg/kg bw. At the 750 µg/kg bw dose level, statistically significant increases in centromeric attenuation and endomitosis were observed. Similar findings were reported at the highest dose of 1000 µg/kg bw, in addition to increased incidence of chromatid gaps and breaks. The incidence of breaks was not increased at the mid-dose, and no dose–response was observed for centromeric attenuation. In the repeated-dose study, results after day 1 dosing were not similar to the results observed in the single intraperitoneal dose study as there was no statistically significant increase in centromeric attenuation or endomitosis at any dose. Overall, the dose–response was inconsistent after day 1, 10 and 20 of dosing. In addition, animals showed partial recovery following the final dose. In sperm, while the incidences of X–Y univalents and chromosomal breaks were increased in both single- and repeated-dose groups, there was no consistent dose–response. Under the conditions of the repeated-dose study, the changes were reduced after the recovery period but were not considered reversible. Amorphous and small heads and coiled tails were the primary changes in sperm morphology (Hassanane et al., 2000).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

Dietary administration

Female White Leghorn chickens (n = 15/treatment; 36 weeks old) were fed ad libitum a culture of *F. roseum* (Alaska 2-2) containing DAS (details not provided)

at a concentration of 15 parts per million (ppm) and other unidentified toxins at culture levels of 0, 1% or 2% of the diet (equivalent[11] to 0, 0.007 and 0.015 mg/kg bw per day of DAS) for 8 weeks followed by a control diet with no toxins for 6 weeks. Chickens were inseminated weekly with pooled sperm from males given normal diets. No significant differences in feed consumption, body weight or egg weight were observed. During the treatment period, egg production was significantly depressed in both treatment groups, within 2 weeks of treatment in the 2% group and within 4 weeks of treatment in the 1% group. In addition, fertility and hatchability of fertile eggs were also reduced in the 2% fed group. The majority of embryo mortality occurred with 7 days of incubation. Production levels recovered when control diets were fed for 6 weeks (Allen et al., 1982).

In a second experiment by Allen et al. (1982), female White Leghorn chickens ($n = 5$/treatment; 50 weeks old) were fed, ad libitum, diets containing 0 or 0.5 mg/kg feed purified DAS (details not provided; equivalent[11] to 0.03 mg/kg bw per day) or 3% *F. roseum* culture (equivalent[11] to 0.03 mg/kg bw per day of DAS) for 4 weeks followed by a 2-week period of control diet. During the treatment period, the hatchability of fertile eggs was gradually reduced by up to 24% in the DAS-fed group by the 4th week and 99% in the *F. roseum* culture–fed group within 1 week compared with controls. The observed effect was reversible when chickens were fed the control diet for 2 weeks. The authors concluded that DAS is only partially responsible for reduced hatchability in *F. roseum*–contaminated feed and only had a moderate effect on egg production and fertility in Leghorn hens (observed in experiment 1).

Naturally mated broiler breeders (20 females and four males per treatment) were fed diets containing 4,15-DAS (purity not specified; prepared from cultures of *F. sambucinum* NRRL 13495 using the method of Richardson & Hamilton, 1987), at concentrations of 0, 1.25, 2.5 or 5 mg/kg feed (equivalent[12] to 0, 0.04, 0.09 and 0.18 mg/kg bw per day) for 3 weeks (weeks 67–69 of age). A restricted feeding regimen of 154 g per bird per day was used when feeding diets containing 4,15-DAS. Basal diets were analysed to confirm the absence of aflatoxins and DAS. Following treatment, all birds were fed control diets for 3 additional weeks. All nest-laid eggs were collected twice daily. Fertility was consistently improved at 5 mg/kg feed, and intermittently at 2.5 and 1.25 mg/kg feed. The effect disappeared upon removal of 4,15-DAS. Hatchability of fertile eggs was comparable to controls and increased only slightly in treated groups at

[11] Dietary intake of DAS was calculated using the feed consumption reported in the study and a reference average body weight for single comb White Leghorn hens, aged 36–54 weeks, of 1.72 kg (http://www.isapoultry.com/en/products/isa/isa-white/).

[12] Dietary intake of DAS was calculated using the restricted feed consumption data and a reference average body weight of 4.4 kg for broiler chickens of both sexes aged 67–69 weeks and of 3.3 kg for males and 2.3 kg for females aged 25–27 weeks (de Avila et al., 2003).

week 68. Egg production was not affected by treatment with 4,15-DAS (Brake, Hamilton & Kittrell, 1999, 2002).

Female broiler breeders were fed diets containing 4,15-DAS (purity not specified; prepared from cultures of *F. sambucinum* NRRL 13495 using the method of Richardson & Hamilton, 1987) at concentrations of 0, 5, 10 or 20 mg/kg feed (equivalent[12] to 0, 0.33, 0.67 and 1.3 mg/kg bw per day) for 3 weeks (weeks 25–27 of age) using a restricted feeding regimen of 154 g per bird per day. Basal diets were analysed to confirm the absence of aflatoxins and 4,15-DAS. Egg production was only decreased at the 20 mg/kg feed level. In a follow-up experiment, when female broiler breeders were fed the same 4,15-DAS-contaminated diets for 2 weeks (weeks 24–25 of age), no significant effect on egg production or egg quality was observed. The authors concluded that short-term consumption of 4,15-DAS-contaminated feed had little effect on egg production (Brake, Hamilton & Kittrell, 2002).

Broiler breeders (25 females and 10 males/treatment) were fed diets containing 4,15-DAS (purity not specified; prepared from cultures of *F. sambucinum* NRRL 13495 using the method of Richardson & Hamilton, 1987) at concentrations of 0, 5, 10 or 20 mg/kg feed (equivalent[12] to 0, 0.18/0.26, 0.36/0.52 and 0.73/1.04 mg/kg bw per day for males and females, respectively) for 3 weeks (weeks 25–27 of age) according to a restricted diet of 114–125 g per bird per day depending on age. Basal diets were analysed to confirm the absence of aflatoxins and DAS. At termination of 4,15-DAS feeding at 27 weeks of age the hens were artificially inseminated with pooled sperm from the treated males. Following treatment, the hens were fed control diets for an additional 2 weeks. All eggs were collected daily. The hens' spermatozoal storage capacity was assessed in addition to the weighing and macroscopic examination of the male testes.

The volume of sperm produced was decreased at 20 mg/kg feed (0.04 mL versus 0.12, 0.18 and 0.13 mL at 0, 5 and 10 mg/kg feed, respectively). Female-related fertility was increased at 5 and 10 mg/kg feed and male-related fertility was decreased at 10 and 20 mg/kg feed (with a greater effect at 10 mg/kg feed). Egg production only decreased at 20 mg/kg feed and by the third week of treatment. The effect persisted for 2 weeks following 4,15-DAS treatment. Female-related hatchability was not affected by 4,15-DAS treatment, but there was a decrease in hatchability attributed to a male effect at 10 mg/kg feed. Although semen quality was not assessed, the authors suggested that the quality of semen at 10 mg/kg feed was poorer compared to that at 20 mg/kg feed, given the greater effect on male-related fertility at 10 mg/kg feed and the effect on hatchability. At necropsy, no differences in relative testes weights were found, but many treated males had small, fluid-filled cysts on the testes (Brake, Hamilton & Kittrell, 1999).

Intraperitoneal administration

Mice

Male CD-1 mice (*n* = 5–10/time point; 21–34 g bw) were treated with a single intraperitoneal injection at doses of 1, 5, 10 or 15 mg/kg bw of 4,15-DAS (purity not specified) in 10% aqueous DMSO. Mice were killed 1 hour or 14 days after treatment, and the sequence of morphological changes in the testes was examined. Detailed description was only provided for animals treated with 15 mg/kg bw. Lesions were reported to decrease in severity and frequency at lower doses but were present in some animals at doses as low as 5 mg/kg bw. Animals given 1 mg/kg bw were reported to have no detectable changes in the tissue. At 15 mg/kg bw of 4,15-DAS, testicular weights were significantly decreased 3 days after exposure and remained low for 14 days following treatment. Changes were observed in most mice by 6 hours, consisting of margination and clumping of chromatin in spermatogonia. Syncytia were seen at 24 hours after treatment. There was progressive depletion of germinal epithelium and mature spermatids; at 7 and 14 days the seminiferous tubules had large irregular lumina and variable cell populations. The authors noted that, overall, testicular damage progressed in severity and was still marked 2 weeks after exposure. The functional significance of these changes was not determined (Conner et al., 1986).

Rat

Male Lewis rats (*n* = 5/treatment; 12 weeks of age) were treated with 1.7 mg/kg bw of 4,15-DAS (purity not specified) dissolved in 10% aqueous DMSO administered as a single intraperitoneal injection. Control rats received the vehicle only. The number of spermatids in the testis, daily sperm production, epididymal sperm reserves, transit time for sperm through the epididymis and morphology of the testis and epididymis were evaluated 1, 3, 7, 30, 60 and 90 days after treatment. Decreases in body weight of approximately 12% were observed 3–7 days following treatment, but these were comparable to controls by day 30. Testicular weight was decreased by day 60; by day 90 testicular weight was only 73% of the control weight. Spermatid content and sperm production were reduced by day 30 and by day 90 were only 37% of control values. These results were not appreciably altered when normalized for the loss of testicular weight. The frequency of hypocellular seminiferous tubules was increased. The hypocellular tubules had few or no germinal epithelial cells and consisted almost entirely of vacuolated Sertoli cells. The authors noted that there was a relatively long period between exposure and the observed testicular changes and that there was no evidence of recovery. The authors concluded that the observed effects reflect injury to germinal cells early in the maturation sequence. Other effects of 4,15-DAS on the reproductive tract included alterations in epididymal transit

times, as reflected by decreased epididymal sperm reserves occurring prior to a decrease in sperm production (Conner et al., 1990).

(b) Developmental toxicity

Oral administration

Gentles et al. (1993) administered a single oral dose of DAS to mice on gestation days 9–11; however, only the abstract of the study report, which provides insufficient information for evaluation, was available to the Committee.

Intraperitoneal administration

Pregnant female ICR mice (24–30 g) were administered a single intraperitoneal injection of 4,15-DAS (purity not specified) at 0, 1, 1.5, 2, 3 or 6 mg/kg bw dissolved in 1:9 mixture of propylene glycol/saline on one of gestation days 7–11. Control animals were either treated with the solvent vehicle or left untreated. Body weights and general appearance of pregnant mice were monitored daily. The dams were killed on day 18 of pregnancy. The numbers of implants, resorptions and live fetuses were counted. Live fetuses were weighed and examined for external malformations. Every third fetus was examined for skeletal defects and the remaining fetuses were examined for visceral anomalies.

Maternal toxicity was observed at 6 mg/kg bw (death and vaginal bleeding) and 3 mg/kg bw (death) but not at lower doses. There was no effect on total number of implantations, but resorptions increased with dose and with day of injection (from 7–34% on gestation days 7–11 at 1 mg/kg bw to 100% on all tested gestation days at 6 mg/kg bw). Fetal body weight of the live fetuses was significantly depressed at all doses and was more depressed with increasing dose. Fetal malformations, both external (exencephaly, omphalocele, hydrocephaly, short snout, protruding tongue and meningoencephalocele) and skeletal (anomalies of the skull, sternebrae, vertebrae, vertebral centra and ribs), were observed especially when 4,15-DAS was given on gestation day 9. On gestation day 9, external and skeletal malformations were observed at all doses. At 1 mg/kg bw, the lowest dose tested, 16% and 64% of fetuses had external gross malformations and skeletal defects, respectively. No internal soft-tissue defects were reported (Mayura et al., 1987).

2.2.6 Special studies

(a) Gastrointestinal effects

Dietary administration

Female Wistar rats were fed diets containing 4,15-DAS (purity not specified) at 10 or 20 mg/kg feed (equivalent to 0.5 and 1 mg/kg bw per day) for 10 weeks

to examine the effect of simultaneous treatment with methylbenzylnitrosamine, which induces oesophageal cancer, or whether potentiation of cancer could be mediated via inhibition of the DNA repair protein *O*-6-methylguanine-DNA methyltransferase. The oesophagus was the only tissue examined macroscopically and histologically. Rats fed 20 mg/kg feed all died by week 7 of treatment. At 10 mg/kg feed, the rats began to die at week 10. A reduced growth rate was reported in all treated animals. No macroscopically visible protrusions of the oesophagus were observed after 10 weeks at 10 mg/kg feed, but a thickening of the epithelium was noted. The authors concluded that simultaneous treatment with methylbenzylnitrosamine for 10 weeks did not potentiate oesophageal tumours and DAS treatment did not alter the level of *O*-6-methylguanine-DNA methyltransferase (Craddock, Sparrow & Henderson, 1986; Craddock, Hill & Henderson, 1987).

In a follow-up study, 10 female Wistar rats were fed diets containing DAS (details not provided) at 10 mg/kg feed (equivalent to 0.5 mg/kg bw per day) for 9 months. Only a small number of parameters were assessed and reported. Deaths occurred at weeks 6, 10, 11 (2 animals), 12 and 24 after the start of treatment. Only four rats survived for 9 months. The histology and DNA replication of the oesophagus were normal, but in two of the four surviving rats, the forestomach showed hyperplasia, with oedema and capillary buds in the oedematous region. The thymuses of all four surviving rats were small (Craddock, Hill & Henderson, 1988).

Oral administration

Craddock, Hill & Henderson (1988) examined cell replication in the oesophagus and stomach of female Wistar rats following single intubated doses of DAS (details not provided) at 0.0125, 0.06, 0.125, 0.5 or 2.0 mg/kg bw in DMSO or repeated weekly intubations of DAS at 2 mg/kg bw in DMSO for 6–8 weeks. Limited parameters were assessed and reported in these studies.

Compared with controls, a single intubated dose of 2.0 mg/kg bw caused an increase in cell replication 1 day after treatment in the oesophagus and in the squamous and glandular stomach. Replication increased further by day 2 but returned to control levels by day 4. At lower doses no increase in replication was evident in the oesophagus, but the effect was observed in the squamous and glandular stomach at 0.5, 0.125 and 0.06 mg/kg bw, with the maximum increase observed 1 day after treatment. The degree of staining at 0.0125 mg/kg bw was comparable to that in the controls.

Following weekly intubations with 2 mg/kg bw of DAS, 2/12 rats survived 8 doses, 4/5 rats survived 7 doses and 1/2 survived 6 doses. Death occurred within the first few weeks after dosing. The only organ that showed macroscopic

abnormality was the thymus; these abnormalities were described as very small or virtually non-existent. When the surviving animals were killed after 9 months, no change in the histology or in DNA replication was observed in the oesophagus or stomach.

Male Sprague Dawley rats (n = 15; 180–210 g bw) were administered DAS (details not provided) by intubation at a dose of 1 mg/kg bw in corn oil for 2 days. Rats were killed on day 3 and the fundic zone of the stomach was examined to evaluate the changes in glycoproteins. Staining with alcian blue to detect complex carbohydrates and assays using lectin conjugates known to react with specific terminal sugar residues of oligosaccharide chains showed increased alcianophilia in various parts of the fundic glands and enhanced labelling with *Lotus tetragonolobus* for α-L-fucose and *Glycine max* for α-D-*N*-acetylgalactosamine in the surface epithelium and in the foveolae, while *Triticum vulgaris* for β-D-*N*-acetylglucosamine binding appeared in the lower mucous neck cells. The authors concluded that these results indicate incomplete or abnormal synthesis or secretion of mucus glycoproteins following DAS exposure due to the large degree of heterogenicity in oligosaccharide chains (More, Galtier & Eeckhoutte, 1990).

(b) Haematological effects

In vivo

Oral administration

Male Wistar rats (n = 30; 200 g bw) received 0 or 1 mg/kg bw DAS (details not provided) or 1 mg/kg bw NEO dissolved in DMSO by gastric intubation, 3 times weekly for 5 weeks (equivalent to 0.43 mg/kg bw per day). Five rats per group were killed on days 0, 7, 14, 21, 28 and 35, and haematological effects of DAS and NEO were examined. Treated animals had a roughness and discoloration of the fur and two rats treated with DAS had severe diarrhoea. For both toxin groups, body weights were comparable to controls. Of the haematological parameters examined, comparable observations were made for both toxins. Erythrocyte counts were affected the most by treatments: in contrast to controls in which erythrocyte counts increased gradually upon maturation, the counts were lower in treated rats than controls from day 7 onward. Haematocrit and haemoglobin values were also lower than controls. Although the number of platelets was not significantly different from controls, the size distribution showed a clear shift towards the larger platelets following DAS treatment. No effects were observed on differential leukocyte counts, mean cell volumes and mean cell haemoglobin levels. The major pathological lesions associated with treatments were atrophy (moderate or mild) and necrosis of the actively dividing cells of the bone marrow, thymus, spleen, lymph nodes and gastrointestinal tract. They were generally mild

and noted mainly after 2–4 weeks of treatment, with some regression of effects in the bone marrow, thymus and lymph nodes at week 5. The authors suggested this result may indicate increased detoxifying capability upon repeat exposure (Janse van Rensburg, Thiel & Jaskiewicz, 1987).

Intraperitoneal administration

CD-1 mice ($n = 8$; 3 weeks of age) were administered a single intraperitoneal dose of DAS (details not provided) of 10 mg/kg bw dissolved in DMSO to examine the time required for haematological measurements to return to pretreatment values. Blood samples were collected on days 1, 3, 7, 14, 21, 28 and 42 following DAS exposure. Spleens were weighed and total spleen cells per gram of tissue were analysed on day 1 and then weekly up to day 42. Total WBC counts were significantly decreased ($P < 0.0005$) compared with controls at day 3, but were comparable with controls at day 7. A significant decrease in platelet numbers, to one third that of controls, was observed at day 1; platelet numbers had recovered by day 7. Haemoglobin and haematocrit had decreased on days 1 and 3 but returned to pretreatment values 3 weeks after exposure. No significant differences in differential WBC counts were observed 1 week after DAS exposure, although decreases were noted in all bone marrow cell types between days 1 and 7 after exposure. Spleen weight and total cells per gram of tissue were significantly decreased on day 1 following exposure. Microscopic examination of spleen of treated mice showed marked hyperplasia in the red pulp and the number of young proliferating lymphocytes and megakaryocytes was markedly increased (Suphiphat et al., 1989).

In vitro

WBC progenitors (colony forming unit–granulocyte and macrophage; CFU-GM) from human umbilical cord blood and rat bone marrow were cultured in the presence of 4,15-DAS (from 10^{-10} to 10^{-6} mol/L), T-2 toxin (from 10^{-11} to 10^{-6} mol/L), HT-2 toxin (from 10^{-9} to 10^{-6} mol/L) or DON (from 10^{-8} to 10^{-6} mol/L) for 14 days. The relative cytotoxicity to human and rat CFU-GM was T-2 toxin ≥ 4,15-DAS > HT-2 toxin > DON (Parent-Massin, Fuselier & Thouvenot, 1994; Parent-Massin & Thouvenot, 1995).

Lautraite et al. (1997) also cultured CFU-GM from human umbilical cord blood and rat bone marrow in the presence of 4,15-DAS (from 5×10^{-10} to 10^{-8} mol/L) for 14 days. Compared with human CFU-GM, rat CFU-GM was more sensitive to 4,15-DAS; compared with late-forming cells, early-forming cells were more sensitive to 4,15-DAS. IC_{50} values on day 14 were 7.6×10^{-9} mol/L for human CFU-GM and 6.2×10^{-9} mol/L for rat CFU-GM. These results support that DAS is myelotoxic for WBC progenitors.

RBC progenitors (erythroid burst forming unit; BFU-E) from human umbilical cord blood were cultured in the presence of 4,15-DAS (from 5×10^{-10} to 10^{-7} mol/L), T-2 toxin (from 10^{-10} to 10^{-8} mol/L), HT-2 toxin (from 10^{-10} to 10^{-7} mol/L) or DON (from 10^{-8} to 2.5×10^{-7} mol/L) for 14 days. 4,15-DAS, T-2 toxin and HT-2 toxin induced a strong cytostatic effect on human BFU-E treated with the highest concentrations. The cytostatic effect was associated with a cytotoxic effect (presence of cellular fragments). Total cytotoxicity as previously reported for the CFU-GM was not observed. DON did not induce cytotoxicity or cytostaticity on BFU-E cultures. No specific morphological changes were observed in any of the BFU-E cultures. In the presence of low concentrations (5.3×10^{-9} and 5×10^{-10} mol/L) of 4,15-DAS, haemoglobin synthesis was inhibited (Rio, Lautraite & Parent-Massin, 1997).

Platelet progenitors (colony forming unit–megakaryocyte; CFU-MK) from human umbilical cord blood were cultured in the presence of 4,15-DAS (from 5×10^{-10} to 10^{-7} mol/L), T-2 toxin (from 10^{-10} to 10^{-8} mol/L), HT-2 toxin (from 10^{-10} to 10^{-7} mol/L) or DON (from 10^{-8} to 2.5×10^{-7} mol/L) for 12 days. T-2 toxin was the most cytotoxic for CFU-MK. At concentrations of 10^{-7} mol/L 4,15-DAS was cytotoxic for CFU-MK. At 4,15-DAS concentrations of 5.3×10^{-9} mol/L a decrease in large colony numbers was compensated by an increase in small colony numbers causing a cytostatic effect. 4,15-DAS had no effect at concentrations of 5×10^{-10} mol/L. DON had no cytotoxic effects at any of the tested concentrations. The results indicate that platelet production could be decreased by a cytotoxic effect on CFU-MK and a cytostatic effect that causes a decrease in megakaryocyte production and consequently platelets (Froquet, Sibiril & Parent-Massin, 2001).

Grandoni et al. (1992) showed that various trichothecenes, including DAS (details not provided), inhibited phospholipid metabolism in stimulated bovine platelets, suggesting a mechanism for the observed mycotoxin-associated effects on platelets.

(c) Immunological effects

In vivo

Oral administration

In mice treated orally with DAS (details not provided) at 3 mg/kg bw for 2 days before intraperitoneal inoculation with *Listeria*, the monocytogenes showed increased mortality and splenic *Listeria* counts. The thymus weight was reduced, and lymphocytes were depleted from the thymus cortex and from splenic lymphoid follicles and periarteriolar lymphoid sheaths. A single dose of DAS at 4 mg/kg bw 6 days before the *Listeria monocytogenes* challenge did not affect mortality. Mice treated with DAS and subsequently inoculated with *Listeria*

had higher neutrophil levels than the *Listeria*-infected control mice (Ziprin & Corrier, 1987).

Intraperitoneal administration

Intraperitoneal treatment of male Swiss mice (n = 5; 4–6 weeks old) with DAS (details not provided) in a 6% ethanol in saline solution at doses of 0–2 mg/kg bw for 7 days resulted in a dose-dependent reduction in the thymus weight and an inhibited response to sheep red blood cells (sRBCs). A significant decrease in thymus weight was observed at a dose of 1.5 mg/kg bw. Antibody production decreased before a significant change in the thymus weight was observed. Liver and spleen weights did not differ from controls. Compared with T-2 toxin, DAS was less active in inducing thymus atrophy and antibody suppression (Rosenstein et al., 1979).

The mortality rate of Swiss mice (OF1) (n = 10/treatment; 6 weeks old; 20–25 g bw) was examined after infection with *S. typhimurium* (single intraperitoneal exposure to 15 ± 5 bacteria per 0.2 mL saline) either before or after intraperitoneal administration of 4,15-DAS (purity not specified; 5.3 mg/kg bw and 2.7 mg/kg bw 10 and 9 days or 4 and 3 days prior to infection versus 5.3 mg/kg bw and 2.7 mg/kg bw 3 and 24 hours or 4 and 5 days after infection). Animals were observed daily for 21 days following treatment. When 4,15-DAS was administered prior to infection, the mortality rate was comparable to controls, and when 4,15-DAS was administered after infection, an increased mortality rate was observed (Bottex, Martin & Fontanges, 1990).

The authors further studied the effect of 4,15-DAS either before or after antigenic stimulation using the direct plaque-forming cell (PFC) assay on the splenic lymphocytes of the mouse. Mice were immunized with a 5% suspension of sRBCs per mouse. The number of PFCs per spleen represented the mean of the PFC response per batch of 10 mice. Mice were administered intraperitoneal doses of 4,15-DAS at 5.3 mg/kg bw on day 1 followed by 2.7 mg/kg bw the next day either 5 days before or 1 day after antigenic stimulation. The PFC assay was performed 4 days later. The results were similar to the mortality rates observed previously, that is, administration of 4,15-DAS before antigenic stimulation did not affect the antibody response, but the antibody response was significantly decreased when 4,15-DAS was administered after antigenic stimulation (Bottex, Martin & Fontanges, 1990).

In vitro

Using mitogen-stimulated murine splenic and thymic lymphocytes, Lafarge-Frayssinet et al. (1979) showed that DAS (details not provided) reversibly inhibited the stimulation of both T- and B-cells and suppressed their ability to

synthesize anti-sRBC antibodies. While at high concentrations a direct cytostatic action was found, at low concentrations an opposite effect was observed.

In mitogen-stimulated human lymphocytes, 4,15-DAS effectively inhibited proliferation and immunoglobulin production (immunoglobulin allotypes IgA, IgG and IgM) in a dose-dependent manner with limited sensitivity between individuals. However, low levels of 4,15-DAS exposure (4×10^{-10} mol/L) could also result in enhanced proliferative responses, as well as elevated immunoglobulin production (especially IgA). Combinations of 4,15-DAS with T-2 toxin, NIV or DON resulted in additive or antagonistic interactions on lymphocyte proliferation. Combinations of DON with either T-2 toxin or 4,15-DAS resulted in inhibition that was significantly lower than, or similar to, the toxicity produced when cells were exposed to only 4,15-DAS or T-2 toxin. In contrast, the combination of T-2 toxin and 4,15-DAS tended to inhibit the proliferative responses more effectively than would be expected from the inhibition produced when the toxins were applied singly. However, a nonlinear response was also observed when the concentration of a single toxin was increased. There was no clear evidence of a synergistic effect in this study (Thuvander, Wikman & Gadhasson, 1999).

Mitogen stimulation was used to examine the time-course recovery of lymphocytes from exposure to DAS (details not provided). The following mitogens were added to lymphocyte cell suspensions: T-cell mitogens (concanavalin A and phytohaemagglutinin A), B-cell mitogen (bacterial lipopolysaccharide) and T-cell-dependent B-cell mitogen (pokeweed mitogen). Cultures were incubated for 72 hours. To determine the response of cells to mitogen, the difference between mitogen-stimulated and unstimulated cells was expressed as the differential uptake of tritiated thymidine ([^{3}H]-TdR) in counts per minute and the ratio of the mean counts per minute of stimulated cultures versus unstimulated cultures. Both concanavalin A and phytohaemagglutinin A stimulation indices returned to control levels 3 weeks following DAS exposure. Lipopolysaccharide- and pokeweed mitogen–stimulation indices had not returned fully to control levels after 6 weeks, indicating that DAS affected the B-cell series more than the T-cells (Suphiphat et al., 1989).

Murine peritoneal macrophages were preincubated with 4,15-DAS at concentrations of 0.1–1 µg/mL. At concentrations that did not affect the cell viability, 4,15-DAS suppressed microbicidal activity of phagocytic cells (reduced phagocytosis at 2 ng/mL, reduced microbicidal activity at 1 ng/mL, reduced superoxide anion production at 1 ng/mL and reduced phagosome–lysosome fusion at 0.1 ng/mL), which indicates that the inhibition of the killing mechanism arises from both oxidative and non-oxidative pathways (Ayral et al., 1992).

Qureshi, Brundage & Hamilton (1998) carried out experiments on macrophages from chickens. Monolayers of cell type were exposed to 0, 12.5 and

25 µg/mL of 4,15-DAS for 1 hour. Treatment with 4,15-DAS resulted in decreased viability of macrophages, causing the suppression in macrophage phagocytic function and therefore altering the first line of immunological defence in chickens.

(d) Modified forms and metabolites

Glucoside forms of DAS have been reported in corn products (Nakagawa et al., 2013a,b); however, no toxicological information is available about these modified forms. In addition, DAS-M1 has been identified in potatoes after thermal treatment and increased acidity. In an in vitro wheat germ assay to investigate protein synthesis inhibition and an in vivo *S. cerevisiae* assay to investigate growth inhibition, DAS-M1 was reported to be approximately 100 and 50 times less potent than DAS, respectively (Shams et al., 2011).

Structure–activity analysis conducted by Thompson & Wannemacher (1986) indicates that type A trichothecenes (identified in the in vitro and in vivo studies conducted with 4,15-DAS, described in section 2.1.2) with an acetyl group at R_3 are the most potent and that removal of this acetyl group results in a pronounced decrease in potency, whereas removal of the acetyl group at R_2 results in a smaller loss of activity (Table 4). Biological data indicate that metabolism in the liver involves deacetylation first at R_2 and then at R_3 followed by conjugation with glucuronic acid. In addition, Wu et al. (2013) indicate that the 12,13-epoxide ring is a crucial toxic group and that the opening of the epoxy group is a critical detoxification reaction. De-epoxidation has been observed to occur in the gastrointestinal tract of rats, pigs and cattle but not dogs or chickens.

The structure–activity predictions for toxicity are consistent with the reported potency ranking for in vitro cytotoxicity and protein synthesis inhibition: 4,15-DAS > MAS > NEO >> SCP (see section 2.1.3(c)). 4,15-DAS was less toxic than 15-MAS after intraperitoneal administration in mice but toxicity was comparable in chickens after oral administration (see Table 5 for a comparison of in vivo LD_{50} values).

Comparable toxicity of 4,15-DAS and 15-MAS was also demonstrated after short-term dietary exposure. Ademoyero & Hamilton (1991a) reported LOELs equivalent to 1, 0.5 and 0.3 mg/kg bw per day for SCP, 4,15-DAS and 15-MAS based on the incidence of oral lesions in male broiler chicks fed contaminated diets for 21 days.

Toxicological data available for NEO were reviewed by RIVM (2002). It was concluded that there is insufficient information to derive a reference dose or make conclusions on the toxicokinetic and toxicological properties of NEO, but that the available information indicates that the mechanism of action is similar to that of other trichothecenes given the structural and toxic effect similarities reported in the available data (limited detail as information reported in reviews

Table 4
Chemical structure of DAS and identified metabolites

	R_1	R_2	R_3	C-8
4,15-DAS	OH	OAc	OAc	H
15-MAS	OH	OH	OAc	H
4-MAS	OH	OAc	OH	H
SCP	OH	OH	OH	H
NEO	OH	OAc	OAc	OH

DAS: diacetoxyscirpenol; MAS: monoacetoxyscirpenol; NEO: neosolaniol; SCP: scirpentriol

Table 5
Comparison of LD_{50} of 4,15-DAS and identified metabolites

Species	Route	Compound	LD_{50} (mg/kg bw)	Reference
Mouse	i.p.	15-MAS	4.5	Thompson & Wannemacher (1986)
		NEO	14.8	
		4,15-DAS	15.3	
Chicken	Oral	4,15-DAS	2.0	Richardson & Hamilton (1990)
		15-MAS	2.5	
		SCP	9.3	
Chicks	Oral	4,15-DAS	5.9	Mirocha et al. (1985)
		15-MAS	3.4	

bw: body weight; DAS: diacetoxyscirpenol; i.p.: intraperitoneal; LD_{50}: median lethal dose; MAS: monoacetoxyscirpenol; NEO: neosolaniol; SCP: scirpentriol

or abstracts only). The reported toxic effects include rapid increase of leukocytes and lymphocytes accompanied by increased β-globulin and decreased γ-globulin and radiomimetic cellular injury and karyorrhexis in the small intestine and bone marrow at lethal intraperitoneal doses in mice (Ueno et al., 1973a,b); decrease in WBC counts and ataxia in hind legs of cats receiving repeat subcutaneous administration (Ueno et al., 1973b); emesis, diarrhoea and anorexia in cats (Ueno et al., 1973b); asthenia, inappetance, diarrhoea and coma in chicks at lethal doses and decreased body-weight gain and feed consumption at sublethal doses (Chi et al., 1978); and emesis in ducklings (Ueno et al., 1983). In addition, Janse van Rensburg, Thiel & Jaskiewicz (1987) reported that NEO and DAS had comparable effects on haematological parameters assessed after 1 mg/kg bw gavage dosing of rats 3 times a week for 5 weeks.

(e) Related contaminants

Since Kimura et al. (2007) identified that 4,15-DAS is formed in *Fusarium* species at a side branch of the T-2 toxin synthetic pathway, the available in vitro

and in vivo data for 4,15-DAS and T-2 toxin, as well as combined effects, were considered. 4,15-DAS and T-2 toxin are structurally similar and undergo similar biotransformation steps involving hydrolysis, hydroxylation, de-epoxidation and glucuronidation following oral administration. T-2 toxin is rapidly converted to HT-2 toxin and other metabolites common to both T-2 toxin and HT-2 toxin in the gut. Therefore, the toxicity of T-2 toxin in vivo is considered to include that of HT-2 toxin (Annex 1, reference *152*).

Consistent with trichothecenes in general, at a biochemical and cellular level 4,15-DAS, T-2 toxin and HT-2 toxin have an inhibitory effect on protein synthesis by binding to ribosomes, have an inhibitory effect on RNA and DNA synthesis, are toxic to cell membranes, and induce apoptosis particularly in lymphatic and haematopoietic tissue (SCF, 2001). (Refer to Table 1 in section 2.1.3(c) for a comparison of functional groups, cytotoxicity and protein synthesis inhibition.)

In vitro

When comparing the cytotoxicity of 4,15-DAS and T-2/HT-2 toxins in in vitro assays with WBC progenitors (Parent-Massin, Fuselier & Thouvenot, 1994; Parent-Massin & Thouvenot, 1995), RBC progenitors (Rio, Lautraite & Parent-Massin, 1997) and platelet progenitors (Froquet, Sibiril & Parent-Massin, 2001), both T-2 toxin and 4,15-DAS were consistently cytotoxic, with T-2 toxin consistently the most potent followed by 4,15-DAS and HT-2 toxin. Using mitogen-stimulated human lymphocytes, the effects on proliferative responses and immunoglobin production were assessed; T-2 toxin was found to be 3–4 times more potent than 4,15-DAS (Thuvander, Wikman & Gadhasson, 1999).

Acute exposure

Oral administration

Hoerr, Carlton & Yagen (1981a,b) exposed male broiler chicks (n = 10; 7 days old) to T-2 toxin or 4,15-DAS by oral gavage doses of 1, 2, 3, 4, 5 or 6 mg/kg bw or in combination. The toxins administered alone or in combination usually caused death within 24 hours. Affected birds typically lost 10% of their body weight and had clear or white opaque liquid faecal droppings. Decreased spontaneous activity was noted prior to death. The 72-hour single oral LD_{50} for T-2 toxin was 4 mg/kg bw and for 4,15-DAS was 5 mg/kg bw.

Intraperitoneal and subcutaneous administration

A comparison of mouse LD_{50} values by the intraperitoneal and subcutaneous routes of administration by Thompson & Wannemacher (1986) did not identify

Table 6
Comparison of type A trichothecenes in mice

Type A trichothecene	LD_{50} (mg/kg bw)	
	Subcutaneous	Intraperitoneal
T-2 toxin	3.3	9.1
HT-2 toxin	6.7	10.1
4,15-DAS	19.5	15.3
NEO	9.7	14.8

bw: body weight; DAS: diacetoxyscirpenol; LD_{50}: median lethal dose; NEO: neosolaniol

substantial differences between the type A trichothecenes via the intraperitoneal route of administration. In general T-2 toxin was the most potent (Table 6).

Following the intraperitoneal treatment of male Swiss mice with DAS or T-2 toxin at doses of 0.5–2 mg/kg bw for 7 days, DAS was found to be less active than T-2 toxin in inducing thymus atrophy and antibody suppression (Rosenstein et al., 1979).

Dermal administration

Schiefer, Hancock & Bhatti (1986) compared the acute topical toxicity of type A trichothecenes 4,15-DAS, T-2 toxin and HT-2 toxin individually and as mixtures. Single doses of 5, 10, 20, 30 or 40 mg/kg bw trichothecene were topically applied to shaved skin of groups of 10–20 male CD-1 mice. 4,15-DAS resulted in mortality in 0, 15%, 25%, 70% and 95% of the animals, respectively. Of the type A trichothecenes, 4,15-DAS was less lethal than T-2 toxin but slightly more lethal than HT-2 toxin.

Intravenous administration

In contrast to the relative lethality observed in broiler chickens and mice (Hoerr, Carlton & Yagen, 1981a,b; Schiefer, Hancock & Bhatti, 1986; Thompson & Wannemacher, 1986) and the immunological effects reported by Rosenstein et al. (1979), Coppock et al. (1989) suggested that 4,15-DAS is approximately 5 times more toxic than T-2 in pigs on the basis of haematological effects reported following intravenous injection. Although Coppock et al. (1989) did not give the exact reason for this suggestion, the segmented neutrophil plots from pigs following exposure to either 0.5 mg/kg bw 4,15-DAS (Coppock et al., 1989) or 0.6 mg/kg bw T-2 toxin (Lorenzana et al., 1985) show a greater dip in segmented neutrophil counts in pigs exposed to 4,15-DAS than those exposed to T-2.

Short-term exposure

Comparisons of the critical effects of 4,15-DAS and T-2 toxin and HT-2 toxin are limited by differences in the study designs and the limited number of comparable studies in the available databases.

The critical effects identified following short-term dietary exposure to T-2 toxin and HT-2 toxin were immunotoxicity and haematotoxicity (Annex 1, reference *152*). The critical effects of T-2 toxin and 4,15-DAS in dietary pig studies are shown in Table 7. The LOAEL of 0.03 mg/kg bw per day for changes in WBC counts identified in the 3-week dietary study in pigs (Rafai et al., 1995) was used to derive the provisional maximum tolerable daily intake (PMTDI) for T-2 toxin and HT-2 toxin. This LOAEL was considered to be close to the NOAEL, as the effects on blood cell counts were subtle and reversible and other studies in pigs showed no effects at this dose (Annex 1, reference *152*). In short-term dietary pig studies conducted with 4,15-DAS, no effects on blood cell counts were observed at doses up to 0.4 mg/kg bw per day, the highest dose tested (Weaver et al., 1981; Harvey et al., 1991).

The comparison of growth inhibition is limited as this is a nonspecific effect that may be due to effects on the central nervous system, reduced feed consumption or other toxic parameters. However, the studies conducted with 4,15-DAS indicate that the reduced weight gain is likely to be associated with feed refusal as feed efficiency was not affected; although feed consumption was reported to be reduced, this was not as clear in the T-2 toxin database.

Although oral lesions were observed in studies conducted with birds and pigs after dietary administration of 4,15-DAS or T-2 toxin (Weaver et al., 1981; Shlosberg, Klinger & Malkinson, 1986; Diaz et al., 1994; Sklan et al., 2003), the occurrence is considered to be due to the presence of the mycotoxin in the feed resulting from localized contact and not from systemic exposure. Therefore, a potency comparison was not considered appropriate for a dietary risk assessment.

Evidence from other animal studies supports that 4,15-DAS and T-2 cause similar immunotoxic and haematotoxic effects following oral exposure. In mice, depletion of lymphocytes from lymphoid tissues was observed after a gavage dose of 4,15-DAS at 3 mg/kg bw per day for 2 days (Ziprin & Corrier, 1987) or a single gavage dose of T-2 at 4 mg/kg bw (Corrier & Ziprin, 1986). In addition, decreased haemoglobin, haematocrit and RBCs as well as atrophy and necrosis of the bone marrow, thymus, spleen and lymph nodes were observed in rats administered an oral dose of 4,15-DAS at 1 mg/kg bw 3 times a week for 5 weeks (equivalent to 0.43 mg/kg bw per day) (Janse van Renburg, Thiel & Jaskiewicz, 1987).

Table 7
Critical effects identified in short-term dietary pig studies for T-2 and 4,15-DAS

	Critical effects LOAEL/NOAEL (mg/kg bw per day)		
Compound	**Growth inhibition**	**Immunological effects**	**Haematological effects**
T-2 toxin (HT-2 toxin)	0.04 (LOAEL) – lowest dose tested Pig, 8 weeks Reduced body-weight gain[a] (Weaver et al., 1978a)	0.03 (LOAEL) – lowest dose tested Pig, 3 weeks Decreased antibody titre, decreased leukocyte count and T-lymphocytes, decreased lymphocyte proliferative response to mitogen stimulation Rafai et al. (1995)	0.06 (LOAEL) Pig, 3 weeks Decreased haemoglobin; 0.1 decreased RBCs Rafai et al. (1995)
4,15-DAS	0.23 (LOAEL) Pig, 9 weeks Reduced body-weight gain[b] Weaver et al. (1981)	0.1 (NOAEL) Pig, 4 weeks No effect on leukocyte count, no effect on lymphocyte proliferative response to mitogen stimulation Harvey et al. (1991)	0.4 (NOAEL) – highest dose tested Pig, 9 weeks No effect on haemoglobin or RBCs Weaver et al. (1981)
	0.1 (LOAEL) Pig, 4 weeks Reduced body-weight gain[c] Harvey et al. (1991)	0.4 (NOAEL) – highest dose tested Pig, 9 weeks No effect on leukocyte count Weaver et al. (1981)	

bw: body weight; DAS: diacetoxyscirpenol; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; RBC: red blood cell

[a] Reduced body-weight gains were not statistically significant, but the treatment group gained 4.1 kg less than controls; feed consumption was significantly less ($P < 0.05$) than controls for the first week only. Feed efficiency was not significantly different.

[b] Reduced body-weight gains were statistically significant compared with controls (details not provided). Feed consumption was statistically significantly reduced compared with controls. Feed efficiency was not significantly different.

[c] Body-weight gains were significantly reduced compared with controls. Feed consumption details were not provided but animals were observed to eat less. Feed efficiency was not significantly different.

Combined effects

Studies that address the effects of combined exposure to 4,15-DAS and T-2 toxin are limited. Thompson & Wannemacher (1986) examined 4,15-DAS, T-2 toxin, DON and verrucarin A in equimolar combinations of 2, 3 or 4 toxins for the effect on the 50% protein synthesis inhibition in Vero cells. The results indicated dose additivity for 4,15-DAS and T-2. No combination of any of the four toxins showed a synergistic action.

Thuvander, Wikman & Gadhasson (1999) demonstrated that combinations of 4,15-DAS with T-2, NIV or DON resulted in additive or antagonistic interactions on lymphocyte proliferation. Combinations of DON with either T-2 or 4,15-DAS were antagonistic, resulting in an inhibition that was significantly lower than, or similar to, the toxicity produced when cells were exposed to only 4,15-DAS or T-2 toxin. In contrast, the combination of T-2 and 4,15-DAS tended to inhibit the proliferative responses more effectively

than would have been expected from the inhibition produced when the toxins were applied singly; however, a nonlinear response was also observed when the concentration of a single toxin was increased. There was no clear evidence of a synergistic effect in this study.

Hoerr, Carlton & Yagen (1981b) determined the LD_{50} in broiler chickens following single and multiple doses of 4,15-DAS and T-2 toxin and in combination. The combined LD_{50}, depending on the ratio of T-2 to 4,15-DAS, ranged from 1.03 to 3.25 mg/kg bw, indicating an additive effect in terms of lethality.

Schiefer, Hancock & Bhatti (1986) compared the acute topical toxicity of trichothecenes individually and as mixtures using CD-1 mice. When part of a mixture, 4,15-DAS appeared to slightly decrease the lethality of the more potent T-2 toxin and increase the lethality of the much less potent 3-acetyldeoxynivalenol (3-AcDON). However, the combination of T-2 toxin, 4,15-DAS and 3-AcDON produced a greater effect than might be predicted from a strictly additive model.

Diaz et al. (1994) fed laying hens diets containing pure 4,15-DAS or T-2 toxin alone (2 mg/kg feed) or in combination (4 mg/kg feed) for 24 days. The basal diet was analysed for the presence of various toxins and only a small amount of DON (0.3 mg/kg feed) was detected. The effects of the combined exposure were additive for reduced feed consumption and incidence of oral lesions. Although the study authors considered the combined effect on decreased egg production to be synergistic, egg production varied considerably over the experimental period and it is not clear if the decrease in egg production in the combined toxin group in the last 5 days of the study is a more-than-additive effect. No significant changes in body weight and only mild changes in plasma enzymes were observed.

In addition to analysing the combined effects of 4,15-DAS and T-2 toxin, studies have examined the combined effects of 4,15-DAS and OTA, aflatoxin and fumonisin B_1 in chickens, turkeys, lambs or pigs. Results indicate that 4,15-DAS and OTA or fumonisin B_1 have an additive or less-than-additive effect in chickens and turkeys for body-weight decreases, feed refusal, oral lesions, organ weight changes and haematological parameter changes (Kubena et al., 1994, 1997b). The combined effect of 4,15-DAS and aflatoxin in chickens (Kubena et al., 1993) and lambs (Harvey et al., 1995) was synergistic for decreased body weight but additive or less-than-additive for organ weight changes and haematological parameter changes. In contrast, the combined effect of 4,15-DAS and aflatoxin was additive or less-than-additive in pigs for all parameters assessed including body weight and were considered to mostly represent the toxicity of aflatoxin (Harvey et al., 1991).

Overall, 4,15-DAS, T-2 toxin and HT-2 toxin appear to cause similar effects at the biochemical and cellular level and there are similarities in toxic effects with T-2 being observed to be more potent when comparing in vitro and in vivo

end-points. However, due to differences in study design and a limited number of comparable studies the data are insufficient for establishing relative potencies. Of the studies addressing the combined effects of these toxins, a consistent additive dose effect was observed; however, the nature of the combined effect has not been elucidated and it is not clear whether the toxins work via identical mechanisms at the biochemical and cellular level.

2.3 Observations in domestic animals/veterinary toxicology

Fusarium species have been known to be associated with a number of animal toxicoses. However, only rarely has a direct connection been established between toxicoses and specific mycotoxin(s). Animal mycotoxicoses associated with trichothecene-producing *Fusarium* species include, among others, the haemorrhagic syndrome (*F. sporotrichioides* and *F. poae*), Akakabi-byo (red mould disease or scabby grain intoxication; *F. graminearum*), feed refusal and emetic syndromes (*F. graminearum*), ill-thrift and oral and other gastrointestinal lesions (D'Mello et al., 1999; RIVM, 2002).

Mostrom & Raisbeck (2012) noted that the hallmark clinical sign of trichothecene toxicosis in animals is feed refusal, which has led to speculation that animals may not voluntarily consume enough contaminated ration to cause marked poisoning; however, when the only available feedstuffs are contaminated with trichothecenes, poisoning may be the result (CAST, 2003). Discrepancies observed between controlled experiments and field studies may be attributed to the presence of unidentified mycotoxins or additional fungal metabolites in the naturally contaminated grain and the additive and synergistic effects between the known and unidentified mycotoxins (D'Mello et al., 1999; Mostrom & Raisbeck, 2012).

Clinical signs typically include feed refusal and weight loss, emesis, oral and gastrointestinal lesions, immunomodulation, coagulopathy and haemorrhage, and cellular necrosis of mitotically active tissues such as intestinal mucosa, skin, bone marrow, spleen, testis and ovary (D'Mello et al., 1999; Mostrom & Raisbeck, 2012). The two case reports identified in the literature that identify DAS as one of the mycotoxins associated with toxicoses are summarized below.

Eight cases of death were reported in 200 adult bovines on a farm in Brazil that added citrus pulp into the diets. Clinical signs included alopecia of the head and neck, moderate bloody diarrhoea, signs of light, yellowish and/or reddish discoloration of the ocular mucosa, increased hepatic area, dehydration, lack of milk production, prostration, weakness, decumbency and death after 10–15 days. Serum levels of urea, creatinine, AST and γ-glutamyltransferase were moderately increased. Discrete hypochromic anaemia was diagnosed in one case.

At necropsy, liver and kidneys were found to be congested and haemorrhages were seen in the abomasum and bowel. Examination of the citrus pulp for fungi revealed *Aspergillus* sp., *Penicillium* sp. and *Mucor* sp. Chemical analysis by thin-layer chromatography detected 7 mg/kg of DAS (Galhardo et al., 1997).

Two cases of death were reported out of 10 Brahma chickens (2 months old) fed a combination of corn, barley and mineral premix. Clinical signs of depression and loss of appetite were observed a few days prior to death. Feed was analysed for mycotoxins. *Fusarium* spp. (400 000 in 1 g corn) and T-2 toxin (0.7 mg/kg), DAS (0.5 mg/kg) and DON (not quantified) were detected. Histopathological analysis of tissues revealed vascular dystrophy of the liver, necrosis and depletion of lymphocytes in the bursa of Fabricius as well as multiple necrosis in the glandular stomach and gut (Konjevic et al., 2004).

2.4 Observations in humans

2.4.1 Biomarkers of exposure

Three human biomonitoring studies were identified in the literature. Two studies were conducted in Belgium and one in Spain. Urine samples were analysed for up to 33 potential mycotoxin biomarkers of exposure. DAS was not quantified in any of the urine samples. Metabolites of DAS were not analysed (Rodriguez-Carrasco et al., 2014a; Heyndrickx et al., 2015; Huybrechts et al., 2015). Taking into consideration that 4,15-DAS is rapidly metabolized and the primary route of excretion differs between species, it is unknown whether analysing for DAS in the urine would provide a biomarker of exposure.

2.4.2 Biomarkers of effects

No information about potential biomarkers was available.

2.4.3 Clinical observations

In the 1970s and early 1980s, 4,15-DAS (under the name anguidine) was investigated for its potential as a cancer chemotherapeutic agent. Multiple phase I and phase II clinical trials were conducted on patients with diagnosed tumours using short treatment periods and intravenous administration (Vidal, 1990; Kornienko et al., 2015). Ultimately, the lack of sufficient evidence for efficacy against tumours and the adverse effects observed resulted in 4,15-DAS being discontinued from further clinical trials. The following section briefly summarizes the relevant toxicological information from these clinical studies.

Five phase I and nine phase II clinical trials exposed approximately 640 patients to 4,15-DAS by intravenous infusion at rates ranging from once per week for several weeks up to once per day for 5 consecutive days followed by a

treatment-free period of 3–4 weeks before beginning another treatment course. Observations from the earliest phase I studies (Goodwin et al., 1978) indicated that toxicity was substantially greater in patients when this was delivered as a bolus intravenous push than when delivered using slower intravenous infusion. A later study found that intermittent intravenous infusion elicited greater toxicity than continuous infusion (Murphy et al., 1978). The earlier phase I trials also found that when liver function was compromised the observed adverse effects were increased compared with patients without impaired liver function (Murphy et al., 1978; Belt et al., 1979; Goodwin et al., 1983). Therefore, all subsequent trials used intravenous infusion rates ranging from 30 minutes to continuous infusion and a lower starting dose for patients with metastasis to the liver or who otherwise had indications of liver dysfunction. The treatment doses in these trials ranged from 0.1 to 10 mg/m^2 (equivalent[13] to 0.0027–0.27 mg/kg bw); however, most patients were given doses of between 3.0 and 5.0 mg/m^2 (equivalent[13] to 0.081–0.135 mg/kg bw).

Mild nausea was reported at doses as low as 1.5–2.4 mg/m^2 (equivalent[13] to 0.041–0.065 mg/kg bw) (Murphy et al., 1978; DeSimone, Grecco & Lessner, 1979) with more significant effects reported at doses of 3.0 mg/m^2 or above (equivalent to 0.081 mg/kg bw) (Diggs, Scoltock & Wiernik, 1978; Murphy et al., 1978; Yap et al., 1979; Thigpen, Vaughn & Stuckey, 1981; Bukowski et al., 1982; Adler et al., 1984; DeSimone et al., 1986). The most consistently observed effects that appeared to exhibit a dose-dependent increase in frequency and severity included nausea and vomiting, myelosuppression, hypotension, fever, and, less frequently, CNS disturbances (confusion, hallucination, drowsiness and seizures), erythema of the skin, alopecia and stomatitis. Myelosuppression was generally characterized as decreased levels of lymphocytes (leukopenia) and platelets (thrombocytopenia). Hypotension ranged from mild to severe (in which cases anti-hypotensive interventions were required). Severe occurrences of myelosuppression, hypotension and/or severe vomiting were frequently the cited reason for discontinuation of treatment.

2.4.4 Epidemiological studies

Historical outbreaks associated with *Fusarium* species include alimentary toxic aleukia (*F. sporotrichioides* and *F. poae*; closely related to the haemorrhagic syndrome in animals), Urov or Kashin–Beck disease (*F. poae*) and Akakabi-byo (*F. graminearum*). Although some follow-up investigations have identified the involvement of other trichothecenes (type A: T-2 toxin and/or type B: DON, NIV,

[13] Doses were presented in mg/m^2 and converted to mg/kg bw by dividing by a body surface area–scaling factor for adult humans (k_m = 37), from the USFDA (2005) guidance.

fusarenon X [FusX]) in these illnesses, DAS has not been positively identified (Beardall & Miller, 1994; RIVM, 2002).

The clinical pathology of alimentary toxic aleukia, as described by Joffe (1974), describes the effects of exposure to potent trichothecenes. Of the four stages of alimentary toxic aleukia identified, the first stage occurred shortly after ingestion of contaminated grain and lasted 3–9 days. Symptoms included a burning sensation of the mouth, tongue, oesophagus and stomach and inflammation of the gastrointestinal mucosa accompanied by vomiting, diarrhoea, salivation, dizziness and tachycardia. The initial clinical appearance of leukopenia was also observed during stage 1. Stage 2 was termed the latent or leukopenic stage as patients reported feeling normal and functioning normally, despite major changes in the haematopoietic system, including progressive leukopenia with granulocytopenia and a relative lymphocytosis. Anaemia, icterus and lowered immune resistance to infections were typical of this stage and some reports describe abnormalities in the central and autonomic nervous functions. Stage 2 lasted from 2 to 8 weeks, with eventual recovery if exposure stopped. If exposure continued, leukopenia worsened and thrombocytopenia and decreased fibrinogen resulted in anaemia and petechial haemorrhages on the skin of the trunk, lateral surfaces of the arms, the thighs, face and head during stage 3. Nasal, gastric and intestinal haemorrhages were also noted. Necrotic lesions could appear in the throat, gums, buccal mucosa, larynx and vocal cords with secondary bacterial infections. Lymph node enlargement was observed, and death from stenosis of the glottis was reported. If the person survived, stage 4 consisted of a convalescent period of 2 or more months while the bone marrow recovered.

3. Analytical methods

3.1 Chemistry

4,15-DAS is a type A trichothecene (Fig. 3(1); Shams et al., 2011; Tamura et al., 2015). All trichothecene mycotoxins have the core 12,13-epoxytrichothec-9-ene structure (Fig. 3(2)). However, different trichothecene analogues have different patterns of substitution around this core structure. Type B trichothecenes such as DON and nivalenol have a carbonyl group at C-8, whereas type A trichothecenes can have a hydroxyl group (e.g. NEO), an ester function (e.g. T-2 toxin) or no functional group at all (e.g. 4,15-DAS) at carbon atom 8 (C-8) of the core 12,13-epoxytrichothec-9-ene structure molecule.

A key metabolite in the trichothecene type A pathway is calonectrin ((3β)-12,13-epoxytrichothec-9-ene-3,15-diyl diacetate (Fig. 3(3)), which is metabolized

Fig. 3
Chemical structures of 4,15-DAS (1), 12,13-epoxytrichothec-9-ene (2), calonectrin (3), 3,15-DAS (4) and 3,4,15-triacetoxyscirpenol (5)

	R_1	R_2	R_3
(1) 4,15-Diacetoxyscirpenol	OH	OAc	OAc
(2) 12,13-Epoxytrichothec-9-ene	H	H	H
(3) Calonectrin	OAc	H	OAc
(4) 3,15-Diacetoxyscirpenol	OAc	OH	OAc
(5) 3.4.15-Triacetoxyscirpenol	OAc	OAc	OAc

by *Fusarium* spp. via 3,15-DAS (Fig. 3(4)) and 3,4,15-triacetoxyscirpenol (Fig. 3(5)) into 4,15-DAS (Fig. 3(1)) (Desjardins, Hohn & McCormick, 1993; Kimura et al., 2007; Alexander, Proctor & McCormick, 2009).

3.2 Description of analytical methods

3.2.1 Introduction

Developments in available analytical techniques and equipment allow simultaneous detection of a broad range of mycotoxins at low levels in so-called multi-methods, as opposed to the targeted methods for the detection of one mycotoxin that were used before (Berthiller et al., 2005; Mol et al., 2008). The multi-methods are very useful in monitoring programmes and surveys. However, when low limits of quantification (LOQs) are required, as in the case of data collection for risk assessment purposes, the multi-method can be adapted to specific performance characteristics of certain mycotoxins or targeted methods can be used (Lopez et al., 2016). Only one report of a method for targeted 4,15-DAS detection was found (Omurtag et al., 2007) and one report for an antibody-based screening method (Hack, Klaffer & Terplan, 1989). No performance criteria

for the analysis of 4,15-DAS or its modified forms were found in the literature. Therefore, the method criteria in CODEX STAN 193-1995 and its amendments (CAC, 1995) for DON can be followed, or the performance criteria for T-2 toxin, HT-2 toxin and DON in Regulation (EC) No. 401/2006 EU and its amendments (European Union, 2006, 2014).

No harmonized methods were found for 4,15-DAS, nor performance criteria for analytical methods, certified reference materials or proficiency tests. In addition, no analytical standards were found for the modified forms of 4,15-DAS.

3.2.2 Screening tests

Several polyclonal antibody-based enzyme immunoassays for 4,15-DAS were developed (Klaffer, Martlbauer & Terplan, 1988; Mills et al., 1988). One aspect that requires careful consideration is test specificity (cross-reactivity). Most immunoassays for trichothecenes have moderate or even strong cross-reactivity with closely related analogues.

The enzyme-linked immunosorbent assay (ELISA) test by Klaffer, Martlbauer & Terplan (1988) consisted of antibodies obtained after immunization of rabbits with 4,15-DAS–hemiglutarate–human serum albumin. It reached a detection limit of 100 ng/mL and showed cross-reactivity for 3α-acetyl-DAS, 4,15-DAS, T-2 toxin, NEO and 15-acetoxyscirpenol.

Mills et al. (1988) produced a high-titre, high-specificity antiserum against 4,15-DAS in rabbits for use in an ELISA test. The test was successfully applied to cereal samples with a detection limit of 300 ng/g. Type B trichothecenes, such as DON and nivalenol, were not recognized by the antibodies. Such high specificity appears to be a general characteristic of antitrichothecenes (Mills et al., 1988). Monoclonal antibodies to 4,15-DAS were produced by a hybridoma with a detection limit of 16 ng/mL when applied in an ELISA test (Hack, Klaffer & Terplan, 1989). Cross-reactivity was noted for 3α-acetyl-DAS, diacetylverrucarol, NEO, T-2 tetraol tetraacetate, fusarenon X, T-2 toxin and HT-2 toxin. However, it is unknown if any of these products is used on a commercial base.

Tangni et al. (2010), on the other hand, assessed the cross-reactivity of antibodies used in some commercial kits specifically designed to determine DON in cereals and cereal products against some fusariotoxins. The three ELISA kits, AGRAQUANT, DON EIA and VERATOX, the lateral flow device (LFD) ROSA LF-DONQ and the fluorescent polarization immunoassay (FPIA) MYCONTROLDON showed low cross-reactivity with 4,15-DAS.

3.2.3 Quantitative methods

4,15-DAS is often analysed and quantified together with other trichothecenes (both type A and B) and/or other types of mycotoxins in so-called multi-methods.

Various combinations of solvents have been used to extract 4,15-DAS (in combination with other mycotoxins) from grain, food and feeds. Acetonitrile and water (Schollenberger et al., 2005; Tanaka et al., 2006; Bryła et al., 2014; Flores-Flores & Gonzalez-Penas, 2015) or methanol and water at different ratios were mostly described as extractants. Ethyl acetate was also used to extract 4,15-DAS and other trichothecenes (Njumbe Ediage, Van Poucke & De Saeger, 2015). The extraction mixture can be acidified with either formic acid or acetic acid. Sospedra et al. (2010) found the acetonitrile/methanol mixture to be the optimum for extracting 4,15-DAS and other trichothecenes from wheat flours. A variation of QuEChERS (Quick Easy Cheap Effective Rugged and Safe) extraction, in which the extraction is enhanced by the presence of magnesium sulfate and sodium chloride or sodium acetate, has also been applied (Sospedra et al., 2010; Vaclavik et al., 2010; Lu et al., 2013; Rodriguez-Carrasco et al., 2014b). Extraction has been performed mainly by high-speed blending or mechanical shaking.

After extraction, the sample is centrifuged and an aliquot is taken for either further purification or direct analysis by liquid chromatography–mass spectrometry (LC-MS) using the "dilute-and-shoot" approach. The "dilute-and-shoot" procedure consists of diluting the sample (extract) with solvent or mobile phase. The diluted extract is then injected into the chromatographic system without a further sample preparation step. Dilution reduces the effect of the matrix interferences. The "dilute-and-shoot" approach is very common in multi-mycotoxin analysis (Sulyok, Krska & Schuhmacher, 2007; Monbaliu et al., 2009).

The main procedures used for clean-up are solid-phase extraction (SPE), dispersive SPE (d-SPE), column chromatography and multifunctional (Mycosep) clean-up columns. Interfering lipids can be removed by extracting the sample extract with *n*-hexane or another nonpolar solvent (Krska, Baumgartner & Josephs, 2001).

Beds based on alumina/charcoal or combinations of alumina/charcoal/EXtrelut have been long used to separate 4,15-DAS and other trichothecenes from food product matrices (Bryła et al., 2014).

Schollenberger et al. (1998) developed a clean-up procedure using a combination of Florisil and cation-exchange cartridge for the analysis of trichothecenes in complex matrices. High polar interferences are removed by precipitation with ethyl acetate followed by SPE with Florisil cartridge. The subsequent clean-up procedure using a cation-exchange resin selectively removes matrix interferences whose polarity is similar to trichothecenes. This procedure has been successfully applied to maize and maize products (Schollenberger et al., 2012), edible oil (Schollenberger et al., 2008), soy food (Schollenberger et al., 2007), grains and feed (Schollenberger et al., 2006) and foods of plant origin (Schollenberger et al., 2005) in Germany.

Application of non-acidified extracts of (red) sorghum on SPE amino cartridges leads to significant removal of the matrix interferences, resulting in a colourless transparent extract. Acidification of the extraction solvent is not advised when using SPE amino columns since it may trigger a redox reaction (possibly, reduction) of tannins, which drastically reduces their binding capability to the amino SPE cartridges. The Oasis HLB cartridge was not optimal to clean up sorghum extracts since extraction recovery for 4,15-DAS (and other trichothecenes) was lower than 50%. Furthermore, matrix interferences (tannins in red sorghum) were not strongly impacted by this column as observed by the red colour of the eluate (Njumbe Ediage, Van Poucke & De Saeger, 2015). However, Oasis HLB cartridges were appropriate to clean up wheat extracts spiked with 2 mg/kg of 4,15-DAS.

SPE cartridges of C-18 and graphitized carbon black have been applied to extracts from lyophilized coffee beverages of different compositions. The performance of both cartridges to remove pigments differs, even when using the same clean-up procedure, due to different matrix interferences. The best recoveries are obtained by clean-up via clarification with Carrez solutions (Garcia-Moraleja et al., 2015a).

d-SPE, in which the sorbent is added to an aliquot of the extract, is commonly used after QuEChERS extraction. By using a much smaller quantity of sorbent and avoiding the cartridge format, d-SPE saves time and solvent compared with the traditional SPE approach. No preconditioning of cartridges is needed, and the sorbent bed cannot dry out. Unlike column-based formats, all of the sorbent interacts equally with the matrix in d-SPE. For the determination of 4,15-DAS, different mixtures of d-SPE sorbents, such as magnesium sulfate + PSA (primary/secondary amine) (Sospedra et al., 2010; Vaclavik et al., 2010) and magnesium sulfate with C-18 (Rodriguez-Carrasco et al., 2014b), have been applied. C-18 removes apolar compounds, while PSA interacts with chemicals by hydrogen bonding and removes more polar compounds, such as fatty acids, other organic acids and, to some extent, sugar and pigments.

Mycosep columns contain a variety of adsorbents, for example, charcoal, celite and ion-exchange resins. They enable rapid sample purification – within 10–30 seconds. A major advantage of this column is the absence of the time-consuming rinsing steps required in SPE. In addition, nearly all analytical interfering substances are retained on the column, whereas 4,15-DAS (and other trichothecenes) are not adsorbed on the packing material. Mycosep 226 (Tanaka et al., 2006), Mycosep 227 (Schothorst & Jekel, 2001; Lopez et al., 2016) and Mycosep 229 (Tamura et al., 2015) have been successfully applied in 4,15-DAS analysis.

When preparing 4,15-DAS and other trichothecenes for analysis by gas chromatography, the sample treatment includes a derivatization step in

order to increase the sensitivity of the analytes, thus lowering LODs and LOQs. Derivatization agents used are a mixture of *N*,*O*-*bis*(trimethylsilyl)acetamide, trimethylchlorosiloxane and trimethylsilylimidazole, commercially supplied under the name of TriSil-TBT (Schothorst & Jekel, 2001; Rodriguez-Carrasco et al., 2012; Escriva et al., 2016); trifluoroacetic anhydride (Perkowski & Basinski, 2002; Tan et al., 2011); and 0.4 mol/L imidazole pentafluoropropionic anhydride (Nielsen & Thrane, 2001). The derivatization agent is added to the dry extract and the reaction usually proceeds at room temperature, 40 °C or 60 °C (Nielsen & Thrane, 2001; Fuchs et al., 2002; Labuda et al., 2005). The derivatized extract can be diluted with organic solvent, such as hexane, dichloromethane or iso-octane, and washed with buffers of phosphate, anhydrous sodium, acetonitrile-toluene or 5% sodium hydrogen carbonate (Nielsen & Thrane, 2001; Fuchs et al., 2002; Labuda et al., 2005; Tan et al., 2011; Rodriguez-Carrasco et al., 2012; Escriva et al., 2016). Finally, the organic layer is transferred to an autosampler vial for gas chromatography analysis.

The derivatized extract can also be evaporated to dryness under nitrogen and then re-dissolved in iso-octane (Perkowski & Basinski, 2002) and washed with water (Schothorst & Jekel, 2001) before the organic layer is transferred to analysis.

The analysis of derivatized 4,15-DAS by gas chromatography is usually performed on a standard column of (5% phenyl) methylpolysiloxane (DB-5, HP-5 or equivalent). Other types of columns, such as DB-17 combined with a more polar phase of 50% phenyl–50% methylpolysiloxane, are also described in the literature (Schothorst & Jekel, 2001). The chromatographic column used for trichothecene analysis is usually 30 m long, but the internal diameter and the film thickness can vary. Several detectors can be coupled to gas chromatography for trichothecene analysis. A flame ionization detector is a possibility (Schothorst & Jekel, 2001), although mass spectrometry (Schollenberger et al., 2008; Tittlemier, Gaba & Chan, 2013) and tandem mass spectrometry are currently the most popular options (Nielsen & Thrane, 2001; Rodriguez-Carrasco et al., 2014b; Escriva et al., 2016). When mass spectrometry or tandem mass spectrometry are used, the ions (*m*/*z*) or transitions that are monitored depend on the derivatization agent used during sample treatment. The LOQ for 4,15-DAS by gas chromatography–flame ionization detection (GC-FID), estimated as the concentration in a sample that gives a signal-to-noise ratio of 9, was 75 µg/kg (Schothorst & Jekel, 2001). By GC-MS/MS, LOQs, following the same criteria, can be lowered to 1 µg/kg (Lopez et al., 2016).

HPLC with diode array detector (DAD) needs low wavelengths for analysis of 4,15-DAS due to its low absorption intensity. It has the advantage of providing both multiwavelength and spectral information in a single chromatographic run. Lacking conjugated unsaturation, 4,15-DAS only exhibits end absorbance near 200 nm. This lack of structural specificity means that 4,15-DAS can be detected and identified by HPLC-DAD at relatively high concentrations only. Omurtag et

al. (2007) quantified 4,15-DAS at a wavelength of 205 nm in cereal samples with an LOQ of 800 μg/kg.

Reports on methods that use liquid chromatography coupled with (tandem) mass spectroscopy (LC-MS and LC-MS/MS) for the analysis of 4,15-DAS have increased over the last few years. LC-MS allows the simultaneous determination of a high number of mycotoxins without derivatization. The use of MS/MS with triple quadrupole mass analysers, in which ions are filtered in two stages dependent on their molecular weight, improves selectivity and allows the determination of 4,15-DAS, together with other mycotoxins, in complicated matrices such as food, feed or biological samples. 4,15-DAS is usually separated from other compounds on a C-18 column (reversed phase) with a wide variety of reported dimensions and commercial brands, and using ammonium acetate or formate buffers in the mobile phases. The use of methanol instead of acetonitrile in the organic mobile phase (phase B) results in higher intensities (Berthiller et al., 2005). 4,15-DAS is measured in positive ionization mode as an ammonium adduct, being 384.2 > 307.2 (quantifier) and 384.2 > 247.2 (Bryła et al., 2014; Flores-Flores & Gonzalez-Penas, 2015; Njumbe Ediage, Van Poucke & De Saeger, 2015) or 384.2 > 105.1 (qualifier) (Berthiller et al., 2005; Garcia-Moraleja et al., 2015a), the transitions monitored for detection and quantification purposes. Although most of the reported literature concerned with the determination of 4,15-DAS (and other mycotoxins) by LC-MS reports electrospray ionization (ESI) as a source for their ionization, the signal for 4,15-DAS has been demonstrated to increase with atmospheric pressure chemical ionization (APCI) (Berthiller et al., 2005; Zachariasova et al., 2010). LOQs ranging from 1 to 5 μg/kg for 4,15-DAS can be achieved by LC-MS/MS.

Ion trap analysers in LC-MS/MS systems have also been successfully applied to determine 4,15-DAS in cereals with an LOQ of 1 μg/kg. However, ion traps cannot hold too many ions or they interact, degrading the analyser sensitivity and/or precision. Samples must therefore be carefully cleaned to eliminate any unwanted ions from the sample matrix (Bryła et al., 2014).

High-resolution mass spectrometry (HRMS), including Orbitrap mass spectrometry (Orbitrap-MS) or time of flight–mass spectrometry (TOFMS), has been successfully applied to determine 4,15-DAS in food matrices. HR-TOFMS instruments provide enhanced full mass range spectra sensitivity and accuracy and have the advantage that quantification can be performed on any ion in the acquired mass range. It has been successfully applied to determine 4,15-DAS in various food commodities, with an LOD of 0.3 μg/kg (Tanaka et al., 2006). Orbitrap-MS has been successfully applied to determine 4,15-DAS in beer and in cereals with similar detection levels as HR-TOFMS (Zachariasova et al., 2010; Tamura et al., 2015). With high resolution and always using the ammonium adduct, 4,15-DAS is detected with an m/z of 384.20168, although the accurate mass can differ depending on the instrument used.

Direct analysis in real time (DART) coupled with high-resolution Orbitrap-MS has been recently applied for the detection/quantification of mycotoxins, including 4,15-DAS, in cereals (Vaclavik et al., 2010). DART-HRMS can be carried out under ambient conditions, without chromatographic separation, which provides a remarkably high throughput of analyses. Due to the relatively high signal fluctuation of ion intensities obtained by repeated DART measurements, an internal standard must be employed for compensation. Using this technique in the presence of ammonia vapour to enhance sensitivity, 4,15-DAS could be easily detected in positive mode as an ammonium adduct with an exact mass of 384.2017. The technique was applied on spiked maize and wheat samples with acceptable recoveries for 4,15-DAS, from 80% to 90%.

3.2.4 Quantitative analysis of biologically modified 4,15-DAS

(a) Analysis of conjugates formed by plants or fungi

Accurate quantification of biologically modified forms of 4,15-DAS in foods, conjugated by plants or by fungi (Rychlik et al., 2014), is still rather difficult due to the lack of appropriate analytical standards and certified reference materials. However, separation, identification and semiquantification of plant or fungal conjugated forms are possible. Plant or fungal conjugated forms often occur in lower concentrations than the free mycotoxin; therefore, more sophisticated methods that could achieve lower LOQs are required.

Glucoside forms of 4,15-DAS and other plant or fungal conjugated forms have been reported in food commodities (EFSA, 2014). Nakagawa et al. (2013b) identified two glucoside forms in maize, 15-MAS-3-glucoside and 15-MAS-4-glucoside. The same authors also identified DAS-glucoside in maize powder reference material on the basis of accurate mass measurements of characteristic ions and fragmentation patterns using high-resolution LC-Orbitrap-MS analysis in the positive polarity (Nakagawa et al., 2013a). Although the absolute structure was not clarified, DAS-3-glucoside seemed to be the most probable structure. The maize samples were extracted with acidified (acetic acid) acetonitrile/water and the extract was cleaned with Bond Elut Mycotoxin column. The glucoside forms were separated on a C-18 column (Nakagawa et al., 2013a). DAS-3-glucoside and 15-MAS-3-glucoside were detected as ammonium adducts with an accurate mass of 546.2532 (Nakagawa et al., 2013a) and 504.2439 (Nakagawa et al., 2013b), respectively.

(b) Functionalized phase I metabolites and conjugates formed by animals

Accurate quantification of the functionalized phase I metabolites and conjugates formed by animals (phase II metabolites) of 4,15-DAS (Rychlik et al., 2014) is difficult due to the lack of analytical standards and certified reference materials.

However, separation, identification and semiquantification of these phase I and phase II metabolites are feasible.

The 4,15-DAS metabolites from phase I and phase II metabolism in liver microsomes of rat, chicken, pig, goat, cow and human (in vitro) and found in urine and faeces of rats and chickens (in vivo) were identified by UHPLC-Q/TOF operated in the positive ionization mode (Yang et al., 2015). Separation was performed on a C-18 column. 4,15-DAS metabolites were first identified by the accurate extracted ion chromatograms obtained by processing the full-scan mass spectrometry data and using expected metabolite ions with 5 ppm mass tolerance. For the unpredicted metabolites, MS/MS data of samples and controls were used. Table 8 shows a summary of the compounds identified.

Heyndrickx et al. (2015) used a syringe filter for clean-up of urine followed by LC-MS/MS identification of 4,15-DAS, among other mycotoxins and mycotoxin metabolites. Four mobile phases were used for the separation of 32 mycotoxins in the ultra-performance liquid chromatography (UPLC) system. Identification was carried out in positive mode using a Xevo TQ-S (Waters, Manchester, United Kingdom) UPLC-MS/MS equipped with an ESI source.

Rodriguez-Carrasco et al. (2014a) developed a GC-MS/MS method for the detection of, among 14 other mycotoxins, 4,15-DAS in human urine samples. After urine samples underwent d-SPE, the extract was dried, derivatized with BSA+TMCS+TMSI and analysed on an Agilent 7890A gas chromatography system coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source. Separation was achieved on an HP-5MS 30 column.

(c) Quantitative analysis of chemically modified 4,15-DAS

The analysis of a chemically modified form of 4,15-DAS after thermal treatment (Rychlik et al., 2014) is described in one paper by Shams et al. (2011). 4,15-DAS was transformed into DAS-M1 (Fig. 4) after thermal treatment of inoculated potatoes. DAS-M1 was structurally elucidated with proton NMR ([^{1}H]NMR), [^{13}C]NMR and two-dimensional NMR. DAS-M1 was extracted from slurries of potato tubers with acetonitrile. After drying and re-dissolving, DAS-M1 was characterized by LC-HRMS and determined by LC-MS/MS. Chromatographic separation was achieved on a C-18 column. For HRMS experiments, a linear trap quadrupole (LTQ)-Orbitrap-XL high-resolution mass spectrometer was used. Two major ions were visible in the spectrum at m/z 402.2124 and m/z 407.1677, which corresponded to the ammonium and sodium adducts, respectively. For LC-MS/MS, a QTRAP system with an atmospheric pressure chemical ionization (APCI) source was used. DAS-M1 was quantified in positive mode with quantifier and qualifier transitions–based ammonium adducts: 402.3 > 325.2 as quantifier and 402.3 > 367.2 as qualifier (Shams et al., 2011).

Table 8
Summary of the 4,15-DAS metabolites detected in samples from in vivo and in vitro experiments

Metabolite	Composition	[M+NH4]+	Major fragments
4,15-DAS	$C_{19}H_{30}NO_7$+	384.202 2	348, 349, 307, 289, 247, 229,199
4-MAS	$C_{17}H_{28}NO_6$+	342.191 7	342, 324, 307, 265, 247, 217, 199, 157
15-MAS	$C_{17}H_{28}NO_6$+	342.191 7	342, 307, 265, 247, 229, 107
SCP	$C_{15}H_{26}NO_5$+	300.180 6	300, 283, 265, 247, 229, 199
8β-OH-DAS	$C_{19}H_{30}NO_8$+	400.196 6	400, 365, 305, 245, 215, 185
NEO	$C_{19}H_{30}NO_8$+	400.196 6	400, 365, 305, 245, 215, 185
7-OH-DAS	$C_{19}H_{30}NO_8$+	400.196 6	400, 365, 323, 305, 263, 245, 227, 197
DAS-3-GlcA	$C_{25}H_{38}NO_{13}$+	560.233 8	560, 367, 349, 307, 289, 247, 229, 199
15-MAS-3-GlcA	$C_{23}H_{36}NO_{12}$+	518.223 2	518, 501, 325, 307, 265, 247, 229, 107
15-MAS-4-GlcA	$C_{23}H_{36}NO_{12}$+	518.223 2	518, 325, 307, 265, 247, 229

DAS: diacetoxyscirpenol; GlcA: glucuronic acid; [M+NH4]: metabolite plus ammonium adduct; MAS: monoacetoxyscirpenol; NEO: neosolaniol; SCP: scirpentriol
Source: Yang et al. (2015)

Fig. 4
DAS-M1

(6) DAS-M1

4. Sampling protocols

No published information on sampling protocols specifically for 4,15-DAS was found. However, it is assumed that 4,15-DAS, like other trichothecenes such as DON, T-2 toxin and HT-2 toxin, can be unevenly distributed.

As described by JECFA at the seventy-second meeting, on sampling protocols for DON, generation of meaningful analytical data requires the sampling stage to be as representative as possible (Annex 1, reference *199*). The

sampling stage frequently represents the greatest contribution to the overall variance of the result.

In general, sampling protocols specify, among various items, the size of lots, sublots, incremental samples, aggregate samples, laboratory samples and test portions. In generating contamination data for regulatory control purposes, official control laboratories are required to ensure that their samples are obtained using official sampling protocols.

The sampling problem for mycotoxins has been addressed by statistical means and the drawing up of sampling protocols. A manual aimed at addressing sampling protocols and written for both food analysts and regulatory officials and explaining some of the statistical issues was produced as part of the Joint FAO/IAEA Programme, Nuclear Techniques in Food and Agriculture (Whitaker et al., 2010). This was developed by the Food and Agriculture Organization of the United Nations (FAO) into the Mycotoxin Sampling Tool (http://tools.fstools.org/mycotoxins/). A training video for practical applications has also been produced by FAO in collaboration with the Italian National Institute of Health (http://www.soluzionepa.it/produzioneaudiovisivi.html; FAO, 2007). The Italian Ministry of Health together with the Istituto Superiore di Sanità and the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana has also produced a general video on sampling from a European Union viewpoint (www.iss.it/myconews/?lang=2&id=86&tipo=16; ISS, 2015).

The setting of maximum levels for mycotoxins in various foods by the Codex Alimentarius Commission and regulatory limits on mycotoxins in foods by the European Union has been accompanied by relevant sampling protocols. The best known and most easily accessible sampling protocols are those from the Codex Alimentarius standard CODEX STAN 193-1995 and its amendments, in which sampling protocols are compiled for several mycotoxins, although not specifically for 4,15-DAS (CAC, 1995). The European Union has sampling protocols for the purpose of official control of the levels of mycotoxins in foodstuffs, as described in Regulation (EC) No. 401/2006 and its amendments (European Union, 2006, 2014). The Regulation indicates the number of incremental samples to be taken, depending on the food commodity and the weight of the lot or a given batch of the commodity. In some instances, such as that for official control of DON, T-2 toxin and HT-2 toxin, the analytical performance of the test method is also specified (EC, 2006).

5. Effects of processing

5.1 Sorting, cleaning and milling

No information was found on the effects of sorting, cleaning and milling on the content of 4,15-DAS in food.

Several papers have been published on the distribution of T-2 toxin and HT-2 toxin in the various fractions after sorting, cleaning and milling of raw cereals. T-2 toxin and HT-2 toxin are type A trichothecenes, like 4,15-DAS (Collins & Rosen, 1981; Scudamore et al., 2007; Pettersson, 2008; Schwake-Anduschus et al., 2010; EFSA, 2011; Karlovsky et al., 2016). EFSA (2011) concluded that processing of grains results in lower T-2 toxin and HT-2 toxin concentrations in grain for human consumption, since the levels of T-2 toxin and HT-2 toxin were higher in unprocessed grains than in grain products for human consumption. EFSA (2011) found that during the milling process, T-2 toxin and HT-2 toxin are not destroyed but unevenly redistributed between fractions, mainly in the bran fraction.

5.2 Thermal processing

The thermal treatment of 4,15-DAS standards in aqueous solution (100 °C for 1 hour and 121 °C for 4 hours) decomposed 26% and 100%, respectively, of the toxin and resulted in the formation of a major degradation product, DAS-M1 (see Fig. 4 above). The conversion from 4,15-DAS to DAS-M1 was increased considerably by either higher temperatures or more acidic pH conditions (Shams et al., 2011). Also, cooking potatoes inoculated with *F. sambucinum* reduced 4,15-DAS levels and increased DAS-M1 levels with cooking time. The use of a pressure cooker favoured the formation of DAS-M1, even at neutral pH.

EFSA concluded that the type A trichothecenes T-2 toxin and HT-2 toxin are relatively stable during baking and cooking (EFSA, 2011).

5.3 Alkaline treatment and fermentation

No information was found on effects of alkaline treatment or fermentation on 4,15-DAS content of food. Incubation of bovine rumen microorganisms or bovine faecal microorganisms with 4,15-DAS rapidly degrades 4,15-DAS to 15-MAS, de-epoxy MAS, SCP and de-epoxy-SCP (Swanson et al., 1987, 1988). This fermentation treatment cannot be applied to food.

6. Prevention and control

There are a few data available on specific intervention measures to prevent 4,15-DAS contamination. Since 4,15-DAS is produced by *Fusarium* spp., management strategies to prevent contamination of crops with 4,15-DAS may focus on preventing *Fusarium* infection and growth in crops in the whole production chain and on decontamination procedures of harvested crops.

6.1 Preharvest control

No specific strategies to prevent 4,15-DAS contamination under field conditions were found. As mentioned before, measures to prevent contamination of crops in the field with other type A trichothecenes may apply to 4,15-DAS. Several of these measures are discussed in the following sections.

6.1.1 Crop management strategies

It is assumed that healthy plants are more resistant to infection. Therefore, agronomic practices that keep plants healthy will probably be effective at preventing *Fusarium* infection and trichothecene accumulation. This includes, among other measures, sowing and harvesting at the appropriate time (Jouany, 2007; Edwards, 2009a,b,c; Eeckhout et al., 2013).

Crop debris from the previous growing season can serve as an inoculum for the following crops. Removal, burning and burial of crop residues therefore serve as strategies counteracting *Fusarium* contamination in the next crop (Jouany, 2007). Edwards (2009a,b,c) showed that ploughing results in lower concentrations of T-2 toxin, HT-2 toxin and other type A trichothecenes in grains.

Crop rotation is critical to prevent debris from *Fusarium*-susceptible crops serving as a source of inoculum (Ferrigo, Raiola & Causin, 2016). It is advised to rotate *Fusarium*-susceptible host crops such as cereals (maize, wheat, barley) with non-host crops such as beets, onions, beans, clover, alfalfa, vegetables and chicory (Eeckhout et al., 2013). Barley and oats as previous crops exacerbate T-2 toxin and HT-2 toxin concentrations in following crops (Edwards, 2009a,b,c).

Use of existing cereal or potato crop cultivars resistant to *Fusarium* may reduce fungal infection and, possibly, 4,15-DAS contamination of the crop (Goral et al., 2012; Hua-Li et al., 2014).

Careful use of fertilizers (Etzerodt et al., 2015; CAC, 2016; Hofer et al., 2016) as well as appropriate water management to prevent drought stress (irrigation scheme) will limit *Fusarium* infection of the crop (Jouany, 2007; VKM, 2013; Ferrigo, Raiola & Causin, 2014, 2016).

6.1.2 Plant protection products to reduce fungal growth and mycotoxin production

Use of plant protection products should be carefully considered since they are not always effective against *Fusarium* (da Cruz Cabral, Pinto & Patriarca, 2013). Various registered fungicides are commercially available. Strobilurins and azole-based fungicides have been shown to be effective against *Fusarium* head blight (Loos et al., 2005; Wollenberg et al., 2016) and T-2 toxin and HT-2 toxin production by *F. langsethiae* (Mateo et al., 2013) but nothing is known on their effects on 4,15-DAS contamination.

Weeds serve as hosts to *Fusarium* (Jenkinson & Parry, 1994; Postic et al., 2011; Altinok, 2013) and compete for space, nutrients and sunlight with the crop plants. However, application of some herbicides, particularly glyphosate, leads to an increase in fusaria responsible for *Fusarium* head blight in wheat, including the 4,15-DAS producer *F. langsethiae* (VKM, 2013). Therefore, the use of such herbicides should be avoided.

Currently under development are several biocontrol practices aimed at either outcompeting toxigenic *Fusarium* species with, for example, the fungus *Trichoderma* (Ng et al., 2015) or inhibiting biosynthesis of mycotoxins with, for example, *Spirulina* extracts (Pagnussatt et al., 2014).

6.2 Postharvest control

Information is available on storage conditions that favour growth of *Fusarium* species. However, no specific intervention measures to prevent 4,15-DAS formation in storage were found in the literature.

In general, grains must be harvested with a low moisture content or else immediately dried to the safe moisture level of less than 14%, depending on the commodity, before storage (Jouany, 2007). Other measures include avoiding temperature fluctuation to prevent condensation of water, controlling for pests at the storage facilities and avoiding moisture build-up in the stored product.

Temperatures below 15 °C in temperate regions contribute to safe storage (VKM, 2013) since growth of the 4,15-DAS-producing fungi and mycotoxin synthesis are inhibited under these conditions (Champeil, Fourbet & Doré, 2004). Maintaining such a safe humidity and temperature throughout the duration of harvest, transport and storage is therefore recommended. Increasing carbon dioxide concentration to above 10% during storage is generally known to retard fungal growth (Weidenborner, 2013).

Salts of weak acids such as sodium benzoate, calcium propionate and potassium sorbate and essential oils from plants may inhibit the growth of several postharvest fungal pathogens including *Fusarium* species in in vitro conditions

(da Cruz Cabral, Pinto & Patriarca, 2013). They are currently being investigated as biocontrol agents.

6.3 Decontamination

A few papers were found in the literature on measures to lower the amount of 4,15-DAS in the harvested crops. All of these measures are still in experimental stages, except for the use of adsorbents; none are allowed in the treatment of food.

6.3.1 Use of irradiation

Gamma and electron-beam irradiation at low doses of 4–5 kGy reduced the number of fungal spores and growth of *Fusarium* spp. (Kottapalli et al., 2003; Aziz & Moussa, 2004). Jouany (2007) reported that doses higher than 10 kGy are required to eliminate *Fusarium* toxins from maize, chickpeas and groundnut seeds. Solar radiation and solar photocatalytic (TiO_2) irradiation were effectively used in inactivating the spores of *F. solani, F. verticillioides, F. oxysporum, F. equiseti* and *F. anthophilum* in water (Sichel et al., 2007).

6.3.2 Use of chemicals

Chemicals such as bases, acids, oxidizing agents, aldehydes or bisulfite gases have been reported to degrade trichothecenes in cereals to products that are less toxic than the primary compounds (He et al., 2010). However, most of the reports focus on DON, NIV and T-2 toxin. Young, Zhu & Zhou (2006) used saturated aqueous ozone at concentrations of about 25 ppm to degrade 10 trichothecenes, including 4,15-DAS and MAS, leaving no residue. Bauer et al. (1987) used a combination of physical and chemical treatments to reduce 4,15-DAS in animal feed. In the chemical treatment, calcium hydroxide monomethylamine was the degrading agent and greater reduction was seen at higher temperatures and moisture content.

6.3.3 Use of microorganisms

Microbial decontamination of mycotoxins in harvested products can become an important strategy of eliminating mycotoxins from feeds and possibly from food. Several experimental studies have been carried out on microbial transformation and degradation of 4,15-DAS (Ueno et al., 1983; Swanson et al. 1987, 1988; Westlake, Mackie & Dutton, 1987; Matsushima et al., 1996).

One particular product allowed on the market in the European Union is Mycofix Plus, which was primarily designed to eliminate *Aspergillus* and *Fusarium* toxins from feed (EFSA, 2009). Mycofix Plus contains adsorbing (algae and plant extracts) and biotransforming materials (the yeast *Trichosporon*

mycotoxinivorans and *Eubacterium* BBSH 797). The yeast detoxifies OTA and zearalenone. Although the anaerobic *Eubacterium* BBSH 797, isolated from rumen fluid, cleaves the 12,13 epoxide ring and has been shown to reduce the effects of DON in sows and dairy cows and T-2 toxin in broiler chickens (EFSA, 2009), no information is available on the activity of *Eubacterium* specifically on 4,15-DAS.

6.3.4 Use of enzymes

Specific enzymes that are able to degrade *Fusarium* toxins have been purified from microbial sources, but there is no information on the effectivity for 4,15-DAS degradation. The aforementioned commercially available Mycofix Plus contains epoxidase as a specific trichothecene-degrading component (EFSA, 2009).

6.3.5 Use of adsorbents

Adsorbents in animal feed may lower the bioavailability and therefore the exposure to mycotoxins. However, care must be taken not to introduce other contaminants, for example, dioxins, when using clays.

Trichothecenes are non-ionizable molecules with a bulky epoxy group; these characteristics do not favour adsorption to a plane surface and they are adsorbed by very few materials – activated charcoals, zeolites, yeast cell walls and modified synthetic polymers (EFSA, 2009). *Lactobacillus rhamnosus*, *Propionibacterium freudenreichii* and bifidobacteria can bind various *Fusarium* toxins including 4,15-DAS (Whitlow, 2006; Dalie, Deschamp & Richard-Forget, 2010). Phillips et al. (1990) showed that activated carbon effectively binds 4,15-DAS. Several currently available commercial products claim to prevent trichothecenes from being absorbed from the intestinal tract: zeolite (octadecyldimethyl benzyl ammonium exchanged–clinoptilolite-heulandite tuff), yeast cell walls (MTB-100) and algae and plant extracts (Mycofix Plus) (EFSA, 2009).

A multi-mycotoxin binder, produced by Tranquil et al. (2013) and intended for use in animal feed, is composed of modified plant lignocellulose, natural clay, artificial clay, organic polymers, activated charcoal and yeast cell wall polysaccharides. It has proven to have the capacity to adsorb a wide range of mycotoxins, including OTA, T-2 toxin, DON, nivalenol, zearalenone, aflatoxins and fumonisins. Reduction of 4,15-DAS in the gastrointestinal tract has not yet been demonstrated.

6.3.6 Emerging technique

Molecular imprinting technology allows for synthesizing molecularly imprinted polymers (MIPs) that have the capacity to recognize and bind specific template molecules (Vasapollo et al., 2011). This technology was applied by Yiannikouris et

al. (2013) who developed molecularly imprinted polymers (MIPs) that recognize and sequester several mycotoxins including 4,15-DAS from animal rations. This technique is promising for the production of very effective and specific synthetic mycotoxin adsorbents.

6.4 Hazard analysis and critical control point (HACCP) and integrated mycotoxin management system

One approach for effectively managing mycotoxins is based on HACCP. This is the simultaneous application of all the strategies along the value chain. While there is a HACCP framework for trichothecene control with regard to *Fusarium* head blight (Aldred & Magan, 2004) and other integrated management strategies have been tested against *Fusarium* species (Jouany, 2007; Bojanowski et al., 2013; Schisler et al., 2015; Degraeve et al., 2016), no HACCP-based integrated management system against 4,15-DAS is described in the literature.

7. Levels and patterns of contamination in food commodities

Data on 4,15-DAS contamination of food were submitted to the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database by the relevant government authorities and were derived from about 80 papers published mainly between 2000 and 2016 (WHO, 2016).

However, the number of data submitted was quite limited, and the number of detected data (i.e. above the LOD) represents a low proportion, that is, 2.3% detected data in the GEMS/Food contaminants database, which contains 16 845 data points. The main food commodities reported to be contaminated with 4,15-DAS were cereals and cereal-based foods. Few other food commodities were analysed and, overall, few contaminated samples were detected (i.e. they were not detected or 4,15-DAS was below the LOD).

7.1 Surveillance data

7.1.1 Data from GEMS/Food contaminants database on occurrence of 4,15-DAS

The GEMS/Food contaminants database (WHO, 2016) was assessed on 31 August 2016 for 4,15-DAS: food commodities involved; number of samples analysed; number of samples with contamination levels above the LOD; maximum level.

Data from five WHO regions were present in the GEMS/Food contaminants database: African Region, Region of the Americas, European Region, Eastern Mediterranean Region and Western Pacific Region. The South-East Asia Region was not represented in the database for 4,15-DAS contamination.

A total of 16 843 records on 4,15-DAS were retrieved from the database. The main food group reported with 4,15-DAS contamination were the cereals and cereal-based products. A more precise analysis shows that raw kernels and flour of wheat, oat, barley, rice, rye, maize or sorghum are the products most often reported to be contaminated with 4,15-DAS. Bread is also contaminated from time to time. 4,15-DAS was reported in one sample of pizza and two samples of potato chips. Contamination data on 4,15-DAS in food commodities submitted to the GEMS/Food contaminants database are summarized in Table 9.

(a) African Region

A total of 1083 analyses appear in the GEMS/Food contaminants database on samples from African countries. Of these, 154 (14%) were positive for 4,15-DAS. Food commodities from this region have the highest level of contaminated samples. Reports from three countries are included in the GEMS/Food contaminants database: Burkina Faso, Ethiopia and Mali. Sorghum is the only food commodity for which data are available. The contamination level is generally low: less than 10 µg/kg food. Maximum levels are 31 µg/kg for Mali, 35 µg/kg for Burkina Faso and 109 µg/kg for Ethiopia.

(b) Region of the Americas

The GEMS/Food contaminants database contains 2400 records for cereals and cereal-based foods, and food for infants. In this region, the only country contributing data on 4,15-DAS in foods was Canada. No 4,15-DAS was detected in the samples.

(c) European Region

Eight countries from the European Region contributed data on 4,15-DAS measurements in foods to the GEMS/Food contaminants database: Czech Republic, Finland, France, Germany, Slovenia, Spain, Sweden and the United Kingdom. The number of samples reported was 11 868, with 178 samples (1.5%) contaminated above the LOQ (0.3–250 µg/kg). The main food commodities tested and shown positive for 4,15-DAS were cereals and cereal-based food. Numerous food groups were tested, but except for cereals and cereal-based food, almost no contaminated samples were detected; for example, only one of 204 samples of food for infants was positive for 4,15-DAS. The general level of contamination

Table 9

Summary of data on concentrations of 4,15-DAS in commodities from the GEMS/Food contaminants database

Food commodities / Statistic	African Region	Region of the Americas	European Region	Eastern Mediterranean Region	Western Pacific Region
Alcoholic beverages					
N	–	–	3	–	16
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	10	–	1.4
Cereals					
N	1 083	1 941	10 677	450	608
% <LOD	86	100	98.4	95.8	99.7
Mean LB (µg/kg)	3.1	0	0.03	0.24	0.002
Mean UB (µg/kg)	5.5	10	8.7	2.6	4.3
Composite foods					
N	–	–	109	–	56
% <LOD	–	–	100	–	98.2
Mean LB (µg/kg)	–	–	0	–	0.005
Mean UB (µg/kg)	–	–	4.5	–	2.4
Fats and oils					
N	–	–	113	–	4
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	7.2	–	2.5
Food for infants					
N	–	450	204	–	8
% <LOD	–	100	99.5	–	100
Mean LB (µg/kg)	–	0	0.000 9	–	0
Mean UB (µg/kg)	–	10	8.1	–	0.4
Fruits					
N	–	–	90	–	8
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	26.6	–	2.5
Herbs and spices					
N	–	–	16	–	8
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	10	–	2.5
Legumes and pulses					
N	–	3	51	–	220
% <LOD	–	100	100	–	99.6
Mean LB (µg/kg)	–	0	0	–	0.04
Mean UB (µg/kg)	–	10	11.5	–	5

Table 9 (continued)

Food commodities / Statistic	African Region	Region of the Americas	European Region	Eastern Mediterranean Region	Western Pacific Region
Meat					
N	–	–	95	–	39
% <LOD	–	–	97	–	100
Mean LB (µg/kg)	–	–	0.014	–	0
Mean UB (µg/kg)	–	–	4	–	2.5
Milk and dairy					
N	–	–	9	–	12
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	10	–	20
No alcoholic beverages					
N	–	–	–	–	8
% <LOD	–	–	–	–	100
Mean LB (µg/kg)	–	–	–	–	0
Mean UB (µg/kg)	–	–	–	–	2.5
Nuts and oilseeds					
N	–	1	184	–	4
% <LOD	–	100	99.5	–	100
Mean LB (µg/kg)	–	0	0.125	–	0
Mean UB (µg/kg)	–	10	14.6	–	2.5
Other					
N	–	–	18	–	4
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	9.5	–	2.5
Products for nutritional uses					
N	–	–	30	–	–
% <LOD	–	–	100	–	–
Mean LB (µg/kg)	–	–	0	–	–
Mean UB (µg/kg)	–	–	9.9	–	–
Snacks and desserts					
N	–	–	200	–	24
% <LOD	–	–	99.5	–	92
Mean LB (µg/kg)	–	–	1.25	–	0.45
Mean UB (µg/kg)	–	–	8.26	–	0.83
Starchy roots					
N	–	5	7	–	15
% <LOD	–	100	100	–	100
Mean LB (µg/kg)	–	0	0	–	0
Mean UB (µg/kg)	–	10	10	–	11

Food commodities / Statistic	African Region	Region of the Americas	European Region	Eastern Mediterranean Region	Western Pacific Region
Stimulant beverages					
N	–	–	20	–	4
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	10	–	2.5
Sugar and confectionery					
N	–	–	11	–	4
% <LOD	–	–	100	–	100
Mean LB (µg/kg)	–	–	0	–	0
Mean UB (µg/kg)	–	–	10	–	2.5
Vegetables					
N	–	–	31	–	–
% <LOD	–	–	100	–	–
Mean LB (µg/kg)	–	–	0	–	–
Mean UB (µg/kg)	–	–	10	–	–

DAS: diacetoxyscirpenol; LB: lower bound; LOD: limit of detection; <LOD: percentage of data points with a level lower than the LOD; mean LB: mean value of contamination (LB approach); mean UB: mean value of contamination (UB approach); *N*: number of data points in the database; UB: upper bound

was low with only eight samples above 10 µg/kg, with a maximum of 251 µg/kg for a snack food sample.

(d) Eastern Mediterranean Region

The only country of the Mediterranean region that submitted data on 4,15-DAS in food was Sudan, with 450 data points on sorghum. Of these sorghum samples, 19 were contaminated (4.2%) with 4,15-DAS above the LOD, up to a maximum of 19 µg/kg.

(e) Western Pacific Region

Four countries from the Western Pacific Region contributed 1042 data points to the GEMS/Food contaminants database: China (Hong Kong Special Administrative Region [SAR]), Japan, New Zealand and Singapore. A total of 0.6% quantified data (6 samples) are indicated for potato chips, cereals and composite food with cereals, with a maximum of 8 µg/kg for chips. Many food categories were tested, for example, nuts, food for infants, milk and beverages.

7.1.2 Literature search on peer-reviewed publications on occurrence of 4,15-DAS in food

In spring 2016, the peer-reviewed literature on occurrence data for 4,15-DAS in food commodities was assessed, including food commodities involved; number

of samples analysed; number of samples with contamination levels above the LOQ; and maximum level. All the reviewed data are summarized in Tables A1-1 to A1-4 in Appendix 1.

(a) African Region

Malawi

One study was found on the analysis of 4,15-DAS in maize beer in Malawi. No 4,15-DAS was detected in the samples.

Nigeria

Three studies were found on occurrence of 4,15-DAS in maize in Nigeria. The higher occurrence (18.6%) was found in stored maize. No 4,15-DAS was detected above the LOD in the second study and 4,15-DAS was detected in 9% of the samples in the third study. 4,15-DAS contamination was 51 µg/kg or less.

(b) Region of the Americas

Argentina

Four papers were found on 4,15-DAS contamination of beer, wheat and grasses from Argentina. Incidence of 4,15-DAS contamination was low (less than 3%), except in one older publication (10%). No 4,15-DAS was found in beer, and it had a low incidence in grasses. The highest values correspond to wheat in the older paper: mean concentration of 792 µg/kg.

Brazil

Of the three publications concerning 4,15-DAS in food commodities in Brazil, just one sample of wheat (out of 20 samples tested) was found contaminated with a high level of 4,15-DAS (600 µg/kg). The food commodities concerned were wheat and corn.

Canada

Four papers were found on the occurrence of 4,15-DAS in Canadian food samples between 1987 and 2005, only in cereals. The incidence of the occurrence of 4,15-DAS was very low (<0.4%), except in the older papers (37.7%). However, some high contamination levels were found for 4,15-DAS in corn (up to 1000 µg/kg).

Mexico

Two publications were concerned with the quantification of 4,15-DAS in maize in Mexico. However, one of the two papers (Pena Betancourt et al., 2015) presented the contamination level as a sum of all trichothecenes type A, and these data

are therefore not usable. No positive samples for 4,15-DAS ware reported in the other paper.

USA

Two papers were found on the occurrence of 4,15-DAS in food commodities from the USA: one on soybeans and soy products, and one in water (streams). Occurrence of 4,15-DAS was high in soy products (up to 35%) and contamination was also high (up to 230 µg/kg). In water streams, few samples were positive and the contamination level was always lower than the detection limit. These streams were not chosen for the possible use of the water for human consumption, but in agricultural areas, in order to evaluate transfer from agriculture to water.

(c) **South-East Asia Region**

India

Three publications on 4,15-DAS occurrence in food commodities came from India. One study (Chakrabarti & Ghosal, 1986) found 4,15-DAS in bananas at a very high contamination value of 14 mg/kg, but these results came from bananas "with heavy mycelial infestation", and therefore cannot be considered as representative. Another study, on rice (Sempere Ferre, 2016), found a 16% incidence of 4,15-DAS at high contamination levels of 200 µg/kg. In the third study many food commodities were analysed but no samples were found to be contaminated with 4,15-DAS above the LOD.

Pakistan

One published paper on 4,15-DAS contamination of maize found 4,15-DAS occurring in 9.2% of the 65 samples, at high contamination levels (mean: 516 µg/kg).

(d) **European Region**

Austria

One paper was published in 2002 on 4,15-DAS contamination in cereals in Austria. Only traces of 4,15-DAS were detected in oat grains.

Belgium

One paper was available on 4,15-DAS contamination in food commodities in Belgium (Njumbe Ediage, Van Poucke & De Saeger, 2015). Very few samples were tested in order to evaluate a new multi-analyte LC-MS/MS method. Eight samples of sorghum imported from Africa were tested; five were positive for 4,15-DAS with a range between 7 and 66 µg/kg.

Croatia

One paper was published in 2006 on grain food commodities in Croatia. Despite the low sensitivity of the analytical method (TLC), a high level of incidence was found: 27.6%. The samples were collected over a 7-year period (1998–2004). This was one of the rare examples in this analysis of a high occurrence and high concentrations.

Finland

A scientific cooperation among European Member States published a report on the occurrence of *Fusarium* mycotoxins in different European countries in 2003, including Finland, which reported analyses of samples for 4,15-DAS. No positive samples were detected.

France

Two published studies examined 4,15-DAS contamination. One study was derived from the second French total diet study (ANSES, 2011), and was included in the GEMS/Food contaminants database. As a result, it is included in the discussion about the European Region (section 7.1.1). The only positive sample was one bread sample (out of 14 bread samples) with a contamination level between the LOD and LOQ. The second study was from the same SCOOP (2003) report as the Finland study, and had a great number of detected (but not quantified) samples. The results for this study are given in ranges (<LOD, <25, <50, <75, <100, <150, <200, >200 µg/kg). Thus, it is not clear if the samples were quantified or only detected, for example, for wheat, contamination was reported as less than 25 µg/kg with an LOQ equal to 20 µg/kg.

Germany

Twelve published papers were found on the analysis of 4,15-DAS in food commodities in Germany. Overall, there was a low incidence and a low level of contamination. One study (Gottschalk et al., 2007) on oat flakes showed a high incidence of 4,15-DAS contamination (between 67% and 100%) but with a low mean contamination (0.11 µg/kg and 0.04 µg/kg for conventional and organically grown oats, respectively).

No samples with 4,15-DAS concentrations above the LOQ were found for wheat (3 studies), oats (2 studies) or peas (1 study). Different parts of maize were analysed with various results. One sunflower oil sample was contaminated with a detectable concentration (out of 12), while no soybean or corn oil samples were found positive for 4,15-DAS (out of 98 samples). One potato sample tested positive for 4,15-DAS contamination (out of 85 fruit and vegetable samples).

Italy

Seven papers were found from over the last few years on 4,15-DAS contamination in food commodities in Italy, all on cereals except for one on baby food. The incidence of 4,15-DAS in the food commodities was low, as were the contamination levels, with the exception of data reported by older publications.

Lithuania

One paper was found on 4,15-DAS contamination of different cereals in Lithuania. The incidence of 4,15-DAS contamination was low (0.5% for wheat, rye and barley combined), and the contamination level was less than 5 µg/kg.

Netherlands

Three papers were found on 4,15-DAS contamination of food commodities in the Netherlands, among them one study on beer and the other two on cereals. 4,15-DAS was detected in only a few samples of maize.

Norway

One publication, from 1999, was found on 4,15-DAS contamination in foods in Norway. The results represent a 3-year (1996–1998) survey of loads and silos. Only one sample out of 449 was positive. Even then, the authors described the concentration of 4,15-DAS as amounting to "traces in one sample".

Poland

Four papers were published on 4,15-DAS contamination of food commodities in Poland since 2000, all of them on cereals. The measured levels were low, although the incidence of contamination was higher than in food commodities in other European countries.

Romania

One paper on 4,15-DAS contamination in cereals in Romania was published in 1998. The incidence of 4,15-DAS contamination was very low and the level of contamination approximated the LOD.

Russian Federation

One recently published paper on 4,15-DAS contamination of animal feed and forage in Russian Federation referred to a total of 15 single compound samples of clover, grass and alfalfa and 29 mixtures (clover-grass, alfalfa, timothy). This was the only study that used the ELISA method to measure contamination. Both incidence and level of contamination of 4,15-DAS were high.

Slovenia

One paper from Slovenia was published in 2010, on 4,15-DAS contamination in grain. 4,15-DAS was not detected above the LOD in any of the 66 samples. However, both the LOD and the LOQ were high.

Spain

Six papers published on 4,15-DAS contamination in food commodities in Spain took into account products such as cereal, coffee, baby food and tiger nuts. The incidence of 4,15-DAS contamination was very low except in coffee, which the authors analysed as coffee beans and not the beverage.

Turkey

One paper was found on 4,15-DAS occurrence in processed cereals and pulse products from Turkey. No 4,15-DAS was detected in the samples.

United Kingdom

Four papers were published on 4,15-DAS occurrence in food commodities in the United Kingdom covering many food commodities including a range of grains and grain-based foods. No 4,15-DAS was detected above the LOD in any of the samples.

Europe

The one study, published in 2015, on 4,15-DAS occurrence in beer from different countries in Europe detected no 4,15-DAS above the LOD.

(e) **Eastern Mediterranean Region**

Egypt

Two publications were found on 4,15-DAS contamination of food commodities in Egypt. One paper on sugar cane concluded that there was "some" 4,15-DAS but without quantification. The other paper, on corn-based foods, found contamination with 4,15-DAS to be low, at 10%, with a 14% incidence, depending on the variety of maize.

Israel

One study on the quantification of 4,15-DAS in corn and wheat in Israel did not detect 4,15-DAS in any of the 15 samples.

Morocco

One study was found on 4,15-DAS occurrence in rice in Morocco. No 4,15-DAS was detected in the samples.

Tunisia

One study was conducted on the quantification of 4,15-DAS in different cereals, with three positive samples and a maximum concentration of 97 µg/kg.

(f) **Western Pacific Region**

Japan

Two publications presented results from Japan on 4,15-DAS occurrence in cereals and beverages. No 4,15-DAS was detected in beverages, although some positive results were found in cereals, but always below the LOQ (5 µg/kg).

New Zealand

One paper, published in 1989, on 4,15-DAS contamination in maize found a high incidence of 4,15-DAS (30%) and a high level of 4,15-DAS contamination (1000 µg/kg).

7.1.3 Co-occurrence of DAS, T-2 toxin and HT-2 toxin

A profile of various mycotoxins often occurs in various food commodities (Perkowski & Basinski, 2002). This can result from the production of a profile of mycotoxins by one fungus (Mateo, Mateo & Jiménez, 2002). However, data on the profiles of mycotoxins produced by a fungal strain are often the result of laboratory experiments using artificial media (Rocha et al., 2015), and it is not known if the fungus behaves in the same way under field conditions. Different strains from the same fungal cultivar can co-occur in field conditions, for example, *F. graminearum* and *F. proliferatum,* as can different fungal cultivars. This can be another reason for co-occurrence of mycotoxins in food commodities (Lazzaro et al., 2015).

The multi-methods used in the analysis of mycotoxins allow for the analysis of their profiles and produce data on their co-occurrence in individual samples to assess co-exposure. Since results on individual samples are seldom reported in the literature, databases such as the GEMS/Food contaminants database are of immense importance for assessing co-occurrence of mycotoxins in individual samples.

(a) Data from GEMS/Food contaminants database on co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin

The GEMS/Food contaminants database was assessed on 11 October 2016 for samples analysed for all three of 4,15-DAS, T-2 toxin and HT-2 toxin. A total of 1159 samples from Finland (53), France (278), Germany (392) and the United Kingdom (436) were found with a record for all three mycotoxins. The results are shown in Table 10.

As the results show, there are few records on co-occurring 4,15-DAS, T-2 toxin and HT-2 toxin. No co-occurrence of the three mycotoxins was reported for the samples from Finland, France and Germany. Of the 436 samples from the United Kingdom, 21 were positive for all three mycotoxins. All the positive samples were raw oats with husk. One other United Kingdom sample contained 4,15-DAS but no T-2 toxin and no HT-2 toxin.

This analysis of the data from the GEMS/Food contaminants database for co-occurrence may be limited by the fact that T-2 toxin and HT-2 toxin were not included in the call for data for the current meeting. Therefore, the results are likely not representative. Despite this, the data that were available show some evidence of co-occurrence and as such may warrant further investigation in the future if full datasets for all three mycotoxins become available.

(b) Open literature search on co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin in food

A limited literature search was conducted in Web of Science for articles on the co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin published from 2000 to 2016. The results are summarized in Table 11.

Several papers were retrieved on co-occurring mycotoxins. Only very few reported the results for the individual samples and were useful for the assessment of co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin.

One paper was found on contamination of oats in Poland with type A trichothecenes (Perkowski & Basinski, 2002). Twelve out of the 99 samples (12%) were contaminated with 4,15-DAS and nine of those samples were co-contaminated with either T-2 toxin, HT-2 toxin or both. Five samples were contaminated with only T-2 toxin, 14 samples with only HT-2 toxin, and three samples with both.

A study on the occurrence of type A trichothecenes in oats revealed that 37 of the 43 samples (86%) were contaminated with 4,15-DAS as well as with T-2 toxin and HT-2 toxin (Gottschalk et al., 2007). All the samples were co-contaminated with NEO.

Only one of 45 soy products tested (2%) was contaminated with 4,15-DAS (Schollenberger et al., 2007). There was no co-occurrence with T-2 toxin

Table 10

Data from GEMS/Food contaminants database on co-occurrence of 4,15-DAS, T-2 toxin and HT-2 toxin in food commodities from Finland, France, Germany and the United Kingdom

Country	No. of samples analysed	No. of positive samples	Concentration (µg/kg)								
			4,15-DAS			T-2 toxin			HT-2 toxin		
			Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
Finland	53	0	0	0	0	0	0	0	0	0	0
France	278	0	0	0	0	0	0	0	0	0	0
Germany	392	0	0	0	0	0	0.4	27.4	0	1.0	45.1
United Kingdom	436	21	0	0.2	18	0	9.2	964	0	19.8	460
Overall	1 159	21	0	0.1	18	0	3.6	964	0	7.8	460

DAS: diacetoxyscirpenol; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; Max.: maximum detected concentration; Mean: mean detected concentration; Min.: minimum detected concentration; No.: number

Table 11

Data on co-occurrence of 4,15-DAS with T-2 toxin and/or HT-2 toxin

Food	No. of samples analysed	No. of samples with 4,15- DAS above LOQ	No. of samples with 4,15-DAS, T-2 toxin and HT-2 toxin co-occurring	Reference
Oats	99	12	3	Perkowski & Basinski (2002)
Fine oat flakes	31	22	22	Gottschalk et al. (2009)
Oat flakes	43	37	37	Gottschalk et al. (2007)
Oat flakes	23	15	15	Gottschalk et al. (2009)
Oat kernels	19	9	9	Gottschalk et al. (2009)
Oat bran	12	9	9	Gottschalk et al. (2009)
Oat-based infant food	13	4	4	Gottschalk et al. (2009)
Rye flour	15	0	0	Gottschalk et al. (2009)
Whole rye flour	9	0	0	Gottschalk et al. (2009)
Soy-based products	45	1	0	Schollenberger et al. (2007)
Whole wheat flour	11	0	0	Gottschalk et al. (2009)
Wheat bran	10	2	2	Gottschalk et al. (2009)

DAS: diacetoxyscirpenol; LOQ: limit of quantification; No.: number

and/or HT-2 toxin although three other samples were contaminated with HT-2 toxin. MAS was detected in four soy food samples and HT-2 toxin co-occurred in three of these samples.

4,15-DAS was not detected in 11 samples of whole wheat flour but was present in two out of 10 wheat bran samples (20%), while all 21 samples were contaminated with T-2 toxin and HT-2 toxin (Gottschalk et al., 2007, 2009). MAS was detected in all of the whole wheat flour samples and wheat bran samples.

No 4,15-DAS was detected in 15 rye flour samples, or in nine whole rye flour samples, whereas all the samples were contaminated with T-2 toxin and HT-2 toxin. Twenty of 31 fine oat flake samples (71%), 15 of 23 oat flake samples (65%), nine of 19 oat kernel samples (47%), nine of 12 oat bran samples (75%) and four of 13 oat-based infant food samples (31%) were contaminated with 4,15-DAS, while each of these samples was also contaminated with T-2 toxin and HT-2 toxin. All of the 12 oat bran samples were also contaminated with MAS and NEO.

7.2 Distribution curves

Distribution could not be determined due to the high number of left-censored data.

7.3 Data on annual variation in contaminant levels

No conclusions could be drawn from the data on annual variations in contamination levels. However, since climatic conditions do affect contamination levels of other mycotoxins, it is expected that the incidence and levels of 4,15-DAS show yearly and regional variations.

8. Food consumption and dietary exposure estimates

8.1 Concentrations in food used in the dietary exposure estimates

Very low occurrence of 4,15-DAS contamination is seen (2.3% contaminated food in the GEMS/Food database) except for food commodities in Africa (14%). Analysis of the peer-reviewed literature gave similar indications of contamination: low occurrence and low levels of contamination in general. The highest occurrence and contamination levels were from the older publications with older analytical methods. This might be due to a lower specificity of the analytical methods used. Generally, after the year 2000, the use of MS/MS gives LODs and LOQs below 10 μg/kg.

Analysis for 4,15-DAS has been conducted for a wide range of different types of food products; however, the large majority of detections of 4,15-DAS occur in cereals and cereal-based products: no DAS was detected in beverages, contamination was very rare in fruits and vegetables and no contamination was reported in baby food. The only product (other than cereals) that gives an alert is coffee. However, the relevant report was only from Spain and from two papers from the same laboratory, with large variations between experiments.

8.2 Food consumption data used in the dietary exposure estimates

The Committee calculated international estimates of dietary exposure for a number of regions using consumption data from the GEMS cluster diets and concentration data from the GEMS/Food contaminants database.

8.3 Assessments of dietary exposure

8.3.1 National estimates

Estimates of daily dietary exposure were found in the literature for four countries: Finland, Morocco, Spain and Tunisia. In each case, very few food groups were considered and very low levels of exposure were calculated. The impact of censored data is very important in any case and then the exposure is very dependent on the analytical methods used and their LOD and LOQ. However, results from Finland and Tunisia, which use consumption of all cereals, could be considered a good estimation. Data from Morocco that took into account rice consumption only is likely to underestimate the exposure to 4,15-DAS. The evaluation from Spain is difficult to interpret because it is based on 100% censored data and depends largely on the LOD.

(a) Finland

The dietary exposure to 4,15-DAS by the adult Finnish population was estimated by a European consortium (SCOOP) in 2003. Data are summarized in Table 12. Exposure was calculated using the middle bound method for left-censored data (using LOD/2 or LOQ/6, depending on the LOD and LOQ, for censored data). Data on mean consumption values were provided by the national food safety agency of Finland and contamination data came from 384 samples with a mean detected level of 4%. This explains the similar level of contamination, close to LOD/2. Thus the exposure is mainly guided by consumption and analytical LODs.

(b) Morocco

Serrano et al. (2012) estimated dietary exposure to 4,15-DAS in Morocco to be 0.1 ng/kg bw per day, taking into account contamination values and average rice consumption of 1.0 kg/person per year. The result is likely an underestimate because of the small range of foods used in the exposure calculation (the only food commodity considered was rice and not cereals).

Table 12
Assessment of mean dietary exposure to 4,15-DAS of the population in Finland

Food product	Food consumption (g/person per day)	Mean 4,15-DAS level (µg/kg food commodity)	Dietary exposure (ng/kg bw per day)[a]
Wheat	114.7	6.9	10
Rye	49.1	6.9	4
Oats	4.45	6.9	0.4
Barley	4.45	6.4	0.4
Total dietary exposure	–	–	15

bw: body weight; DAS: diacetoxyscirpenol
[a] Mean body weight was estimated at 77.1 kg.
Source: SCOOP (2003)

(c) Spain

Rodriguez-Carrasco et al. (2013) calculated daily exposure to 4,15-DAS based on their assessments of contamination values. The data are summarized in Table 13. No 4,15-DAS was detected above the LOD (in 119 samples of wheat, 23 samples of rice and 17 samples of maize). The authors chose to use a middle bound method based on an LOD/2 substitution for left-censored data with an LOD of 2.5 µg/kg.

The population groups considered in this study were young children (0–3 years, 10 kg); older children (5–12, 20 kg); and adults (18–65 years; 70 kg). Consumption data on wheat were derived from different Spanish surveys published by the Spanish Agency for Food Safety and Nutrition; consumption data on rice-based and maize-based foods were taken from the most recent food balance data reported by FAO for the Spanish population.

Garcia-Moraleja et al. (2015a) estimated dietary exposure to 4,15-DAS through coffee in adults and adolescents (Table 14) using a lower bound (LB) approach (contamination is 0 when not quantified or detected). Consumption data were provided by the Spanish Agency for Food Safety and Nutrition for long-term exposure (chronic) food consumption statistics based on a survey from 2009 that included 1067 adolescent and adult participants.

(d) Tunisia

Serrano et al. (2012) estimated dietary exposure to 4,15-DAS by the population of Tunisia to be 24.7 ng/kg bw per day based on an all-cereals consumption. Consumption was based on GEMS/Food cluster diets and contamination data were based on three contaminated samples, detected out of 52 samples of barley, sorghum, wheat and maize (LOQ of 5 µg/kg).

Table 13
Assessment of mean exposure of the population in Spain to 4,15-DAS via food

Population age (years)	Consumption (g/day)			Dietary exposure (ng/kg bw per day)
	Wheat	Rice	Maize	
0–3	32.9	31.5	4.1	8.56
5–12	79.4	31.5	4.1	5.76
18–65	25.1	31.5	4.1	1.08

bw: body weight; DAS: diacetoxyscirpenol
Source: Rodriguez-Carrasco et al. (2013)

Table 14
Assessment of dietary exposure to 4,15-DAS via coffee in Spain

Population	Dietary exposure (ng/kg bw per day)	
	Mean	P95
Adults ($n = 981$)	0.185	0.858
Adolescents ($n = 86$)	0.043	0.376

bw: body weight; DAS: diacetoxyscirpenol; P95: 95th percentile
Source: Garcia-Moraleja et al. (2015a)

8.3.2 International estimates

An evaluation of daily dietary exposure to 4,15-DAS was made for different geographical areas using contamination data from the GEMS/Food database and consumption data from GEMS/Food cluster diets (17 cluster diets termed G01 to G17). The WHO regions analysed were the African Region, the Region of the Americas, the European Region, the Eastern Mediterranean Region and the Western Pacific Region, the only regions with contamination data. The relevant cluster diets were G13 for the African Region and the Eastern Mediterranean Region; G07, G08, G11 and G15 for the European Region; and G10 for the northern regions of the Americas and for the Western Pacific Region.

Three issues complicated the exposure assessment for 4,15-DAS:

- The data showed very high censorship, higher than 95%, except for Africa (86%);
- There was in general a high LOQ (often higher than 10 µg/kg); and
- Generally, only a limited range of food commodities were sampled and analysed (only sorghum for the African and Eastern Mediterranean regions), and when many food categories were analysed, a very high percentage (~90% or more) of data came from cereals.

Because of these limitations, the estimated dietary exposures must be considered with caution. Exposure estimates were only calculated for the general population and have a very high degree of uncertainty, as consumption data for specific groups are scarce.

One scenario for assessment of exposure of the population to 4,15-DAS used the LB approach, where all samples below the LOD were assigned a zero concentration before deriving mean concentrations for each food group.

Three scenarios for exposure to 4,15-DAS were calculated using the upper bound (UB) approach in cases where sufficient data were available, as for the African, Eastern Mediterranean, European and Western Pacific regions. For the Americas, only one scenario was calculated because the global level of censorship (100%) did not allow the use of scenarios 2 and 3. The data are summarized in Table 15.

For scenario 1, dietary exposure was calculated at level 1 of consumption (whole consumption divided into 18 groups) and a group was used for exposure calculation if at least one contamination analysis was done on this group (even if there was no detection).

For scenario 2, dietary exposure was calculated at level 1 of consumption, but a food group was used only if at least one sample was contaminated at a level above the LOD in that group (generally cereals and cereal-based food, nuts and pulses, confectionery).

For scenario 3, dietary exposure was calculated at level 2 of consumption (61 food groups) with the same condition as scenario 2.

No estimation for dietary exposure to 4,15-DAS was made by combining contamination levels from the GEMS/Food database and published results on diets. Because the two data sources gave similar results, adding data from publications will not contribute any further information. The only food that could be questioned is coffee, which does not appear in the GEMS/Food database estimates. However, considering the higher coffee consumption from the GEMS/Food cluster diets and calculating a UB mean estimate of dietary exposure to 4,15-DAS through coffee consumption for a 70 kg individual, as from Garcia-Moraleja et al. (2015a) data, gives a result of 3 ng/kg bw per day. This value can be considered a conservative estimate for everybody.

For the African and Eastern Mediterranean regions, the only tested product was sorghum. The diet is not at all covered by this product alone, and the dietary exposure might be underestimated, but with no information for 17 food groups out of 18, it is impossible to reach a conclusion.

For the Western Pacific region, the 18 food categories were analysed, but cereal foods represent approximately two thirds of all the samples, and only six analyses were above the LOD. As such, these data can be considered to be representative of the dietary contamination. However, they are mainly linked

Table 15
International estimates of exposure to 4,15-DAS via food for adults

WHO Region	Dietary exposure (ng/kg bw per day)[a]			Left-censorship (%)
	LB mean exposure	UB mean exposure for scenario 1/2/3	LB–UB P90 exposure[b] for scenario 1/2/3	
Africa (Burkina Faso, Ethiopia, Mali)	1.4	20.3/20.3/5	2.8–40.6/40.6/10	86
Americas (Canada)	0	154/na/na	0–308/na/na	100
Eastern Mediterranean (Sudan)	0.4	17/17/4	0.8–34/34/8	96
Europe (Czech Republic, Finland, France, Germany, Slovenia, United Kingdom)	2.8	363/69/41	5.6–726/138/82	98.3
Western Pacific (China [Hong Kong SAR], Japan, New Zealand)	0.4	239/57/6.5	0.8–478/114/13	99.4

bw: body weight; DAS: diacetoxyscirpenol; LB: lower bound; na: not able to be calculated; P90: 90th percentile; SAR: Special Administrative Region; UB: upper bound; WHO: World Health Organization
[a] Body weight used is 60 kg.
[b] Based on Environmental Health Criteria 240 (FAO/WHO, 2009), P90 exposure was estimated by the Committee as twice the mean exposure.

with the detection level. This clearly appears in scenarios 2 and 3, where the only maintained food groups are groups where at least one sample was positive. The more precise value for the UB mean estimate for scenario 3 is then 6.5 ng/kg bw per day.

For the Region of the Americas (Canada only), there were no samples reported with concentrations of 4,15-DAS above the LOD. Almost all contamination data came from cereals or cereals for infants (only nine samples on other food groups). The LOD and LOQ are generally high (10 and 50 µg/kg, respectively). It is difficult to consider that the results are representative of reality, and the results are probably largely overestimated. It was impossible to apply the two alternative scenarios because of no positive results.

For the European Region, the dataset contained results from 18 food categories from eight countries. The main problem is the high level of censorship associated with the high LOQ (80% are higher than 10 µg/kg, most of them being equal to 33 µg/kg). This largely overestimates the dietary exposure. The two alternative scenarios are quite low and not very different from each other because most of the positive samples came from the same subgroup (cereals) with a high consumption level. The best UB mean estimate is 41 ng/kg bw per day, which is relatively high, compared with the results from the Western Pacific Region, because of the high LOQ used.

8.4 Potential effect of limits and their enforcement on chronic dietary exposure

The evaluation of different maximum limits of 4,15-DAS in foods and their effects on estimates of dietary exposure was not required for this evaluation.

9. Dose–response analysis and estimation of toxic/carcinogenic risk

9.1 Identification of key data for risk assessment

9.1.1 Pivotal data from biochemical and toxicological studies

Standard toxicological studies conducted according to established guidelines were not available to characterize the potential toxicity of 4,15-DAS. Although preclinical studies conducted with dogs and rhesus monkeys are available, these studies were designed to identify the highest nontoxic dose for further clinical trials and used intravenous administration over a maximum duration of 5 consecutive days. While these studies provided qualitative information about the toxicity of 4,15-DAS, they were not considered relevant to assessing risk from dietary exposure to 4,15-DAS.

Of the studies employing oral or dietary administration of 4,15-DAS, the typical duration was less than 1 month, only one dose of 4,15-DAS was used and a limited number of parameters were analysed. In addition, the majority of the studies were conducted with fowl, including ducks, chickens and turkeys. The relevance of an avian animal model for a human health risk assessment was considered to be limited because biochemical studies indicate that chickens lack the ability for de-epoxidation of 4,15-DAS in the gut microflora and conjugation reactions in the liver, which are the primary detoxification pathways in mammalian species.

Two short-term dietary studies conducted with pigs were available. A small number of animals were used in these studies, and interpretation of the studies was limited by inadequate reporting of results. The primary treatment-related effects were oral lesions, feed refusal and reduced body weights or body-weight gains, which were consistent with observations in 4,15-DAS feeding studies conducted with other species. The decreases in body weight or weight gain compared with controls were not considered toxicologically significant as feed efficiency was not affected; the effect appeared to be associated with feed refusal, which may have been a secondary effect of the oral lesions. Although the oral lesions were considered to be treatment-related as their onset, incidence and

severity were related to the concentration of 4,15-DAS in the diet, this effect was considered to be the result of localized contact and not systemic exposure. The relevance for human dietary exposure was considered limited.

The gastrointestinal tract and the lympho-haematopoietic system are considered the primary target tissues for toxicity based on in vitro biochemical mode of action studies, acute toxicity studies and preclinical studies and clinical trials conducted with intravenous administration of 4,15-DAS. However, effects on these tissues were not observed following short-term dietary exposure to 4,15-DAS. Moreover, these studies had several limitations and the Committee did not consider them suitable for risk assessment for humans.

9.1.2 Pivotal data from human clinical/epidemiological studies

The information available from human clinical/epidemiological studies was considered insufficient or not relevant for a quantitative risk assessment of dietary exposure to 4,15-DAS.

10. Comments

10.1 Biochemical aspects

4,15-DAS is rapidly absorbed and metabolized, with plasma concentrations of 4,15-DAS and two unconjugated metabolites, 15-MAS and SCP, peaking within 30–60 minutes after gelatine capsule intubation in swine and decreasing to non-detectable amounts 48 hours after dosing (Bauer et al., 1985). Following oral administration of a single radiolabelled 4,15-DAS dose of 0.55 or 0.66 mg/kg bw to rats and mice, respectively, radiolabel was found in the gastrointestinal tract, liver, kidney and tissues of the lympho-haematopoietic system (spleen, thymus, femur bone marrow). Approximately 90–94% of the oral dose was excreted in urine and faeces within 24 hours. The excretion was observed to parallel an associated decline of radiolabel in the tissues. The remaining low levels of radioactivity (up to 3%) plateaued over the following 6 days and were higher in the lympho-haematopoietic tissues than in the other tissues (Wang, Busby & Wogan, 1990). Although the available studies indicate that 4,15-DAS is bioavailable following oral exposure, the extent to which this occurs is not clear. In the pig, the majority of 4,15-DAS was excreted in the faeces as metabolites, with only a small amount of 4,15-DAS or metabolites detected in the urine (Bauer, Gareis & Gedek, 1989). In mice and rats, the majority of the radiolabel was excreted in the urine (Wang, Busby & Wogan, 1990).

In vitro studies indicate that 4,15-DAS is metabolized by gut microflora to several metabolites, including 15-MAS, SCP, de-epoxy MAS and de-epoxy SCP, in rats, cattle and pigs. The de-epoxidation is considered to be an important step in the detoxification of trichothecenes. However, de-epoxidation was not observed in chickens, horses or dogs (Swanson et al., 1987, 1988). In vivo and in vitro studies suggest that metabolism continues in the liver biphasically: in phase I, deacetylation via hydrolysis (first at C-4 and then at C-15) and hydroxylation (at C-7 and C-8), and in phase II, conjugation with glucuronic acid. Glucuronide conjugates of 4,15-DAS were identified in all species tested except chickens (Yang et al., 2015).

10.2 Toxicological studies

4,15-DAS is acutely toxic, with oral LD_{50} values in the range of 2–15 mg/kg bw in mice, rats and chickens. The lowest oral LD_{50} was observed in chickens (Richardson & Hamilton, 1990). The higher susceptibility of chickens to the toxicity of 4,15-DAS is consistent with biotransformation data that demonstrated the deficiencies of chickens for de-epoxidation in the gut microflora and conjugation reactions in the liver (Swanson et al., 1988; Young et al., 2007; Yang et al., 2015).

Effects of acute oral exposure to 4,15-DAS in mice, rats, chickens and pigs included lethargy, diarrhoea, vomiting and flushing of the skin, together with necrosis in the gastrointestinal tract and the lympho-haematopoietic tissues. Limited short-term studies of toxicity were available. In studies in which 4,15-DAS was administered to livestock animals in the diet for periods of up to 9 weeks, oral lesions, feed refusal and reduced body weights or body-weight gains were consistently observed. These studies were not considered suitable for an assessment of risk for humans.

No long-term studies of toxicity or carcinogenicity were available.

Tests for genotoxicity with 4,15-DAS in bacterial or eukaryotic in vitro systems gave uniformly negative results. One in vivo study with 4,15-DAS administered intraperitoneally was available (Hassanane et al., 2000); however, owing to study limitations, it is unclear whether the observed DNA strand breaks and chromosomal aberrations were a consequence of an interaction between the toxicant and genetic material or were secondary to cytotoxicity and inhibition of protein synthesis by 4,15-DAS. The Committee noted that DNA strand breaks and chromosomal aberrations were observed for T-2 toxin and that these effects were observed only at doses known to cause cytotoxicity and inhibition of protein and DNA synthesis (Annex 1, reference *152*).

No reproductive or developmental toxicity studies conducted by the oral route of exposure in mammalian species were available.

Structure–activity predictions for the toxicity of the metabolites of 4,15-DAS and the reported potency ranking for in vitro cytotoxicity and inhibition of protein synthesis indicate that the metabolites are less toxic than the parent compound (Ueno, 1977, 1983; Thompson & Wannemacher, 1986). However, the available comparative data for 4,15-DAS and 15-MAS indicate that adverse effects are induced with similar potency when the compounds are administered by the oral route of exposure (Mirocha et al., 1985; Richardson & Hamilton, 1990; Ademoyero & Hamilton, 1991a). As 4,15-DAS is rapidly converted to 15-MAS and other common metabolites in both the gut microflora and the liver, the toxicity of 4,15-DAS in vivo can be considered to include that of 15-MAS.

Because of the limited availability of data on 4,15-DAS, the Committee considered its similarity with the other type A trichothecenes, T-2 and HT-2, by comparing the available toxicological data for these trichothecenes and reviewing studies of combined effects. The comparison of 4,15-DAS with T-2/HT-2 toxin is supported by the similar chemical structures of type A trichothecenes and evidence that, similar to other trichothecene mycotoxins, 4,15-DAS has been shown to be a potent inhibitor of the initial step of protein synthesis (Mizuno, 1975; Tscherne & Pestka, 1975; Cundliffe & Davies, 1977; Thompson & Wannemacher, 1986); to inhibit DNA synthesis (Cooray, 1984); and to induce apoptosis in T-lymphocytes (Lee, Park & Kim, 2006; Nasri et al., 2006; Jun et al., 2007).

When comparing in vitro cytotoxic effects on blood cell progenitors or mitogen-stimulated human lymphocytes (Parent-Massin, Fuselier & Thouvenot, 1994; Parent-Massin &Thouvenot, 1995; Rio, Lautraite & Parent-Massin, 1997; Thuvander, Wikman & Gadhasson, 1999; Froquet, Sibiril & Parent-Massin, 2001), T-2 toxin was consistently observed to be more potent than 4,15-DAS. A comparison of in vivo effects was limited by differences in study design and a limited number of comparable studies between the available databases. The critical effects identified for T-2 toxin at the fifty-sixth meeting of JECFA were decreased WBC counts, haemoglobin and RBCs and a decreased lymphocyte proliferative response to mitogen stimulation, following short-term dietary exposure of pigs to T-2 toxin at a dose of 0.03 mg/kg bw per day (Rafai et al., 1995). In the short-term dietary pig studies conducted with 4,15-DAS, which involved only a few animals, no effects on blood cell counts were observed at doses up to 0.4 mg/kg bw per day, the highest dose tested (Weaver et al., 1981; Harvey et al., 1991). However, evidence from other animal studies suggests that 4,15-DAS and T-2 toxin cause similar immunotoxic and haematotoxic effects following oral exposure. In mice, depletion of lymphocytes from lymphoid tissues was observed following 2-day administration of 4,15-DAS by gavage at a dose of 3 mg/kg bw per day (Ziprin & Corrier, 1987). Similar observations were reported after T-2 toxin was administered to mice as a single gavage dose of 4 mg/kg bw (Corrier & Ziprin, 1986). In addition, although a comparable rat study was not available for T-2 toxin, administration of oral doses

of 4,15-DAS to rats at 1 mg/kg bw 3 times a week for 5 weeks (equivalent to 0.43 mg/kg bw per day) was associated with decreased haemoglobin, haematocrit and RBC count from day 7 onward. Atrophy and necrosis of the bone marrow, thymus, spleen, lymph nodes and gastrointestinal tract were observed within 2–4 weeks of treatment (Janse van Rensburg, Thiel & Jaskiewicz, 1987).

Although T-2 toxin appears to be more potent than 4,15-DAS in vitro and in vivo, the available data are insufficient for establishing relative potencies.

Of the few studies that considered the combined effects of 4,15-DAS and T-2 toxin, a consistent additive dose effect was observed for end-points such as in vitro inhibition of protein synthesis and lymphocyte proliferation (Thompson & Wannemacher, 1986; Thuvander, Wikman & Gadhasson, 1999), oral lethal doses following acute exposure (Hoerr, Carlton & Yagen, 1981b) and the incidence of oral lesions, feed refusal and decreased egg production following short-term dietary exposure in chickens (Diaz et al., 1994).

10.3 Observations in domestic animals/veterinary toxicology

Fusarium species have been associated with a number of animal toxicoses. However, only two case reports in bovines and chickens were identified in the literature that specifically identified 4,15-DAS as one of the mycotoxins associated with toxicoses. In these cases, the clinical observations included, but were not limited to, diarrhoea, loss of appetite, dehydration, weakness and death (Galhardo et al., 1997; Konjevic et al., 2004). These chickens revealed necrosis in the gastrointestinal tract and bursa of Fabricius (site of haematopoiesis), as well as depletion of lymphocytes (Konjevic et al., 2004).

10.4 Observations in humans

In the 1970s and early 1980s, 4,15-DAS was investigated under the name anguidine for its potential as a cancer chemotherapeutic agent using intravenous infusion dosing at doses ranging from 0.1 to 10 mg/m^2 (equivalent to 2.7–270 µg/kg bw), but these investigations were ultimately discontinued due to the lack of sufficient efficacy against tumours and observations of adverse effects in phase II clinical trials. The reported adverse effects in these trials were consistent with the target sites of toxicity of 4,15-DAS observed in the animal studies. These adverse effects included myelosuppression, which was characterized as decreased levels of lymphocytes and platelets, vomiting and hypotension beginning at doses equivalent to 81 µg/kg bw and reports of mild nausea at lower doses equivalent to 41–65 µg/kg bw (Murphy et al., 1978; DeSimone, Greco & Lessner, 1979; DeSimone et al., 1986).

In historical outbreaks of illness associated with *Fusarium* species where 4,15-DAS was investigated, 4,15-DAS was not detected (Beardall & Miller, 1994; RIVM, 2002).

10.5 Analytical methods

The Committee reviewed and identified specific analytical issues associated with the screening and quantification of 4,15-DAS and modified[14] forms of 4,15-DAS, including 4,15-DAS metabolites in human biomarker studies.

Several screening tests using antibodies have been established for the detection of 4,15-DAS (Hack, Klaffer & Terplan, 1989). However, most of the immunoassays for trichothecenes have moderate or strong cross-reactivity with closely related compounds (Tangni et al., 2010). Results from ELISA tests should always be confirmed using quantitative chromatographic methods. For quantification, 4,15-DAS is commonly extracted from the food matrix by acetonitrile/water or methanol/water; however, dilute-and-shoot extraction in combination with LC-MS/MS is more often used in more recently described methods (Lopez et al., 2016).

Modified forms of 4,15-DAS – 15-MAS-3-glucoside, 15-MAS-4-glucoside and DAS-3-glucoside – were identified in a maize reference material by LC-Orbitrap-MS (Nakagawa et al., 2013b). The degradation product DAS-M1 was identified by LC-high-resolution MS after heating 4,15-DAS in an aqueous solution, and it was quantified by LC-MS/MS (Shams et al., 2011). In human biomonitoring studies, 4,15-DAS was analysed in urine by either LC-MS/MS (Heyndrickx et al., 2015) or GC-MS/MS (Rodriguez-Carrasco et al., 2014c).

The main issue related to analytical methods for the quantification of 4,15-DAS is that 4,15-DAS is usually detected as one of many mycotoxins using multi-mycotoxin methods. This means that the LOQ can vary considerably between methods, possibly resulting in many left-censored data, directly increasing exposure assessment uncertainty.

A challenge to the analysis of 4,15-DAS in food and feed is the lack of harmonized methods, performance criteria for analytical methods, certified reference materials and proficiency tests. Analysis of modified forms of 4,15-DAS, including metabolites, has the additional challenge that not all analytical standards are currently available.

[14] The term "modified" is used to refer to covalently bound metabolites that are produced by fungi, formed through an interaction between 4,15-DAS and matrix constituents in a plant or during food processing, or metabolized in the human or animal body.

10.6 Sampling protocols

No published information on sampling protocols specifically for 4,15-DAS was found. However, as for other trichothecene mycotoxins, it is assumed that 4,15-DAS will be distributed unevenly in a batch of raw materials.

Although no sampling protocols specific to 4,15-DAS were found, some generic guidelines on sampling of mycotoxins are available. The FAO Mycotoxin Sampling Tool on sampling protocols, developed for both food analysts and regulatory officials, can be used (http://tools.fstools.org/mycotoxins/), and sampling protocols are available from the Codex Alimentarius Commission standard CODEX STAN 193-1995 (CAC, 1995). Furthermore, the European Union has sampling protocols for the purpose of official control of the levels of mycotoxins in foodstuffs, as described in Regulation (EC) No. 401/2006 and its amendments (European Union, 2006, 2014).

10.7 Effects of processing

Reports on the effects of food processing on the occurrence of 4,15-DAS in foods were evaluated by the Committee. No papers were found on the distribution of 4,15-DAS in the fractions after sorting, cleaning and milling of cereals. One paper reported that 4,15-DAS in aqueous solution was hydrolysed to DAS-M1 after thermal treatment.

10.8 Prevention and control

There is little information available on specific intervention measures to prevent 4,15-DAS contamination. As 4,15-DAS is produced by *Fusarium* species, management strategies to prevent contamination of crops with 4,15-DAS may focus on prevention of *Fusarium* infection and growth in the whole production chain and decontamination procedures of harvested crops.

Preharvest measures to reduce *Fusarium* infection focus on careful consideration of management strategies and plant protection products to keep plants healthy and the *Fusarium* inoculum low (Jouany, 2007). Soil type (VKM, 2013) and tillage (Oldenburg, Valenta & Sator, 2000) influence survival and propagation of *Fusarium*. Management practices that aim at healthy plants should be implemented by sowing and harvesting at the appropriate time (Jouany, 2007; Eeckhout et al., 2013), careful use of fertilizer (Hofer et al., 2016) and irrigation (Ferrigo, Raiola & Causin, 2014). Use of plant protection products should be carefully considered, as they are not always effective against *Fusarium* (da Cruz Cabral, Pinto & Patriarca, 2013). Currently, several biocontrol practices are

under development aiming at either outcompeting toxigenic *Fusarium* species (Ng et al., 2015) or inhibiting biosynthesis of mycotoxins (Pagnussatt et al., 2014). Crop rotation with non-host crops such as beets, onions, beans, clover, alfalfa, vegetables or chicory will prevent build-up of inoculum (Eeckhout et al., 2013). Use of existing crop cultivars resistant to *Fusarium* may reduce fungal infection and possibly 4,15-DAS contamination of the crop (Goral et al., 2015).

No literature was identified on postharvest strategies to prevent 4,15-DAS contamination, other than a small number of papers on decontamination treatments. Irradiation (Kottapalli, Wolf-Hall & Schwarz, 2006), thermal treatment (Shams et al., 2011) and chemical decontamination (Young, Zhu & Zhou, 2006) showed effects on 4,15-DAS decontamination under experimental settings. Biodecontamination of feed can be carried out using microorganisms (or enzymes) and adsorbents, which, when added to feed, can reduce the bioavailability of 4,15-DAS (EFSA, 2009).

10.9 Levels and patterns of contamination in food commodities

The Committee evaluated data on 4,15-DAS contamination in food that were submitted to the GEMS/Food contaminants database and that were derived from about 80 papers published mainly between 2000 and 2016.

The number of data submitted to the GEMS/Food contaminants database on the occurrence of 4,15-DAS in food was relatively low (16 845 records), and only 2.3% of the records had positive data (above the LOD). The main food commodities reported to be contaminated with 4,15-DAS were cereals and cereal-based foods. Few records on other food commodities were found, and generally no 4,15-DAS was detected in those other food commodities (specified as not detected or below the LOD). The highest prevalence of 4,15-DAS was found in sorghum from Africa (14% from 1083 records), with a highest value of 109 µg/kg. No 4,15-DAS was detected in samples from the Americas (2400 records). Less than 1% (0.6%) of the samples from the Western Pacific Region were contaminated with 4,15-DAS, with the highest level of 8 µg/kg in potato chips. A prevalence of 4% occurred in samples from the Eastern Mediterranean Region, with analyses only for sorghum. In Europe, the prevalence of 4,15-DAS in food was 1.5%, mainly in cereals, as well as one sample of cereal-based food for infants and one snack food sample.

Data from the scientific literature confirmed the low prevalence of 4,15-DAS in food and the relative importance of cereals. Results were mainly from European countries, which reported low prevalence and low concentrations of 4,15-DAS (Schollenberger et al., 2012). In Spain, a high prevalence of 4,15-DAS and high contamination were detected in coffee (non-specified), with levels up

to 400 μg/kg (Garcia-Moraleja et al., 2015a). Very few publications were found for the Americas, except a few papers published before the year 2000. Some papers were found on the occurrence of 4,15-DAS in the Western Pacific Region: low concentrations (<5 μg/kg) and a prevalence of 20% for corn, wheat and barley in Japan (Tamura et al., 2015); and high concentrations (up to 1000 μg/kg) for maize in New Zealand (Hussein et al., 1989). A low background level of 4,15-DAS was found in maize, wheat and barley in Africa (range of prevalence 5–10%), with levels up to 97 μg/kg (Serrano et al., 2012). The prevalence of 4,15-DAS in food samples from India was low, except for sorghum (prevalence of 43%, concentrations up to 70 μg/kg) (Lincy et al., 2008). One paper from Pakistan reported a 10% prevalence of 4,15-DAS in maize samples, with a mean concentration of 500 μg/kg (Khatoon et al., 2012).

Feed samples from the Russian Federation showed high concentrations of 4,15-DAS (up to 490 μg/kg) and high prevalence (up to 90%). However, these results were from one paper using an ELISA method with no confirmatory analysis (Kononenko et al., 2015).

It can be concluded that the prevalence and contamination level of 4,15-DAS in food samples from various regions in the world are low, based on the results from both the GEMS/Food contaminants database and the scientific literature. Reports of high prevalence and high contamination levels of 4,15-DAS were mainly published before 2000. The main food group contributing to the occurrence of 4,15-DAS is cereals, and most reports are on sorghum. The other food group in which 4,15-DAS was occasionally detected was coffee.

No reports on transfer of 4,15-DAS from feed to food of animal origin were found.

10.10 Food consumption and dietary exposure assessment

The Committee reviewed national dietary exposures to 4,15-DAS from the literature and calculated international estimates of dietary exposure for a number of regions in the world using concentration data from the GEMS/Food contaminants database and consumption data from the GEMS/Food cluster diets.

Some estimates of mean dietary exposure were reported in published papers, such as 15 ng/kg bw per day in Finland (SCOOP, 2003) for consumption of cereals, between 1 and 8.5 ng/kg bw per day in Spain for consumption of cereals by adults and children (Rodríguez-Carrasco et al., 2013), between 0.3 and 0.9 ng/kg bw per day in Spain for consumption of coffee by adolescents and adults (Garcia-Moraleja et al., 2015a), 24.7 ng/kg bw per day in Tunisia for consumption of cereals (Serrano et al., 2012) and 0.1 ng/kg bw per day in Morocco for consumption of rice (Serrano et al., 2012). The estimates for Finland

and Tunisia include the major sources of dietary exposure; however, this is not the case for Morocco, where consumption of rice is very low, and for Spain, where there was no occurrence at all in cereals.

For the international estimates of dietary exposure to 4,15-DAS, considering the very high proportion of non-detected analytical results for 4,15-DAS in foods (from 86% for Africa up to 100% for the Americas), an LB–UB approach was taken by the Committee. Moreover, considering the relatively low number of food commodities with quantified data from all over the world, it was decided to calculate the dietary exposure only for WHO regions for which data were available, and not for all cluster diets (Table 16). The WHO regions analysed for which both concentration data and consumption data were available were Africa (G13 cluster diet with sorghum), Eastern Mediterranean (G13 cluster diet with sorghum), Europe (average of G07, G08, G11, G15 cluster diets with all 18 GEMS/Food commodities), Western Pacific (G10 cluster diets with all 18 GEMS/Food commodities) and the Americas (G10 cluster diet with cereals, food for infants, legumes and pulses, nuts and oilseeds, starchy roots).

In order to limit the uncertainty in its estimates, the Committee decided to refine the UB estimates, taking into consideration the number of food commodities for which concentration data were available from WHO regions. Therefore, three scenarios for exposure to 4,15-DAS were calculated using this UB tiered approach for WHO regions when this was possible (e.g. Africa, Eastern Mediterranean, Europe and Western Pacific).

The best refined international LB–UB mean (high) exposure estimates for adults were 1.4–5 ng/kg bw per day (2.8–10 ng/kg bw per day) for Africa, 0–154 ng/kg bw per day (0–308 ng/kg bw per day) for the Americas (these results have a high level of uncertainty due to no detections at all), 0.4–4 ng/kg bw per day (0.8–8 ng/kg bw per day) for the Eastern Mediterranean, 2.8–41 ng/kg bw per day (5.6–82 ng/kg bw per day) for Europe and 0.4–6.5 ng/kg bw per day (0.8–13 ng/kg bw per day) for the Western Pacific. The Committee noted that the very high degree of censorship (below LOD/LOQ) in the concentration dataset and the relatively high LOQs (particularly for the Americas) have a considerable influence on the results. Thus, there is substantial uncertainty in the estimated dietary exposures, and these need to be interpreted with caution.

10.11 Dose–response analysis

Owing to limitations in the study design of the few available studies on 4,15-DAS, these studies were considered to be inadequate for dose–response modelling.

Table 16
International estimates of exposure to 4,15-DAS via food for adults[a]

WHO Region	LB mean exposure (ng/kg bw per day)	UB mean exposure (scenario 1/2/3) (ng/kg bw per day)	LB–UB P90 exposure[b] for scenario 1/2/3 (ng/kg bw per day)	Left-censorship (%)
Africa (Burkina Faso, Ethiopia, Mali)	1.4	20.3/20.3/5	2.8–40.6/40.6/10	86
Americas (Canada)	0	154/na/na	0–308/na/na	100
Eastern Mediterranean (Sudan)	0.4	17/17/4	0.8–34/34/8	96
Europe (Czech Republic, Finland, France, Germany, Slovenia, United Kingdom)	2.8	363/69/41	5.6–726/138/82	98.3
Western Pacific (China [Hong Kong SAR], Japan, New Zealand)	0.4	239/57/6.5	0.8–478/114/13	99.4

bw: body weight; na: not able to be calculated; LB: lower bound; P90: 90th percentile; SAR: Special Administrative Region; UB: upper bound
[a] Body weight used is 60 kg.
[b] P90 exposure is estimated by the Committee as twice the mean exposure.
Source: FAO/WHO (2009).

11. Evaluation

The Committee concluded that there are insufficient toxicological data available to derive a point of departure for the risk assessment of 4,15-DAS alone. There are limitations in the available short-term toxicity studies and no data from chronic exposure and reproductive and developmental toxicity studies.

4,15-DAS and T-2/HT-2 toxin are structurally similar, and there is evidence that they cause similar effects at the biochemical and cellular levels, have similarities in toxic effects in vivo and have an additive dose effect when co-exposure occurs. Therefore, the evidence was considered sufficient by the Committee to support including 4,15-DAS in the group PMTDI for T-2 and HT-2 toxin established at the fifty-sixth meeting of JECFA (Annex 1, reference *152*). The PMTDI of 0.06 µg/kg bw for T-2 and HT-2 toxin, alone or in combination, was established based on a LOAEL of 0.03 mg/kg bw per day associated with changes in WBC counts following 3 weeks of dietary exposure in pigs (Rafai et al., 1995) and the application of an uncertainty factor of 500. The inclusion of 4,15-DAS in the group PMTDI of 0.06 µg/kg bw is considered to be a conservative approach when taking into consideration the observation that T-2 toxin was consistently more potent than 4,15-DAS when comparing similar in vitro and in vivo end-points.

The Committee noted that there is a paucity of occurrence data for 4,15-DAS and that what data were available to the Committee were frequently left-censored and had relatively high LOQs, thereby increasing the uncertainty in the dietary exposure assessment.

In the 2001 JECFA evaluation, the total dietary exposure to T-2 toxin and HT-2 toxin was estimated only from the GEMS/Food European diet owing to the fact that data on these toxins were not available from regions other than Europe. The total LB mean dietary exposure to T-2 toxin plus HT-2 toxin was estimated to be 16.3 ng/kg bw per day, with wheat, barley and oats being the major dietary sources (Annex 1, reference *152*).

The Committee noted that only LB dietary exposure estimates for Europe were available for the sum of T-2, HT-2 and 4,15-DAS. From these estimates, the sum of the LB dietary exposure estimates for 4,15-DAS of up to 0.0028 µg/kg bw per day and the total dietary exposures estimated for T-2 plus HT-2 of 0.016 µg/kg bw per day results in a LB mean dietary exposure of 0.019 µg/kg bw per day and in a LB high dietary exposure estimated at 0.038 µg/kg bw per day (twice the mean; FAO/WHO, 2009). It was not possible to estimate the UB dietary co-exposure because of the lack of UB data reported for T-2 and HT-2 toxins in the previous 2001 JECFA evaluation together with the substantial uncertainty that is reported for UB estimates of dietary exposure to 4,15-DAS. The Committee concluded that these LB estimates for Europe do not exceed the group PMTDI for T-2, HT-2 and 4,15-DAS.

11.1 Recommendations

The Committee was made aware of new toxicity studies on T-2/HT-2 toxin and therefore recommends an update of the 2001 JECFA evaluation of T-2/HT-2 toxin.

In addition, studies are needed to address the relative potencies of 4,15-DAS and T-2/HT-2 toxin, the species differences with regard to bioavailability following oral exposure, the potential for chronic toxicity from exposure to concentrations in the diet, and the potential for reproductive and developmental toxicity of 4,15-DAS.

The Committee recommends improving the LOQs for 4,15-DAS, particularly when developing multi-mycotoxin methods.

The Committee encourages the development of analytical standards, suitable certified reference materials and proficiency tests to support the analysis of 4,15-DAS and its modified forms, including biomarkers.

The Committee recommends that more food commodities be analysed using methods with appropriate sensitivity that would allow the refinement of its estimates of dietary exposure to DAS, T-2 and HT-2 from all regions.

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Appendix 1

Table A1-1
4,15-DAS levels in cereals[a]

Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean	Min.	Max.	Median	Reference
African Region											
Maize	Nigeria	LC-MS	180	9	–	20	16	1	51	–	Adejumo, Hettwer & Karlovsky (2007)
Maize	Nigeria	GC-MS	106	0	6	–	–	–	–	–	Bankole Schollenberger & Drochner (2010)
Stored maize	Nigeria	LC-MS/MS	70	18.6	0.4	–	10	3	30	–	Adetunji et al. (2014)
Region of the Americas											
Barley	Canada	GC-MS	14	0	–	50	–	–	–	–	Clear et al. (1997)
Barley	Canada	GC-MS	116	0	–	100	–	–	–	–	Campbell et al. (2000)
Corn	Brazil	GC-MS	80	0	40	180	–	–	–	–	Milanez & Valente-Soares (2006)
Corn	Canada	GC-MS	673	0.4	–	100	–	490	1 000	–	Campbell et al. (2000)
Corn-based products	Brazil	GC-MS	78	0	20–60	60–550	–	–	–	–	Milanez, Valente-Soares & Baptista (2006b)
Maize	Mexico	TLC 2D	20	0	–	–	–	–	–	–	Robledo, Marin & Ramos (2001)
Maize	Mexico	LC-MS/MS	2	50	–	–	–	–	–	–	Pena Betancourt et al. (2015)
Oats	Canada	GC-MS	73	0	–	100	–	–	–	–	Campbell et al. (2000)
Wheat	Argentina	TLC	261	10	500	–	792	–	–	–	Quiroga et al. (1995)
Wheat	Argentina	GC-ECD	120	0	–	–	–	–	–	–	Gonzalez et al. (2008)
Wheat	Brazil	GC	20	5	100	–	600	–	–	–	Furlong et al. (1995)
Wheat	Canada	GC-MS	53	37.7	–	50	–	–	<80	–	Abramson, Clear & Nowicki (1987)
Wheat	Canada	GC-MS	99	0	–	100	–	–	–	–	Campbell et al. (2000)
South-East Asia Region											
Barley	India	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)
Maize	India	HPLC	2	0	10	40	–	–	–	–	Lincy et al. (2008)
Maize	Pakistan	TLC	65	9.2	–	250	516	364	750	–	Khatoon et al. (2012)

Table A1-1 (continued)

Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean	Min.	Max.	Median	Reference
Rice	India	HPLC	5	0	10	40	–	–	–	–	Lincy et al. (2008)
Rice	India	HPLC	50	16	–	–	–	100	200	–	Sempere Ferre (2016)
Sorghum	India	HPLC	14	43	10	40	–	14	70	–	Lincy et al. (2008)
Wheat	India	HPLC	2	0	10	40	–	–	–	–	Lincy et al. (2008)
European Region											
Grains	Croatia	TLC	465	27.6	100	–	200	100	1 200	–	Sokolovic & Simpraga (2006)
Cereal-based food	Germany	GC-MS/MS	84	0	14	–	–	–	–	–	Schollenberger, Muller & Drochner (2005)
Maize, oat, rice, rye, spelt, wheat	Italy	LC-MS/MS	48	4.2	–	5	–	65	84	–	Serrano et al. (2012)
Oat, barley, spelt, rye, wheat	Italy	LC-MS/MS	93	0	4	10	–	–	–	–	Juan, Ritieni & Manes (2013)
Wheat, rye + barley	Lithuania	GC-MS	84; 46; 29	0.5	–	–	<5	–	–	–	Keblys, Flaoyen & Langseth (2000)
Barley, wheat, oats	Norway	GC-MS	449	0.2	–	–	<4	–	–	–	Langseth & Rundberget (1999)
Grains	Poland	GC-MS	99	12	–	–	23	–	111	–	Perkowski & Basinski (2002)
Grain	Slovenia	GC-MS	66	0	50	100	–	–	–	–	Jakovac-Strajn et al. (2010)
Cereal	United Kingdom	GC-MS	27	0	–	10	–	–	–	–	SCOOP (2003)
Flour	United Kingdom	GC-MS	8	0	–	10	–	–	–	–	SCOOP (2003)
Barley	Austria	LC-MS	5	0	–	50	–	–	–	–	Razzazi-Fazeli et al. (2002)
Barley	Finland	GC-MS	77	0	5–25	–	–	–	–	–	SCOOP (2003)
Barley malt	Finland	GC-MS	68	0	25	–	–	–	–	–	SCOOP (2003)
Malting barley	France	GC-MS	194	0	30	–	–	–	–	–	SCOOP (2003)
Barley	Germany	GC-MS	240	0	1–5	–	–	–	–	–	Muller et al. (1997a)
Barley	Poland	GC-MS	15	73	–	–	12	–	–	–	Perkowski, Kiecana & Kaczmarek (2003)
Barley	United Kingdom	GC-MS	446	0	–	10	–	–	–	–	Edwards et al. (2009b)
Maize	Austria	LC-MS	5	0	–	50	–	–	–	–	Razzazi-Fazeli et al. (2002)
Corn	France	GC-MS	111	41	20	30	–	<30	<30	–	SCOOP (2003)
Corn	Germany	GC-MS/MS	41	12	14	–	49	–	76	–	Schollenberger et al. (2006)
Maize (whole)	Germany	GC-MS	9	0	–	–	–	–	–	–	Schollenberger, Muller & Drochner (2005)

Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean	Min.	Max.	Median	Reference
Maize flour	Germany	GC-MS	15	0	–	–	–	–	–	–	Schollenberger, Muller & Drochner (2005)
Maize kernel	Germany	GC-MS	24	16.6	–	–	21	–	–	–	Schollenberger, Muller & Drochner (2005)
Semolina	Germany	GC-MS	14	0	–	–	–	–	–	–	Schollenberger, Muller & Drochner (2005)
Maize flour	Italy	LC-MS/MS	1	100	4.5	14	25	–	–	–	Gentili et al. (2007)
Maize oil	Italy	LC-MS/MS	1	0	0.5	1.5	–	–	–	–	Gentili et al. (2007)
Maize	Netherlands	LC-MS/MS	42	5	0.75	2.5	–	<2.5	–	–	Van Asselt et al. (2012)
Corn	Poland	LC-ESI-MS/MS	57	<30	–	1.3	3.6–5.9 (depending on varieties)	–	–	–	Aniolowska & Steininger (2014)
Maize	Romania	LC-EIA	30	3.3	2	–	2.6	–	–	–	Curtui et al. (1998)
Maize	Spain	GC-MS/MS	17	0	2.5	–	–	–	–	–	Rodriguez-Carrasco et al. (2013)
Corn product	United Kingdom	GC-MS	70	0	–	10	–	–	–	–	SCOOP (2003)
Polenta	United Kingdom	GC-MS	8	0	–	10	–	–	–	–	SCOOP (2003)
Oats	Austria	LC-MS	5	40	–	50	–	traces	–	–	Razzazi-Fazeli et al. (2002)
Oats	Finland	GC-MS	72	0	25	–	–	–	–	–	SCOOP (2003)
Oats	Germany	GC-MS/MS	17	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Oat flakes	Germany	LC-MS/MS	43	85	–	–	0.1	–	–	–	Gottschalk et al. (2007)
Oats	Germany	GC-MS	272	0	–	–	–	–	–	–	Muller et al. (1998)
Oats	Poland	LC-MS/MS	178	20	–	–	3.2	–	20.8	–	Twaruzek et al. (2013)
Oats	United Kingdom	GC-MS	458	0	–	10	–	–	–	–	Edwards et al. (2009a)
Rice	Spain	GC-MS/MS	23	0	2.5	–	–	–	–	–	Rodriguez-Carrasco et al. (2013)
Rice	United Kingdom	GC-MS	100	0	–	10	–	–	–	–	SCOOP (2003)
Rye	Finland	GC-MS	33	0	5–25	–	–	–	–	–	SCOOP (2003)
Sorghum	Belgium	LC-MS/MS	8	62.5	1.2	2.5	–	7	66	–	Njumbe Ediage, Van Poucke & De Saeger (2015)
Sorghum	Germany	LC-MS/MS	2	2	1.2	2.5	–	6	91	–	Njumbe Ediage, Van Poucke & De Saeger (2015)
Wheat	Austria	LC-MS	5	0	–	50	–	–	–	–	Razzazi-Fazeli et al. (2002)

Table A1-1 (continued)

Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean	Min.	Max.	Median	Reference
Wheat	Finland	GC-MS	134	0	-	50	–	–	–	–	SCOOP (2003)
Bread	France	GC-MS	14	7	3	10	–	<LOQ	<LOQ	–	ANSES (2011)
Durum wheat	France	GC-MS	97	0	30	60	–	–	–	–	SCOOP (2003)
Soft wheat	France	GC-MS	562	58	30	60	–	<30	<30	–	SCOOP (2003)
Wheat	France	GC-MS	52	100	–	20	–	<25	<25	–	SCOOP (2003)
Wheat	Germany	GC-MS	445	0	–	–	–	–	–	–	Muller et al. (1997b)
Wheat	Germany	GC-MS	276	0	–	–	–	–	–	–	Muller et al. (2001)
Wheat	Germany	GC-MS/MS	41	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Wheat	Italy	LC-ESI-MS	14	21	20	100	<100	–	–	–	Dall'Asta et al. (2004)
Wheat	Italy	LC-MS/MS	28	28	1	–	1.9	0.1	13.1	0.2	Lattanzio et al. (2013)
Wheat grain	Italy	LC-MS/MS	74	0	15	30	–	–	–	–	Juan et al. (2016)
Wheat	Netherlands	LC-MS	86	0	–	5	–	–	–	–	Van des Fels-Klerx et al. (2012)
Wheat	Romania	LC-EIA	25	0	2	–	–	–	–	–	Curtui et al. (1998)
Wheat	Spain	LC-MS	20	0	1.5	5	–	–	–	–	Sospedra et al. (2010)
Wheat-based food	Spain	GC-MS/MS	119	0	2.5	–	–	–	–	–	Rodriguez-Carrasco et al. (2013)
Biscuits	United Kingdom	GC-MS	54	0	–	10	–	–	–	–	SCOOP (2003)
Bread	United Kingdom	GC-MS	56	0	–	10	–	–	–	–	SCOOP (2003)
Wheat	United Kingdom	GC-MS	1 624	0	–	10	–	–	–	–	Edwards et al. (2009c)
Wheat flour	United Kingdom	GC-MS	29	0	–	10	–	–	–	–	SCOOP (2003)
Wheat product	United Kingdom	GC-MS	95	0	–	10	–	–	–	–	SCOOP (2003)
Eastern Mediterranean Region											
Barley, sorghum, wheat, grains from maize and wheat	Tunisia	LC-MS/MS	52	6	–	5	–	6.4	97	–	Serrano et al. (2012)
Corn-based food: yellow corn	Egypt	HPLC	50	10	–	–	11.3	9.8	12.9	–	El-Sayed, Soher & Sahab (2003)
Corn-based food: white corn	Egypt	HPLC	7	14	–	–	22.1	–	–	–	El-Sayed, Soher & Sahab (2003)
Corn	Israel	LC-MS/MS	15	0	0.3	–	–	–	–	–	Shimshoni et al. (2013)

Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean	Min.	Max.	Median	Reference
Rice	Morocco	LC-MS/MS	70	0	–	5	–	–	–	–	Serrano et al. (2012)
Wheat	Israel	LC-MS/MS	15	0	0.3	–	–	–	–	–	Shimshoni et al. (2013)
Western Pacific Region											
Barley tea	Japan	LC-ESI-MS/MS	5	0	–	70 ng/L	–	–	–	–	Suga et al. (2004)
Barley	Japan	LC-MS	9	22	–	5	–	–	<LOQ	–	Tamura et al. (2015)
Corn	Japan	LC-MS	18	28	–	5	–	–	<LOQ	–	Tamura et al. (2015)
Maize	New Zealand	GC-MS TLC	20	30	–	–	–	–	1 000	–	Hussein et al. (1989)
Wheat	Japan	LC-MS	12	17	–	5	–	–	<LOQ	–	Tamura et al. (2015)

ECD: electron capture detector; EIA: enzyme-linked immunoassay; ESI: electrospray ionization; Food: food commodity; GC: gas chromatography; HPLC: high-performance liquid chromatography; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum concentration; Mean: mean concentration; Min.: minimum detected concentration; MS: mass spectrometry; MS/MS: tandem mass spectrometry; No.: number of samples analysed; TLC: thin-layer chromatography; WHO: World Health Organization

[a] Data drawn from open literature.

[b] Analytical method used for quantification.

[c] Number of samples analysed for 4,15-DAS.

[d] Percentage of samples with 4,15-DAS levels above the LOQ.

Table A1-2
4,15-DAS levels in baby food, unspecified [a]

Food commodity	Country	Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
Baby food	Italy	LC-MS/MS	62	0	2	10	–	–	–	–	Juan et al. (2014)
Infant cereal food	Spain	LC-ESI-MS/MS	57	0	15	50	–	–	–	–	Lu et al. (2013)
Infant formula milk	Italy	LC-MS/MS	13	0	2	10	–	–	–	–	Juan et al. (2014)

ESI: electrospray ionization; LC: liquid chromatography; MS/MS: tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum concentration; Mean: mean concentration; Min.: minimum detected concentration; No.: number
[a] Data drawn from open literature.
[b] Analytical method used for quantification.
[c] Number of samples analysed for 4,15-DAS.
[d] Percentage of samples with 4,15-DAS levels above the LOQ.

Table A1-3
4,15-DAS levels in foods other than cereals and baby food[a]

WHO Region / Country /Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
European Region											
Beer											
Netherlands	Beer	GC-FID	51	0	8	25	–	–	–	–	Schothorst et al. (2003)
Europe	Beer	GC-MS/MS	154	0	4	8	–	–	–	–	Rodriguez-Carrasco et al. (2015)
Coffee											
Spain	Caffeinated coffee	LC-MS/MS	52	44	3	5	6.5	5.7	8.5	–	Garcia-Moraleja et al. (2015b)
Spain	Decaffeinated coffee	LC-MS/MS	40	0	3	5	–	–	–	–	Garcia-Moraleja et al. (2015b)
Spain	Milk added coffee	LC-MS/MS	11	0	3	5	–	–	–	–	Garcia-Moraleja et al. (2015b)
Spain	Natural coffee	LC-MS/MS	23	0	3	5	–	–	–	–	Garcia-Moraleja et al. (2015b)
Spain	Torrefacto coffee	LC-MS/MS	69	24.6	3	5	6.5	5.7	8.5	–	Garcia-Moraleja et al. (2015b)
Spain	Coffee	LC-MS/MS-IT	169	7	1.38	5.99	196	195	402	–	Garcia-Moraleja et al. (2015a)

WHO Region / Country	/Food	Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
Pulses											
Germany	Fruits / vegetables	GC-MS/MS	85	1.2	14	–	–	21	–	–	Schollenberger, Muller & Drochner (2005)
Germany	Peas	GC-MS/MS	25	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Soy food (kernel)	GC-MS/MS	45	2.2	14	–	–	21	–	–	Schollenberger et al. (2007a)
Germany	Soy meal	GC-MS/MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Soybean and corn oil	GC-MS	98	0	3	–	–	–	–	–	Schollenberger et al. (2008)
Germany	Sunflower oil	GC-MS	12	8.3	3	–	–	12	–	–	Schollenberger et al. (2008)
Turkey	Processed cereals and pulse products	HPLC then GC-MS	85	0	–	–	–	–	–	–	Omurtag et al. (2007)
Other foods											
Spain	Tiger nuts	LC-MS/MS	83	0	1.5	4	–	–	–	–	Rubert, Soler & Manes (2012)
Region of the Americas											
Beer											
Argentina	Beer	GC	50	0	–	–	–	–	–	–	Molto et al. (2000)
Pulses											
USA	Meal (soybean)	GC-MS	17	35	10	–	–	<10	130	–	Jacobsen et al. (1995)
USA	Oil (soybean)	GC-MS	3	0	10	–	–	–	–	–	Jacobsen et al. (1995)
USA	Whole soybean	GC-MS	20	25	10	–	–	15	230	–	Jacobsen et al. (1995)
Other foods											
USA	Water (streams)	LC-MS/MS	116	5	33 ng/L	–	–	–	<LOD	–	Kolpin et al. (2014)
Eastern Mediterranean Region											
Other foods											
Egypt	Sugar cane	TLC	40	some	–	–	–	–	–	–	Abd-Elaah & Soliman (2005)
South-East Asia Region											
Other foods											
India	Banana	HPLC-TLC	–	–	–	–	14 000	–	–	–	Chakrabarti & Ghosal (1986)

Table A1-3 (continued)

WHO Region / Country / Food		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
Spices											
India	Chilli	HPLC	2	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Turmeric	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Coriander	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Ginger	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Pepper	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Chilli powder	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)

2D: two-dimensional; ESI: electrospray ionization; FID: flame ionization detection; Food: food commodity; GC: gas chromatography; HPLC: high-performance liquid chromatography; IT: ion trap analyser; LC: liquid chromatography; MS: mass spectrometry; MS/MS: tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum concentration; Mean: mean concentration; Min.: minimum detected concentration; No.: number; TLC: thin-layer chromatography; WHO: World Health Organization

[a] Data drawn from open literature.

[b] Analytical method used for quantification.

[c] Number of samples analysed for 4,15-DAS.

[d] Percentage of samples with 4,15-DAS levels above the LOQ.

Table A1-4
4,15-DAS levels in feed[a]

WHO Region / Country / Feed		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
European Region											
Alfalfa											
Russian Federation	Alfalfa	ELISA	5	40	–	–	–	110	200	–	Kononenko et al. (2015)
Russian Federation	Alfalfa timothy mixture	ELISA	5	60	–	–	210	160	250	–	Kononenko et al. (2015)
Clover											
Russian Federation	Clover	ELISA	15	90	–	–	165	79	345	–	Kononenko et al. (2015)
Russian Federation	Clover-grass mixture	ELISA	15	46	–	–	245	93	490	–	Kononenko et al. (2015)

WHO Region / Country / Feed		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
Grass											
Germany	Hay	GC-MS/MS	28	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Russian Federation	Grass	ELISA	9	22	–	–	–	100	170	–	Kononenko et al. (2015)
Russian Federation	Grass	ELISA	9	20	–	–	130	87	175	–	Kononenko et al. (2015)
Maize											
Germany	Corn by-products	GC-MS/MS	13	61	14	–	21	–	21	–	Schollenberger et al. (2006)
Germany	Corn plants	GC-MS/MS	8	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Corn silage	GC-MS/MS	5	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Maize cob	GC-MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2012)
Germany	Maize gluten	GC-MS	11	0	–	–	–	–	–	–	Schollenberger et al. (2005)
Germany	Maize husk	GC-MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2012)
Germany	Maize kernel	GC-MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2012)
Germany	Maize leaves	GC-MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2012)
Germany	Maize rudimentary ears	GC-MS	13	30	14	–	162	–	1 294	–	Schollenberger et al. (2012)
Germany	Maize silage	GC-MS	18	33	–	–	64	–	–	–	Schollenberger et al. (2005)
Germany	Maize stalks	GC-MS	13	0	14	–	–	–	–	–	Schollenberger et al. (2012)
Italy	Maize feed	LC-ESI-MS	7	28	20	–	–	276	847	–	Dall'Asta et al. (2004)
Other feed											
Germany	Lupine	GC-MS/MS	9	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Rapeseed meal	GC-MS/MS	12	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Oilseed by-products	GC-MS/MS	8	0	14	–	–	–	–	–	Schollenberger et al. (2006)
Germany	Other feed	GC-MS	15	46.6	–	–	21	–	–	–	Schollenberger et al. (2005)
Region of the Americas											
Grass											
Argentina	Grasses (from 2011)	LC-MS/MS	106	2	0.25	–	–	–	–	13.6	Nichea et al. (2015)
Argentina	Grasses (from 2014)	LC-MS/MS	69	3	0.25	–	–	–	–	6.49	Nichea et al. (2015)
Soybean											
USA	Hull (soybean)	GC-MS	17	70	10	–	–	<10	130	–	Jacobsen et al. (1995)

Table A1-4 (continued)

WHO Region / Country / Feed		Analytical method[b]	No. of samples[c]	% positive[d]	LOD (µg/kg)	LOQ (µg/kg)	Mean (µg/kg)	Min. (µg/kg)	Max. (µg/kg)	Median (µg/kg)	Reference
South-East Asia Region											
India	Cattle feed	HPLC	2	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Oil cake	HPLC	4	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Poultry feed	HPLC	2	0	10	40	–	–	–	–	Lincy et al. (2008)
India	Pet food	HPLC	1	0	10	40	–	–	–	–	Lincy et al. (2008)

2D: two-dimensional; ELISA: enzyme-linked immunosorbent assay; ESI: electrospray ionization; Feed: feed commodity; GC: gas chromatography; HPLC: high-performance liquid chromatography; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; MS: mass spectrometry; MS/MS: tandem mass spectrometry; Max.: maximum concentration; Mean: mean concentration; Min.: minimum detected concentration; No.: number; TLC: thin-layer chromatography; WHO: World Health Organization

[a] Data drawn from open literature.

[b] Analytical method used for quantification.

[c] Number of samples analysed for 4,15-DAS.

[d] Percentage of samples with 4,15-DAS levels above the LOQ.

Fumonisins (addendum)

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1. Explanation 417
2. Biological data 418
2.1 Biochemical aspects 419
2.1.1 Absorption, distribution and excretion 419
(a) Absorption 419
(b) Distribution 420
(c) Excretion 420
2.1.2 Biotransformation 421
2.1.3 Effects on enzymes and other biochemical parameters relevant to the mechanism of action 423
(a) Ceramide synthases 424
(b) Linking inhibition of ceramide synthase to biological effects 426
(c) Ceramide synthase inhibition and oxidative stress 429
(d) Linking downstream biochemical effects to adverse effects 430
(e) Linking biochemical and adverse effects to potential biomarkers of effect 431
2.2 Toxicological studies 432
2.2.1 Acute toxicity 433
2.2.2 Short-term studies of toxicity 434
(a) Studies using purified FB_1 434
(b) Studies using diets/treatments formulated with FB_1 prepared from partially purified fumonisins, *F. verticillioides* culture material or naturally contaminated maize 437
2.2.3 Long-term studies of toxicity and carcinogenicity 444
2.2.4 Genotoxicity 444

2.2.5 Reproductive and developmental toxicity 447
(a) In vivo studies using purified FB_1 dosed orally 447
(b) In vivo studies using intraperitoneal dosing 450
(c) In vitro studies using purified FB_1 452
2.2.6 Special studies 454
(a) Immunotoxicity 454
(b) Neurotoxicity 460
(c) Combined toxicity of fumonisins with other mycotoxins 463
2.3 Observations in domestic animals/veterinary toxicology 471
2.3.1 Pigs 472
2.3.2 Horses 472
2.3.3 Other farm animal species 472
2.4 Observations in humans 472
2.4.1 Biomarkers of exposure 472
(a) Urinary fumonisin B_1 (UFB_1) 473
(b) FB_1 in breast milk 477
(c) FB_1 in hair 478
2.4.2 Biomarkers of effects 479
2.4.3 Clinical observations in humans 480
2.4.4 Epidemiological studies 480
(a) Oesophageal cancer 481
(b) Hepatocellular cancer (HCC) 481
(c) Childhood growth impairment 482
3. Levels and patterns of contamination in food commodities 485
3.1 Surveillance data 485
3.2 Modified and bound fumonisins 504
4. Food consumption and dietary exposure assessment 507
4.1 Concentrations in food used in the dietary exposure estimates 507
4.1.1 FB_1 508
4.1.2 Total fumonisins 514
4.2 Food consumption data used in the dietary exposure assessments 515
4.3 Assessments of dietary exposure 515
4.3.1 National estimates from the scientific literature 515
(a) Brazil 524
(b) China (including Hong Kong SAR) 524
(c) France 526
(d) Guatemala 526
(e) Japan 527
(f) Malawi 527
(g) Netherlands 528
(h) Portugal 528
(i) Republic of Korea 529
(j) Spain 529
(k) United Republic of Tanzania 530
(l) Viet Nam 530
(m) Zimbabwe 531
(n) Summary 531
4.3.2 National estimates derived by the Committee 532
4.3.3 International estimates 536

5. Dose–response analysis and estimation of toxic/carcinogenic risk 540
5.1 Identification of key data for risk assessment 540
6. Comments 541
6.1 Biochemical aspects 541
6.2 Toxicological studies 543
6.3 Observations in domestic animals/veterinary toxicology 546
6.4 Observations in humans 547
6.5 Levels and patterns of contamination in food 548
6.6 Food consumption and dietary exposure estimates 549
6.7 Dose–response analysis 554
7. Evaluation 554
7.1 Recommendations 556
8. References 556

1. Explanation

Fumonisins are produced by *Fusarium verticillioides* (formerly *F. moniliforme*), *F. proliferatum* and *F. fujikuroi*, as well as some less common *Fusarium* species, for example *F. anthophilum*, *F. dlamini*, *F. napiforme* and *F. thapsinum* (Rheeder, Marasas & Vismer, 2002; Suga et al., 2014). Fumonisin B_2 (FB_2) and fumonisin B_4 (FB_4) are also produced by *Aspergillus niger* (Mogensen, Larsen & Nielsen, 2010). Fumonisins are common contaminants of maize and have also been found in rice.

The B-series of the fumonisins are modified sphingoid bases, including fumonisin B_1 (FB_1) (Chemical Abstracts Service [CAS] No. 116355-83-0), FB_2 (CAS No. 116355-84-1), fumonisin B_3 (FB_3) (CAS No. 136379-59-4) and FB_4 (CAS No. 136379-60-7), which are the major forms found in food and were described previously by the Committee at the seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA; Annex 1, reference *206*). There are also many other fumonisin analogues that can be classified into four main groups, A, B, C and P, which contain two tricarballylic acid (TCA) moieties. Members of the series FBX are different from these because they are esterified by other carboxylic acids, such as *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid. There are also fumonisin analogues that have their 19- or 20-carbon aminopolyhydroxyalkyl chain esterified by fatty acids, such as palmitic acid, linoleic acid and oleic acid. At the time of the 2011 evaluation, there were at least 28 FB_1 isomers that had been isolated and characterized. The hydrolysis of the tricarballylic esters at C-14 and C-15 gives rise to partially hydrolysed fumonisin B or totally hydrolysed fumonisin B in food.

Fumonisins were evaluated by JECFA for the first time at the fifty-sixth meeting (Annex 1, references *152* and *153*) and then re-evaluated at the seventy-fourth meeting (Annex 1, references *205* and *206*). At the seventy-fourth

meeting, the Committee used a short-term dose–response study of liver toxicity in male transgenic mice fed diets containing purified FB_1 (Bondy et al., 2010) to derive a group provisional maximum tolerable daily intake (PMTDI) for FB_1, FB_2 and FB_3, alone or in combination, of 2 µg/kg body weight (bw) on the basis of a lower 95% confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) of 0.165 mg/kg bw per day and an uncertainty factor of 100. Because the derived PMTDI at the seventy-fourth meeting of JECFA was the same as the group PMTDI established at the fifty-sixth meeting of JECFA, based on renal toxicity in a 90-day rat study, the group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, was retained at the seventy-fourth meeting.

Fumonisins were evaluated by the present Committee in response to a request from the Codex Committee on Contaminants in Foods (CCCF) for an updated exposure assessment. The Committee also evaluated toxicological studies that had become available since the previous evaluation in 2011.

A literature search was conducted to identify all available published data since 2010 using the University of Georgia Libraries Galileo databases and the University of Saskatchewan Electronic Library. The search terms included, singly or in combination, "fumonisin", "*Fusarium verticillioides*", "ceramide", "toxicology", "reproduction", "genotoxicity", "acute" and "chronic", among others. The literature search on the occurrence of and dietary exposure to fumonisins was run using three databases (Scopus, PubMed and Ovid) and a cut-off date of 2011.

2. Biological data

There have been several comprehensive authoritative reviews of the data describing the biological activity, toxicology and human epidemiological studies of fumonisins, and in particular FB_1. The fifty-sixth JECFA drew heavily on the World Health Organization (WHO) *Environmental Health Criteria 219: Fumonisin B_1* (IPCS, 2000; Annex 1, references *152* and *153*). Likewise, the seventy-fourth JECFA (Annex 1, reference *205*) drew heavily on the fifty-sixth JECFA and the 2002 International Agency for Research on Cancer (IARC) scientific monograph (IARC, 2002), which stated that FB_1 is possibly carcinogenic to humans (Group 2B).

As with previous evaluations, this review focuses on FB_1. However, studies using naturally contaminated material, fungal culture material or partially purified fungal culture material are included if they have been analysed for fumonisin content. It should be noted that when using either culture material or naturally contaminated foods, the term "fumonisins" includes fumonisins other

than B_1, B_2 and B_3. However, typically, FB_1 can be used as a marker for dietary exposure to fumonisins and other metabolites of fumonisin-producing *Fusarium* (Annex 1, references *205* and *206*).

The literature search to identify all available biological data published since 2010 was conducted using the University of Georgia Libraries Galileo databases and the University of Saskatchewan Electronic Library – specifically, the Thomson Reuters™ Web of Science BIOSIS Citation Index℠, MEDLINE with Full Text (EBSCO), University of Saskatchewan Electronic Library, Directory of Open Access Journals, WorldCat, Toxnet and PubMed (NCBI/NIH). The search terms included, singularly or in combination, were "fumonisin", "*Fusarium verticillioides*", "ceramide", "toxicology", "reproduction", "genotoxicity", "acute", "chronic" and others. There were approximately 1155 unique hits for "fumonisin" from BIOSIS, MEDLINE and PubMed. Titles and abstracts for all positive hits were printed and read, and 270 were deemed to contain information for possible inclusion in section 2 of this monograph, including section 2.4, on observations in humans.

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Since the 2011 JECFA review at the seventy-fourth meeting (Annex 1, reference *205*), only a few new studies describing the absorption, distribution and/or excretion of fumonisins have been published. These as well as information from previous reviews (IPCS, 2000; Annex 1, references *152*, *153*, *205* and *206*) are summarized briefly in the following paragraphs.

(a) Absorption

In all animal species studied, FB_1 absorption is rapid. However, the quantity of FB_1 detected in plasma and tissues after oral administration is very low (negligible to <4% of dose). The bioavailability of FB_2 and FB_3 may be less than that of FB_1. Feeding studies in pigs and rats have shown that FB_1 accumulates in the liver and kidney to a much greater extent than expected based on the relative amounts of FB_1, FB_2 and FB_3 in the diets. This finding is supported by the fact that pure FB_2 did not induce liver toxicity in mice when fed the same dose of FB_1 that induced liver toxicity (Howard et al., 2002). Reduced toxicity of pure FB_2 and FB_3 compared to pure FB_1 was also seen in chick embryos (Henry et al., 2001).

FB_1 does not penetrate the skin (Boonen et al., 2012).

A recent study in broiler chickens confirms the rapid absorption and low bioavailability of orally dosed FB_1 in animal models (Antonissen et al., 2015a).

The lower oral bioavailability of FB_2 has also been noted in ducks and turkeys (Benlashehr et al., 2011), similar to what is seen in rodents.

Hydrolysed FB_1, which can be formed during alkaline processing or in the gut due to microbial metabolism, is much more easily absorbed than FB_1. Hydrolysed FB_1 has also been detected in animal tissues. Although much more readily absorbed than FB_1, hydrolysed FB_1 is much less toxic than the parent compound in vivo (Grenier et al., 2012; Voss et al., 2013, 2017a; Masching et al., 2016).

(b) Distribution

Fumonisins are distributed to most tissues; however, the liver and kidney retain the highest concentrations. FB_1 persists in the kidney much longer than in plasma or the liver; in male Sprague Dawley and Wistar rats, the levels of FB_1 in the kidney can be 10 times the amount in the liver. The preferential accumulation of FB_1 in rat kidney compared with liver has been confirmed in more recent studies (Szabó-Fodor et al., 2008; Harrer, Humpf & Voss, 2015). There is little evidence that fumonisin, at environmentally relevant dosages, crosses the blood–brain barrier. For example, equine leukoencephalomalacia (ELEM) is believed to be a consequence of vascular deregulation (Annex 1, references *205* and *206*). Nonetheless, FB_1 does appear to cross the placenta and enter developing murine embryos, where it can disrupt sphingolipid metabolism (Gelineau-van Waes et al., 2012).

(c) Excretion

After intraperitoneal or intravenous dosing of FB_1, initial elimination from tissues is rapid with extensive enterohepatic recirculation. After oral dosing, peak plasma levels occur within one to several hours. Several studies, using different routes of exposure and different animal species, have shown that fumonisins are excreted primarily in faeces, either unchanged or with the loss of one or both of the TCA side-chains. Several human studies have shown high levels of fumonisin in faeces (most recently, Phoku et al., 2014). In a recent study in rats, Hahn et al. (2015) found that the relative recoveries of FB_1, partially hydrolysed FB_1 (loss of one TCA side-chain) and hydrolysed FB_1 (loss of both TCA side-chains) in the faeces of rats fed diets containing FB_1 at 10 mg/kg diet for 21 days were 93.8%, 5.9% and 0.3%, respectively.

Low levels of FB_1 can be detected in the urine of animals exposed experimentally to fumonisin, including rabbits, rats, pigs, horses, vervet monkeys and, most recently, mice (Riley et al., 2015a). In vervet monkeys, 0.25–1.5% of the oral FB_1 dose was recovered in urine, which is similar to the low levels of urinary excretion in studies with other animals (reviewed in van der Westhuizen

et al., 2013). As summarized in the previous evaluations (IPCS, 2000; Annex 1, references *152*, *153*, *205* and *206*), it has been estimated that pigs exposed to dietary FB_1 at 2–3 mg/kg bw would require a withdrawal period of at least 2 weeks for the FB_1 to be eliminated from the liver and kidney. Several studies have confirmed this finding using the persistence of free sphinganine or sphingoid base 1-phosphates as biomarkers of effect in the kidney, liver and, most recently, mouse blood spots (Riley et al., 2015a). In the mouse, elevated sphinganine and sphinganine 1-phosphate (Sa 1-P) can be detected for at least 5 days after the last dosing with FB_1 and return to a fumonisin-free diet. The urinary FB_1 (UFB_1) was below the limit of detection (LOD) at 72 hours post-dosing. Thus, although elevated levels of FB_1 are rapidly eliminated when animals are returned to FB_1-free diets, the biomarkers of effect can remain elevated for much longer. For example, the 2001 and 2011 evaluations included several studies in which rodents were dosed with high levels of FB_1 that caused large increases in urinary sphinganine. When the animals were transferred to fumonisin-free diets, the urinary sphinganine returned to undetectable levels after several days. However, if the animals were returned to diets with low levels of FB_1, the urinary sphinganine stayed elevated for much longer. Although the FB_1 half-life after oral dosing is not known for certain, the oral half-life in pigs is probably between 8 and 48 hours based on what is known from the parenteral routes, the time required to reach peak levels in plasma (1–7 hours) after gavage and the estimated time for complete clearance from the liver and kidney (2 weeks).

In humans consuming known amounts of fumonisins, UFB_1 is detected soon after exposure begins (first urine sample taken 2.75 hours post-consumption). The amount excreted peaks quickly but is highly variable, and decreases rapidly after consumption ceases (Riley et al., 2012). Total urinary excretion of FB_1 in humans was less than 1% (0.12–0.90%, n = 10) of the cumulative dose, a value similar to that reported in animal studies (reviewed in van der Westhuizen et al., 2013). The estimated half-life was less than 48 hours after consuming diets with fumonisins for 3 consecutive days, and greater than 48 hours but less than 72 hours when consuming for 6 consecutive days (Riley et al., 2012). FB_1 was excreted in the urine much more efficiently than FB_2 or FB_3 based on the relative levels of FB_1, FB_2 and FB_3 in the food consumed (Riley et al., 2012; Torres et al., 2014). Whether FB_1 is better absorbed from the human gut is unknown.

2.1.2 Biotransformation

There is no convincing evidence of fumonisin metabolism by cytochrome P450 (CYP), the microsomal esterase or any other microsomal enzyme. However, studies have shown that cytochrome P450 activity can be altered as a result of

the inhibition of ceramide synthase by fumonisin. For example, in a recent study in human hepatoma HepG2 cells, Chuturgoon, Phulukdaree & Moodley (2014a) showed that treatment with FB_1 at 200 μmol/L significantly downregulated expression of microribonucleic acid miR-27b, while CYP1B1 messenger RNA (mRNA) and protein expression were significantly upregulated. The authors concluded that FB_1-induced modulation of miR-27b may contribute to hepatic neoplastic transformation. However, it should be noted that the dose was the median inhibitory concentration (IC_{50}) for cell viability.

In another recent study using the rat liver hepatoma cell line H4IIE, FB_1 alone or in combination with aflatoxin B_1 increased *cyp*1A transcription and CYP1A activity and upregulated the aryl hydrocarbon receptor (Mary et al., 2015). The effects were seen at concentrations that were not cytotoxic.

These studies suggest that although there are no convincing data for FB_1 biotransformation via cytochrome P450, FB_1 can indirectly affect biotransformation of other compounds.

Fumonisins B_1 and B_2 are hydrolysed (one or both TCA side-chains removed) in the gut by microbial degradation. Both FB_1 and FB_2 and to a much lesser extent their hydrolysed counterparts can inhibit ceramide synthases based on elevation in the sphinganine/sphingosine (Sa/So) ratio and free sphinganine in vivo. While the TCA side-chains contribute to the ability of FB_1 to inhibit ceramide synthases, the primary amino group is required for inhibition, and fumonisin A_1 (*N* -acetylated FB_1) does not cause significant elevation in sphinganine or the Sa/So ratio in rat liver slices. These findings have been confirmed and extended in a recent in vivo study using male Sprague Dawley rats fed diets formulated with equimolar amounts (equivalent to FB_1 at 10 mg/kg diet) of highly purified FB_1, partially hydrolysed FB_1 (one TCA side-chain removed), fully hydrolysed FB_1 (both TCA side-chains removed) and *N*-(1-deoxy-D-fructos-1-yl) FB_1 (deoxy-D-fructose on the primary amino group of FB_1) (Hahn et al., 2015). The results show clearly that all of the investigated fumonisin derivatives were of much lower toxicological relevance than FB_1 based on the lack of any evidence of disrupted sphingolipid metabolism (elevated sphinganine or Sa/So ratio) in urine or kidney and no significant nephrotoxicity. It was also found that the *N*-(1-deoxy-D-fructos-1-yl) FB_1 could be partially metabolized to release free FB_1, presumably by microbial degradation in the gastrointestinal tract, and both the *N*-(1-deoxy-D-fructos-1-yl) FB_1 and FB_1 released in the gut were detected in the faeces and urine, but there was no significant effect on the Sa/So ratio in kidney or signs of nephrotoxicity.

Hydrolysed FB_1 is known to be a substrate for ceramide synthase, which converts it to *N*-acyl-hydrolysed FB_1 derivatives of differing fatty acyl chain lengths in vitro and in vivo. The *N*-acyl derivatives of hydrolysed FB_1 are more cytotoxic in vitro than FB_1 itself. The in vivo biological activity of the *N*-acyl-

hydrolysed FB_1 derivatives was unknown in 2011 (Annex 1, references *205* and *206*).

Recent studies show that FB_1 is also a substrate for ceramide synthase forming *N*-acyl-FB_1 derivatives in vivo and in vitro. The *N*-acyl-FB_1 derivatives are more cytotoxic in vitro than FB_1 (Harrer et al., 2013; Harrer, Humpf & Voss, 2015). Considerably more *N*-acyl-hydrolysed FB_1 than *N*-acyl-FB_1 accumulates in cells. This mimics the relative accumulation of hydrolysed FB_1 and FB_1 (Harrer et al., 2013).

Like the *N*-acyl-hydrolysed FB_1 derivatives, the in vivo toxicity of the *N*-acyl-FB_1 derivatives is not known. However, in male Sprague Dawley rats given pure FB_1 or hydrolysed FB_1 by intraperitoneal injection, 12- to 20-fold more FB_1 accumulated in the kidney than in the liver, whereas 6-fold more hydrolysed FB_1 accumulated in the liver than in the kidney. The much greater accumulation of FB_1 in male rat kidney is expected and has been shown in several studies. Nonetheless, very little of the total FB_1 in the kidney was metabolized to *N*-acyl-FB_1, whereas in the liver approximately half of the total fumonisin consisted of the *N*-acyl-FB_1 derivatives (Harrer, Humpf & Voss, 2015). It should be noted that in male Sprague Dawley rats, the kidney is much more sensitive to FB_1-induced toxicity than the liver, and in the Harrer, Humpf & Voss (2015) study the great majority (>90%) of the total fumonisin in the kidney was unmetabolized FB_1.

Compared to kidney, the liver accumulated much higher levels of hydrolysed FB_1 and *N*-acyl-hydrolysed FB_1. While the role of *N*-acyl-FB_1 derivatives in fumonisin toxicity is problematic, hydrolysed FB_1 has been repeatedly shown to be much less toxic than FB_1 in feeding studies (most recently Grenier et al., 2012; Voss & Riley, 2013; Hahn et al., 2015; Masching et al., 2016; Voss et al., 2017a) and is the basis for the commercially available feed additive known as FUMzyme® that hydrolyses FB_1 in the gut.

2.1.3 Effects on enzymes and other biochemical parameters relevant to the mechanism of action

FB_1 is a naturally occurring 1-deoxysphingoid base. Several families of 1-deoxysphingoid base analogues are produced by fungi and found in plant-based foods consumed by humans (reviewed in Duan & Merrill, 2015). For example, *Alternaria alternata* (AAL) toxins are 1-deoxysphingoid base analogues produced by pathogenic species of *Alternaria* on tomatoes, but surveys of food have not reliably reported on them (Ostry, 2008; Hickert et al., 2016). Tomatoes inoculated with *A. arborescens* contained 13 *A. alternata* toxins (Renaud et al., 2015). Strains of *F. avenaceum* have been reported to produce the 1-deoxysphingoid base 2-amino-14,16-dimethyl-octadecan-3-ol (2-AOD-3-ol) (Uhlig et al., 2005).

However, this has not been independently confirmed and the amounts reported in grains are below or at the LOD with the current technology.

FB_1 is clearly nephrocarcinogenic (male F344/N rats) and hepatocarcinogenic (male BDIX rats and female B6C3F1 mice). The weight of the evidence supports a nongenotoxic mechanism of toxicity and carcinogenicity (Bondy et al., 2012; Müller, Dekant & Mally, 2012). Disruption of lipid metabolism appears to play an important and early role in fumonisin toxicity. FB_1 is a potent and specific inhibitor of ceramide synthases, enzymes in the de novo sphingolipid biosynthetic pathway. The evidence for, and consequences of, fumonisin inhibition of ceramide synthases, and the subsequent disruption of sphingolipid metabolism in vitro and in vivo, have been reviewed in detail in the previous WHO authoritative food safety evaluations (IPCS, 2000; IARC, 2002; Annex 1, references *152*, *153*, *205* and *206*). Several more recent reviews of the mechanisms of fumonisin action focus on specific hypothesized or known end-points of fumonisin toxicity, for example, renal cancer (Müller, Dekant & Mally, 2012; Voss & Riley, 2013), growth impairment (Smith, Stoltzfus & Prendergast, 2012), liver cancer (Gelderblom & Marasas, 2012; Torres et al., 2015), developmental toxicity (Voss et al., 2017b) and involvement in human diseases (IARC, 2012, 2015).

The following brief review is intended to acquaint the reader with a basic understanding of how inhibition of ceramide synthases leads to disruption of sphingolipid metabolism and additional downstream effects on global lipid metabolism. As in the previous sections of this monograph, publications since the seventy-fourth JECFA meeting are the focus of this update.

(a) Ceramide synthases

Six mammalian ceramide synthases are known to attach fatty acids of various chain lengths to the long-chain amino alcohols known as sphingoid bases to form dihydroceramides, the immediate precursors to ceramides (Fig. 1). The most common sphingoid base substrates for the enzymes in animals are sphingosine and sphinganine. All six ceramide synthases are inhibited by FB_1 but ceramide synthase 2 is the predominant ceramide synthase in mouse liver, kidney and lung (Mullen, Hannun & Obeid, 2012). In liver, ceramide synthase 2 has a preference for very long-chain (C-22 and C-24) fatty acyl CoAs (reviewed in Merrill, 2011). The organ and tissue specificity of ceramide synthase expression in liver and lung of fumonisin-treated pigs has recently been investigated (Loiseau et al., 2015). When ceramide synthase is blocked, ceramide and more complex sphingolipid biosyntheses are disrupted and the precursors of ceramides (sphingoid bases) begin to accumulate because they are upstream of ceramide synthase. This is also seen in ceramide synthase 2–null mice where sphinganine is elevated up to 50-fold in liver (Pewzner-Jung et al., 2010a). In fumonisin-treated animals,

Fig. 1

Overview of de novo sphingolipid biosynthesis and turnover and the biochemical effects of ceramide synthase inhibition by fumonisin[a]

[a] Fumonisin (large "X") inhibition of ceramide synthases and the biochemical consequences. Within the cell membrane are two examples of complex sphingolipid interactions with membrane proteins. The structure of the B-series fumonisins is shown. Fumonisin B_1 contains hydroxyl groups at R1, R2 and R3.
Source: Eaton et al. (2010)

increases in sphinganine in target tissues and blood are tightly correlated with the onset and severity of toxic, pathophysiological and histopathological effects in target organs of numerous species (reviewed in IPCS, 2000; IARC, 2002; Annex 1, references *152*, *153*, *205* and *206*). Other consequences of ceramide synthase inhibition by FB_1 are increased levels of sphingosine, sphinganine 1-phosphate, sphingosine 1-phosphate and phosphatidylethanolamine and decreased levels of "downstream" complex sphingolipids, including those associated with membrane receptors or transporters found in lipid rafts (reviewed in Voss & Riley, 2013).

(b) Linking inhibition of ceramide synthase to biological effects

Perturbations of sphingolipid homeostasis by FB_1 are reversible in animals as are its apoptotic and mitotic effects in rodent liver and kidney. There have also been numerous studies in rats, primary hepatocytes and cell lines characterizing changes in various lipid pools (fatty acids, phospholipids, cholesterol) following fumonisin treatment (IPCS, 2000; IARC, 2002, Fig. 2; Gelderblom & Marasas, 2012; Annex 1, reference *153,* "Fumonisins", Tables 1 and 2; Annex 1, references *205* and *206*). Most recently, short-term studies have investigated the effects of two cancer-promoting treatments (FB_1 or 2-acetylaminofluorene/partial hepatectomy) on lipid metabolism and induction of altered lipid phenotypes in rat liver. Both cancer-promoting treatments induced similar changes, including increased phosphatidylethanolamine and cholesterol content, increased levels of palmitic acid (hexadecanoic acid; C16:0) and mono-unsaturated fatty acids in phosphatidylethanolamine and phosphatidylcholine, as well as a decrease in stearic acid (octahexadecanoic acid; C18:0) and long-chain polyunsaturated fatty acids in the phosphatidylcholine fraction of membrane extracts (Riedel et al., 2015). Many of the described changes can be linked back to ceramide synthase inhibition.

The weight of the evidence indicates that the proximate cause for fumonisin-induced toxicity and animal diseases is inhibition of ceramide synthase resulting in a global disruption of lipid metabolism (Annex 1, reference *153*, "Fumonisins", Tables 1 and 2). The precise downstream biochemical and molecular events linking ceramide synthase inhibition to the known fumonisin-induced animal diseases are not well defined. This is in part because many researchers know FB_1 primarily as an invaluable tool for revealing the role of sphingolipids, and in particular ceramide biosynthesis, in the regulation of cellular physiology as it relates to health and disease in plants, animals and humans (IPCS, 2000, Table 4; Mullen, Hannun & Obeid, 2012, Table 6; Annex 1, reference *153*, "Fumonisins", Table 3). However, what follows after FB_1 inhibits ceramide synthase is a global redirection of lipid substrates into other pathways and products that can affect a multitude of signalling systems involved in cellular regulation (Fig. 2). Thus, the risk of using FB_1 as a tool to study sphingolipid functions is that fumonisin inhibition of ceramide synthases causes changes in many lipid metabolites and end-products that are involved in a diverse range of structural and physiological functions in cells (Duan & Merrill, 2015). Focusing on the effects of inhibition of ceramide biosynthesis while ignoring the elevation in sphingoid bases and their metabolites complicates the effort to understand and prioritize the critical downstream events that lead to fumonisin-induced toxicity and increased risk of cancer and other diseases in humans.

Fig. 2

Possible cellular consequences of fumonisin inhibition of ceramide synthases and global disruption of sphingolipid metabolism

Source: Annex 1, reference *206*

In the de novo sphingolipid biosynthetic pathway in animals, the most likely sphingoid base substrate for all ceramide synthases is sphinganine. Sphinganine is formed after the condensation of serine with a fatty acyl-CoA by the enzyme serine palmitoyltransferase. However, if serine availability is limited, or the enzyme is mutated so that it prefers alanine or glycine, then acylation forms 1-deoxysphingoid bases that lack a hydroxyl on the 1-carbon (reviewed in Duan & Merrill, 2015). Formation of sphingoid base 1-phosphates, sphingomyelin and all more complex sphingolipids requires a hydroxyl on the 1-carbon; thus 1-deoxysphingoid bases cannot be phosphorylated and 1-deoxyceramides cannot be processed into more complex sphingolipids. The role of 1-deoxysphingolipids in the biological effects of FB_1 is unknown.

In male mice, 1-deoxysphinganine accumulated in liver and kidney following dietary exposure to FB_1. The levels of 1-deoxysphinganine in liver approached those of the accumulated free sphinganine and were much higher than the levels of sphingoid base 1-phosphates (Bondy et al., 2012). This is not the case in male rats consuming diets containing FB_1. In male rats, 1-deoxysphinganine has not been detected in either liver or kidney, whereas high levels of sphinganine and sphinganine 1-phosphate accumulated in the kidney

(Voss et al., 2011), the most sensitive target organ in male Sprague Dawley and F344N rats. The absence of 1-deoxysphinganine in rat liver and kidney and high levels of 1-deoxysphinganine and low levels of sphinganine 1-phosphate in mouse liver suggest that de novo sphingoid base biosynthesis in rats and mice differ and that these differences might influence their species and target organ-specific responses to fumonisin exposure.

1-Deoxydihydroceramides and 1-deoxyceramides in liver of female mice were rapidly depleted when the mice were treated with FB_1 (Voss et al., 2009). Whether mouse serine palmitoyltransferase has greater plasticity in terms of amino acid specificity or if mouse diets contain insufficient serine to meet the animal's metabolic demands is unknown. However, in the absence of any FB_1 treatment, mice fed diets high in alanine had elevated levels of 1-deoxysphingolipids and developed peripheral neuropathy (Garofalo et al., 2011). It is not known if 1-deoxysphinganine is elevated in humans exposed to FB_1, but there is a human disease (hereditary sensory and autonomic neuropathy type 1, HSAN1) that is linked to a mutation in serine palmitoyltransferase that allows the enzyme to utilize alanine and glycine to produce 1-deoxysphingoid bases, which are neurotoxic in cultured neurons (reviewed in Duan & Merrill, 2015). Whether 1-deoxysphinganine could contribute to adverse effects associated with human consumption of FB_1-contaminated foods is unknown.

Not all 1-deoxysphinganine analogues are equally toxic in vivo. For example, Enigmol is a 1-deoxysphingoid base that has been shown to suppress tumours in mouse models in vivo (Symolon et al., 2011). The antitumour efficacy of Enigmol is attributed at least in part to the fact that it, not surprisingly, cannot be phosphorylated by sphingosine kinase 1, which some consider to be an oncogene. In addition, relative to FB_1, Enigmol is a weak inhibitor of ceramide synthases (Humpf et al., 1998; Symolon et al., 2011). That the enzyme ceramide synthase and its de novo downstream product ceramide as well as the enzyme sphingosine kinase and its cellular product sphingosine 1-phosphate play important opposing roles in tumorigenesis is well documented (Espaillat et al., 2015; Reimann et al., 2015; Suh & Saba, 2015). Paradoxically, the carcinogenic FB_1 is often used to screen cancer chemotherapeutic agents for their ability to produce ceramide de novo to kill cancer cells (IPCS, 2000, Table 4; Annex 1, reference *153*, "Fumonisins", Table 3). A few recent examples of the use of FB_1 to suppress the efficacy of potential cancer therapies are suppression of ceramide-induced apoptosis by picropodophyllin in human Ewing's sarcoma cells (Wu et al., 2016), HDAC inhibitor AR-42–induced anti-colon cancer cell activity (Xu et al., 2015) and enhanced killing of SCC17B human head and neck squamous cell carcinoma cells after photodynamic therapy plus fenretinide (Boppana et al., 2015). Based on this suppression of cancer therapy efficacy, as well as the ceramide biosynthesis inhibition and stimulated sphingoid base 1-phosphate production,

FB_1 would obviously not be a good candidate as a drug for suppressing tumour development and growth. Quite the opposite, decreased ceramide production and increased production of sphingoid base 1-phosphates provide FB_1 with all the biochemical tools necessary to be a potent cancer promoter.

(c) Ceramide synthase inhibition and oxidative stress

In addition to the global disruption of lipid metabolism, increased oxidative stress has been frequently proposed as a biochemical mechanism for fumonisin toxicity in vitro and in vivo (Annex 1, reference *153*, "Fumonisins", Tables 1 and 2). However, the signs of oxidative stress observed in vitro and in vivo are likely to be secondary to ceramide synthase inhibition and the consequent changes in various lipid pools and bioactive lipid metabolites. For example, at the most fundamental physiological level, disruption of sphingolipid metabolism is a potential drain on the energy reserves of cells necessary to maintain the redox balance within affected cells. The first enzyme in the de novo biosynthetic pathway (serine palmitoyltransferase) requires pyridoxal phosphate (vitamin B_6), and the formation of sphinganine requires NADPH (nicotinamide adenine dinucleotide phosphate (reduced)). The uncoupling of sphinganine biosynthesis from ceramide production results in the sustained production of sphinganine with no apparent feedback to stop its biosynthesis. Recent studies using FB_1 suggest that the downregulation of serine palmitoyltransferase activity by ORMDL proteins (orosomucoid-like proteins) requires metabolites that would normally be formed downstream of dihydroceramide (Gupta et al., 2015). Thus the uncoupled production of sphinganine de novo is an energy drain on cells. Likewise, the formation of sphingoid base 1-phosphates via sphingosine kinases consumes adenosine triphosphate (ATP), and the irreversible exit of the sphingoid base 1-phosphates from the sphingolipid metabolic pathway requires vitamin B_6. In addition to depleting cells' high energy resources and critical cofactors, sphinganine itself is a highly cytotoxic biochemical that has been shown to inhibit mitochondrial complex IV activity and increase reactive oxygen species (ROS) generation (Zigdon et al., 2013). In mice, elevated sphingosine accumulates in brain mitochondria following traumatic brain injury and has been linked to decreased activity of cytochrome oxidase, the rate-limiting enzyme in the mitochondrial electron transport chain (Novgorodov et al., 2014) and a potential source of ROS. Sphinganine was equally effective at inhibiting cytochrome oxidase activity in isolated mitochondria (Novgorodov et al., 2014).

The documented changes in membrane lipid composition are likely to create an environment more susceptible to lipid peroxidation and increased disorder in membranes, leading to their increased leakiness, putting additional demands on cell energy reserves and jeopardizing the cellular redox balance. The

potentially important role of dietary factors and oxidative stress in the toxicity of fumonisins is well known (Gelderblom & Marasas, 2012). Many recent studies have reported additional evidence of oxidative stress in animals and cells treated with fumonisin (see section 2.2.4 for examples).

The biochemical–metabolic sequence of events linking ceramide synthase inhibition, elevated sphingoid bases, decreased complex sphingolipid biosynthesis and altered lipid composition of membranes to possible human disease or other adverse effects is not well understood. Many reports have presented evidence for the involvement of oxidative stress, altered cytokine-mediated signalling, altered membrane properties and disruption of the cell cycle in FB_1-induced toxicity (reviewed in Annex 1, references *205* and *206*). The ability of FB_1 to alter the balance of biochemical mediators of cell death and survival/proliferation in target tissues is the likely key to its mechanisms of both toxicity and carcinogenicity.

(d) Linking downstream biochemical effects to adverse effects

Fumonisin disruption of sphingolipid metabolism is defined in IPCS (2000, section 7.3.1.2) as:

> …inhibition of sphingosine and ceramide biosynthesis, depletion of more complex sphingolipids, increase in free sphinganine, decrease in reacylation of sphingosine derived from complex sphingolipid turnover and degradation of dietary sphingolipids, increase in sphingoid base degradation products (i.e. sphingosine (sphinganine) 1-phosphate, ethanolamine phosphate and fatty aldehydes), and increase in lipid products derived from the increase in the sphingoid base degradation products. (Merrill, Liotta & Riley, 1996).

This provides a starting-point for tracing out the downstream effects of ceramide synthase inhibition. Specifically, fumonisin inhibition of ceramide synthases results in reduced ceramide biosynthesis and elevation of sphingoid bases and sphingoid base 1-phosphates in blood and tissues. Ceramide is a known mediator of receptor-dependent cell death, whereas sphingoid base 1-phosphates are known mediators of cell survival via modulation of intracellular and extracellular sphingosine 1-phosphate receptor signalling pathways (Annex 1, reference *206*, "Fumonisins (addendum)", Fig. 2). In mice, ablation of ceramide synthase 2 results in a large reduction in C-22 and C-24 ceramides and an increase in C-16 ceramides and sphinganine in liver (Pewzner-Jung et al., 2010a). These same ceramide synthase 2–null mice had elevated levels of apoptosis and proliferation in the liver and upregulation of cell cycle–related genes, and spontaneously develop liver tumours at about 10 months of age (Pewzner-Jung

et al., 2010b). Ablation of ceramide synthase 2 creates a mouse that cannot synthesize C-22 and C-24 ceramides. The loss of the ability to make these very long-chain sphingolipids has significant effects on the biophysical properties of membranes that are postulated to affect processes such as adhesion and fusion of vesicles (Silva et al., 2012). Inability to synthesize C-22 and C-24 ceramides was also shown to interfere with uptake of palmitate by hepatocytes and liver (Park et al., 2014). When the null mice were fed a high fat diet, they developed hepatic nodules containing fatty droplets (Park et al., 2014). Since the Pewzner-Jung et al. (2010a,b) studies, Zigdon et al. (2013) found that ceramide synthase 2 ablation resulted in chronic oxidative stress that coincided with C-16 ceramide and sphinganine accumulation in mitochondria. The chronic oxidative stress was due to ROS induction caused by impaired mitochondrial complex IV activity and was accompanied by increased levels of antioxidant enzymes, lipid peroxidation, protein nitrosylation and ROS. Many of the changes and effects seen in liver of the ceramide synthase 2–null mice are reminiscent of changes and effects reported in studies in which ceramide synthases are blocked using FB_1. Note that there are six known ceramide synthases and, even though ceramide synthase 2 is the predominantly expressed ceramide synthase in mice, the presence of the five other ceramide synthases likely explains the compensatory increase in C-16 ceramides in the membranes of the null mice that show increased membrane fluidity (Merrill, 2011).

(e) Linking biochemical and adverse effects to potential biomarkers of effect

In the sphingolipid turnover pathway, sphingosine is the sphingoid base that builds up following fumonisin inhibition of ceramide synthases. Sphingosine differs from sphinganine in that it contains a double bond at the 4–5 position; this double bond is inserted when dihydroceramide is desaturated and ceramide is formed. Thus, free sphingosine is only available when ceramide and more complex ceramide-containing sphingolipids are broken down in an agonist-dependent process or degraded for salvage and reuse. The increase in the Sa/So ratio in tissues of fumonisin-treated animals and cells is because the de novo ceramide precursor sphinganine forms and accumulates much more rapidly and to a greater extent than the generation of the degradation/turnover/salvage product, sphingosine. In addition, the lack of feedback by downstream metabolites (Gupta et al., 2015) results in the functional uncoupling of sphinganine biosynthesis from ceramide biosynthesis. There is a similar difference in the sphinganine 1-phosphate/sphingosine 1-phosphate (Sa 1-P/So 1-P) ratio, which also increases with fumonisin treatment (Gelineau-van Waes et al., 2012; Riley et al., 2015a; Gardner et al., 2016a). The elevations in sphinganine, sphinganine 1-phosphate,

Sa/So ratio and Sa 1-P/So 1-P ratio are frequently used biomarkers of effect (inhibition of ceramide synthases) in vitro and in vivo.

Recent studies show that the elevated levels of sphinganine and sphinganine 1-phosphate in tissues following oral exposure to FB_1 are paralleled by increased levels of sphinganine 1-phosphate and, to a lesser extent, sphinganine and sphingosine 1-phosphate in mouse blood spots (Gelineau-van Waes et al., 2012) in a dose-dependent fashion (Riley et al., 2015a). The accumulation of sphinganine 1-phosphate and the increase in the Sa 1-P/So 1-P ratio in the mouse blood spots are dose-dependent and parallel the dose-dependent increases in the liver and kidney, and are positively correlated with the UFB_1 (Riley et al., 2015a). The rationale for collecting blood spots rather than serum or plasma, as done in previous studies, was that red blood cells (RBCs) contain and produce large amounts of sphingosine 1-phosphate and sphinganine 1-phosphate (Fig. 3). It has been shown that RBCs are the main source of the sphingoid base 1-phosphates in the blood (reviewed in Thuy et al., 2014). In addition, RBCs cannot synthesize sphingolipids de novo (Hänel, Andréani & Gräler, 2007; Bode et al., 2010) and lack the ability to de-phosphorylate sphingoid base 1-phosphates (Ito et al., 2007). However, RBCs can phosphorylate sphingoid bases via sphingosine kinases (Yang et al., 1999). Thus, the sphingosine and sphinganine phosphorylated in the RBCs are produced in non-RBC tissues. In animals, large amounts of sphingosine and sphinganine are produced in liver and kidney following FB_1 exposure, and this is the likely source of substrates for the sphingosine kinases in the RBCs of FB_1-treated mice. Thuy et al. (2014) proposed that under normal physiological conditions, RBCs serve as reservoirs of the bioactive sphingoid base 1-phosphates for the purpose of buffering the sphingosine 1-phosphate concentration in the plasma. Most recently, Riley et al. (2015b) showed that there is a positive and statistically significant correlation between fumonisin exposure, using UFB_1, and the sphinganine 1-phosphate and Sa 1-P/So 1-P ratio (biomarkers of effect) in humans consuming diets containing high levels of fumonisins. This is important because it supports the hypothesis that the underlying proximate cause of fumonisin-induced animal diseases is also operational in vivo in humans.

2.2 Toxicological studies

This section summarizes the acute, short-term and long-term toxicological studies in animals exposed to fumonisins B_1, B_2 and B_3, individually or in combination, conducted since the two previous JECFA evaluations. In addition, as in previous evaluations, studies conducted using diets prepared using naturally contaminated maize or *F. verticillioides* culture material with known amounts of fumonisins are also summarized. The focus of this section is new studies conducted in animal

Fig. 3
Red blood cell uptake and metabolism of sphingoid base 1-phosphates[a]

CerS: ceramide synthases; FB_1: fumonisin 1; RBC: red blood cell; Sa 1-P: sphinganine 1-phosphate; So 1-P: sphingosine 1-phosphate; $SphK_{1/2}$: sphingosine kinases
[a] In animals large amounts of sphingosine and sphinganine are produced in liver, kidney and other tissues following fumonisin inhibition of ceramide synthases (CerS). The sphingosine and sphinganine produced in tissues following fumonisin exposure are a likely source of substrates for the sphingosine kinases ($SphK_{1/2}$) in the RBCs following FB intake. However, RBCs can also take up and store sphingosine 1-phosphate (So 1-P) and sphinganine 1-phosphate (Sa 1-P) from endothelial cells. The vertical arrows next to sphinganine and sphingosine represent the relative magnitude of the accumulation when ceramide synthase is inhibited in animal models. The downward arrow next to ceramide indicates the decrease in ceramide biosynthesis in tissues
Source : Riley et al. (2015a)

models to reveal dose–response relationships or studies that reveal novel aspects of fumonisin toxicity that expand the understanding of the biochemical basis for fumonisin toxicity and carcinogenicity and its potential for contributing to animal and human disease.

The toxicological studies reviewed in the two previous evaluations are summarized in Tables 1, 2 and 3 in the fumonisins addendum in the seventy-fourth meeting monographs (Annex 1, reference *206*) and Tables 4, 5 and 6 in the fumonisins monograph in the fifty-sixth meeting monographs (Annex 1, reference *153*).

2.2.1 Acute toxicity

As in the previous evaluations, no studies showed lethality from a single dose of FB_1. The 2011 evaluation described single dose studies of pure FB_1 in mice, rats and pigs. Since 2011, three studies in mice (summarized below) have used a single dose of pure FB_1.

In male ICR mice (n = 6/group; 18–25 g), a single intrathecal injection of 10 nmol FB_1 (calculated as 0.34 mg/kg bw) 3 hours after partial sciatic nerve ligation transiently attenuated tactile allodynia 48 hours after the ligation. The study was repeated with two injections at the same dose 3 hours and 3 days after ligation, with the same results but with more prolonged attenuation of

tactile allodynia and with suppression of microglial cell induction (a source of inflammatory mediators) (Kobayashi et al., 2012).

In male C57BL/6 mice (n = 7–10), a single intraperitoneal injection of FB_1 at 8 mg/kg bw sensitized the mice to pentylenetetrazol-induced seizures (decreased latency and increased frequency of myoclonic jerks) (Poersch et al., 2015). Changes in mitochondrial function were also observed.

In another study in male C57BL/6 mice (n = 5/group), animals were given a single subcutaneous dose of FB_1 (6.75 mg/kg bw) and then exposed to diesel exhaust or clean air for 8 hours (Shaheen et al., 2016). The lungs were collected 12 hours after inhaling the diesel exhaust for 8 hours. Treatment with diesel exhaust only and FB_1 only both upregulated mRNA expression of two pulmonary surfactant proteins. In the diesel-exhaust-plus-FB_1 group, there appeared to be an effect on the expression of the two proteins that suggested additivity.

Shaheen et al. (2016) repeated this study with dosing at the same level on 3 days over a 7-day period (i.e. dosing every other day). The immunohistochemical staining of bronchial epithelial cells suggested that the diesel exhaust induced ceramide production and that ceramide induction was suppressed on day 7 in the group treated with diesel exhaust plus FB_1.

All of these studies provide information supportive of the complexity of the in vivo biological activity of fumonisin, but none provide practical information for establishing dose–response relationships at environmentally relevant levels of FB_1 contamination of foods. Nor were they specifically designed to define the acute toxicity of fumonisin.

2.2.2 Short-term studies of toxicity

The three acute studies described in section 2.2.1 also had dosing times other than a single dose and thus are technically both acute (single dose) and short-term studies. Those studies are not described again in this section.

The short-term studies of toxicity of pure FB_1 published since the previous JECFA evaluation or not reviewed previously are summarized in Table 1.

(a) Studies using purified FB_1

Male Sprague Dawley rats (n = 8/group) were fed diets containing pure FB_1 at 0 or 15 mg/kg feed (equivalent to 0 and 1.5 mg/kg bw per day) with or without AdiDetox (a purported "mycotoxin inactivator") for 7 days. Rats fed FB_1 alone at 15 mg/kg diet had significantly increased ($P < 0.05$) relative liver weight and serum cholesterol. Free sphinganine, free sphingosine and the Sa/So ratio were significantly increased in kidney. The adsorbent partially reduced the FB_1-induced increase in kidney sphinganine and Sa/So ratio (Denli et al., 2015).

Table 1
Summary of short-term studies of toxicity of pure[a]/purified FB_1

Species / description	Length of study	No. per group	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male Sprague Dawley rats, "young", 125 g	7 days	8	Equivalent to 0 or 1.5	15 mg/kg diet	Increased relative liver weight, cholesterol, Sa/So ratio	1.5	–	Denli et al. (2015)
Male F344 rats, 150–180 g, age not stated	46 days total following two different tumour promotion protocols with last 2 weeks on FB_1 diets	5	Equivalent to 0 or 25	250 mg/kg diet	Changes in lipid profiles characteristic of cancer promotion	25	–	Riedel et al. (2015)
Male Sprague Dawley rats, 6 weeks old, 111 g	21 days	4	Equivalent to 0 or 1	10 mg/kg diet	Elevated pathology scores and Sa/So ratio; other pure fumonisin analogues tested but with no toxicity or elevated Sa/So ratios	1	–	Hahn et al. (2015)

bw: body weight; FB_1: fumonisin B_1; LOAEL: lowest-observed-adverse-effect level; No.: number; NOAEL: no-observed-adverse-effect level; Sa/So: sphinganine/sphingosine
[a] "Pure FB_1" means that it was purchased from a reputable supplier (Sigma-Aldrich [MilliporeSigma], Cayman Chemical, Promec, etc.) or provided by a source known to produce >90% pure FB_1.

The Committee noted that the study used only one dose level of FB_1. Thus a no-observed-adverse-effect level (NOAEL) could not be determined.

Male Fischer 344 rats were divided between four groups (n = 5/group): control; FB_1-promotion group; 2-acetylaminofluorene/partial hepatectomy–promotion group; and 2-acetylaminofluorene/partial hepatectomy/FB_1–promotion group. The control group was fed AIN 76A diet for 46 days; the FB_1-promotion group was fed AIN 76A diet for 32 days and then an AIN 76A diet containing pure FB_1 at 250 mg/kg feed (equivalent to 25 mg/kg bw per day) for 14 days; the 2-acetylaminofluorene/partial hepatectomy–promotion group was fed AIN 76A diet for 14 days, gavaged with 2-acetylaminofluorene (20 mg/kg bw per day) on days 15, 16 and 17, subjected to partial hepatectomy on day 18 and then continued on the AIN 76A diet until day 46; the 2-acetylaminofluorene/partial hepatectomy/FB_1–promotion group was treated in the same way as the

2-acetylaminofluorene/partial hepatectomy promotion–group, but the AIN 76A diet contained pure FB_1 at 250 mg/kg feed. On day 46, liver tissue was collected and lipids were extracted.

The purpose of the experiment was to characterize lipid changes during cancer promotion and compare the altered lipid profiles in rats resulting from two different cancer-promoting protocols. Both promotion protocols resulted in what was called a "typical lipid phenotype", characterized by increased levels of phosphatidylethanolamine, cholesterol, palmitic acid (C16:0) and mono-unsaturated fatty acids in the phospholipids, and decreased stearic acid (C18:0) and long-chained polyunsaturated fatty acids in the phosphatidylcholine fraction (Riedel et al., 2015).

The toxicological effects on liver and kidney were not presented, but in a previous 21-day feeding study using pure FB_1 at 250 mg/kg diet, the same group of researchers reported nodular regeneration, fibrosis and ductile endothelial cell proliferation in liver and nephrosis, necrotic epithelial cells, apoptosis, hypereosinophilia and sloughing of epithelial cells in kidney (Annex 1, reference *153*, "Fumonisins", Table 5).

The Committee noted that the study used only one dose level of FB_1. Thus a NOAEL for the effects of FB_1 could not be determined.

Male Sprague Dawley rats (6 weeks old, 111 g) were fed diets containing 13.9 µmol/kg diet (equivalent to FB_1 at 10 mg/kg diet or 1 mg/kg bw per day) of FB_1 (97.2% pure); partially hydrolysed FB_1–a chain (73.2% pure); partially hydrolysed FB_1–b chain (93% pure); fully hydrolysed FB_1 (both chains; 100% pure); and *N*-(1-deoxy-D-fructos-1-yl) FB_1 (97.5% pure) for 21 days. The impurities were other fumonisins. Parts of this study are also summarized in sections 2.1.1 and 2.1.2 and only the toxicological findings are summarized in this section. Liver and kidney samples were collected on day 22 for scoring the pathological effects of the various treatments (n = 4/treatment) using haematoxylin and eosin stain. Briefly, kidney specimens were assigned scores of 0 (for normal); 1 (for a few apoptotic cells in an otherwise normal tissue); 2 (for apoptotic tubular epithelial cells, detachment and sloughing and occasional mitotic figures); and 4 (for severe lesions; not seen in this study).

There was no effect on body weight, and no histopathological effects were observed in liver. The FB_1 group was the only group with a significantly ($P < 0.05$) elevated kidney pathology score compared with the negative control group. Likewise, the Sa/So ratio was only significantly elevated in the urine and kidneys of the group consuming the diets containing FB_1 (Hahn et al., 2015).

The Committee noted that the study used only one dose level of FB_1. Thus, a NOAEL for the effects of FB_1 could not be determined.

(b) Studies using diets/treatments formulated with FB_1 prepared from partially purified fumonisins, *F. verticillioides* culture material or naturally contaminated maize

Short-term studies of toxicity using partially purified fumonisins, *F. verticillioides* culture material or naturally contaminated maize that were not previously reviewed or were published since the previous JECFA evaluation are summarized in Table 2.

Mice

Female mice (strain not indicated; n = 14–15/group; approximately 20 g) were fed a diet containing FB_1 (purity not specified) at 0 or 150 mg/kg feed (22.5 mg/kg bw per day calculated) for 16 weeks. The stomachs were removed and processed for histopathological and histochemical analysis.

Final body weight was reduced, but not significantly, and there was no significant effect on body weight gain. Treatment resulted in decreased parietal cell number and pronounced atrophy in the gastric mucosa mediated through increased apoptosis and decreased proliferation. This study confirms the potential of fumonisins at high dietary concentrations to adversely affect the gastrointestinal tract (Alizadeh et al., 2015).

The Committee noted that the study used only one dose level of FB_1 (unspecified purity). Thus, a NOAEL could not be determined.

Female BALB/c mice (10 weeks old; ~30 g), divided between six groups (n = 15/group), were dosed orally with silymarin and/or FB_1 intraperitoneally for 14 days. The silymarin-only group received silymarin at 100 mg/kg bw per day, daily by oral gavage. Two groups received FB_1 only at 1.5 and 4.5 mg/kg bw every other day for 13 days, and two groups received both silymarin (100 mg/kg bw per day, daily) and FB_1 (1.5 and 4.5 mg/kg bw every other day). The FB_1 was 70% pure, based on information on purity from the FB_1 provider. The negative control group was dosed every day with saline.

The FB_1-induced increase in apoptosis in the liver and upstream mediators of apoptosis were attenuated by the silymarin treatment. The protective effect of silymarin was attributed to its presumed efficacy as an antioxidant. The results provide additional evidence for the role of oxidative stress in tissues exposed to very high levels of fumonisins and confirm the protective effect of silymarin.

The use of partially purified FB_1 and intraperitoneal dosing precluded the use of this study for the dose–response assessment (Sozmen et al., 2014).

Rats

Venancio et al. (2014) divided male Wistar rats (21 days old; weight at study start ~42–47 g) between two groups, a control and a fumonisin-treated group (n = 8/group). The control group was fed the basal diet for 42 days, and the

Table 2
Summary of short-term toxicity studies in mice, rats and pigs[a] using diets containing FB_1 prepared from partially purified fumonisins, *F. verticillioides* culture material or naturally contaminated maize

Species / description	Length of study	No. per group	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female mice (strain not stated), age not stated, 20 g	16 weeks	14 or 15	Equivalent to 22.5	Diet (150 mg/kg diet)	Atrophy, increased apoptosis, decreased proliferation in the gastric mucosa	22.5	–	Alizadeh et al. (2015)
Female Balb/c mice, 10 weeks old, 30 g	14 days	15	1.5 or 4.5	i.p.	Increased apoptosis and mediators of apoptosis. Protection by an antioxidant	1.5	–	Sozmen et al. (2014)
Male Wistar rats, 21 days old, 42–47 g	42 days	8	Equivalent to 0.45	Oral, via the diet (6 mg/kg diet)	Renal toxicity based on elevated water intake and sodium excretion, inflammatory infiltrate, fibrosis and number of altered foci in kidney	0.45	–	Venancio et al. (2014)
Male Sprague Dawley rats, 4–5 weeks old, 100 g	28 days	6 or 7	Equivalent to 0, 3.75, 7.5 or 15	Diet (0, 50, 100, 200 mg/kg diet)	Dose-dependent decreased feed intake and body weight gain and increased serum liver enzymes, bilirubin, urea, uric acid, creatinine and DNA fragmentation. Protection by lactic acid bacteria	3.75	–	Khalil et al. (2015)
Male Sprague Dawley rats, 5 weeks old, 120 g	21 days	5	Equivalent to 0.02, 0.14, 0.27 or 0.32[b]	Diet (0.22, 1.8, 3.6, 4.2 mg/kg diet)	Dose–response for increased mean pathology scores and Sa/So and Sa 1-P/So 1-P ratios. Protection by nixtamalization (alkaline cooking)	0.14	–	Voss et al. (2013)
Male crossbred piglets, 4 weeks old	28 days	6	0.12, 0.24 or 0.36	Diet (0, 3, 6, 9 mg/kg diet)	Dose-dependent moderate to high interstitial inflammation and alveolar oedema in the lungs	0.012	–	Souto et al. (2015)
Male crossbred piglets, 4 weeks old	28 days	18	1.5	Oral bolus	Lower feed conversion ratio and higher liver weight. Increased COX-1 and nNOS in the stomach; increased HSP 70 in jejunum; increased αB-crystallin, COX-1 and HO-1 in colon	1.5	–	Lalles et al. (2010)

bw: body weight; COX-1: cyclooxygenase 1; HO-1: haem oxygenase 1; i.p.: intraperitoneal; LOAEL: lowest-observed-adverse-effect level; No.: number; nNOS: nitric oxide synthase; NOAEL: no-observed-adverse-effect level

[a] Studies in poultry are described in the text.

[b] Naturally contaminated maize-amended diets before nixtamalization had FB_1 levels at 1.8, 3.6 and 4.2 mg/kg diet. After nixtamalization, the FB_1 levels were 0.08, 0.13 and 0.37 mg/kg diet. (Note the basal diet also contained FB and there was significant kidney pathology in the high-dose nixtamalization group.)

fumonisin-treated group was fed the basal diet containing FB_1 prepared using *F. verticillioides* culture material at 6 mg/kg feed; there was no description of the fumonisin analysis of the diet or the culture material.

At 42 days, there was no difference in feed intake or body weight gain (final weight approximately 240 g). Water intake and urinary sodium excretion were significantly elevated in the fumonisin-treated group. Inflammatory infiltrate and mild fibrosis in the outer renal cortex were noted. The number of altered foci was significantly elevated in the fumonisin-treated group. This study confirms the renal toxicity of fumonisin in male rats. Assuming that FB_1 at 1 mg/kg diet is equivalent to 0.075 mg/kg bw, the average dose for this study was 0.45 mg/kg bw per day (Venancio et al., 2014).

The Committee noted that the study used only one dose level of FB_1 from culture material. Thus a NOAEL could not be determined.

Khalil et al. (2015) divided male Sprague Dawley rats (4–5 weeks old; initial weight approximately 100 g) between 12 groups (n = 6 or 7/group). A negative control group was fed a basal diet for 4 weeks. Positive control groups were fed the basal diet containing FB_1 at 50, 100 or 200 mg/kg diet (based on high-performance liquid chromatographic [HPLC] analysis of the diets; equivalent to 3.75, 7.5 and 15 mg/kg bw per day, assuming that FB_1 at 1 mg/kg diet is equivalent to 0.075 mg/kg bw). Two additional positive control groups (no fumonisin) were dosed each day orally with 10^{10} colony-forming units (CFU)/mL of either *Lactobacillus delbrueckii* subsp. *lactis* or *Pediococcus acidilactici*. The source of the fumonisin was culture material produced by an isolate of *F. moniliforme* (now *F. verticillioides*). Six treated groups consumed diets containing FB_1 at 50, 100 or 200 mg/kg (equivalent to 3.75, 7.5 and 15 mg/kg bw per day, assuming that FB_1 at 1 mg/kg diet is equivalent to 0.075 mg/kg bw) plus one of the lactic acid bacterial strains at each FB_1 dose level. At the end of the 4-week feeding period, there was a clear dose–response decrease in feed intake and body weight gain in the FB_1-positive-control groups; both lactic acid bacterial co-treatments had protective effects. Likewise, at week 4 and earlier, there were clear dose-dependent increases in serum liver enzymes and bilirubin in the FB_1-positive-control groups; both lactic acid bacterial co-treatments had protective effects. The protective effects of the lactic acid bacterial co-treatments were also apparent for the FB_1-induced increase in serum levels of urea, uric acid and creatinine at week 4 and DNA fragmentation at weeks 3 and 4 (Khalil et al., 2015).

This study shows the efficacy of lactic acid bacteria in protecting the liver and kidney from fumonisin-induced toxicity in rats. A NOAEL for FB_1 could not be determined because the effects were seen at the lowest dose level.

Voss et al. (2013) divided male Sprague Dawley rats (n = 35; 5 weeks old; ~120 g) between seven groups (n = 5/group) that were fed control or test diets for 3 weeks. Test diets were prepared using naturally contaminated whole kernel maize containing FB_1 at approximately 30 mg/kg maize. The contaminated maize also contained smaller amounts of FB_2 and FB_3 (not quantified). The contaminated maize was diluted with sound control maize containing FB_1 at less

than 0.3 mg/kg maize to prepare three 4 kg batches with "low", "mid" and "high" levels of contamination. Batches of each contamination level were subjected to alkaline cooking (nixtamalization). The nixtamalized maize was washed, freeze-dried and ground. Alkaline-cooked and uncooked maize from each batch were diluted 1:1 with rodent diet (Teklad 2019) containing FB_1 at less than 0.5 mg/kg diet. The control diet was a mixture of control maize and rodent diet (50% weight per weight). All diets were analysed using immunoaffinity column clean-up followed by HPLC quantification of FB_1. The uncooked-low, uncooked-mid and uncooked-high test diets contained FB_1 at 1.8, 3.6 and 4.2 mg/kg diet, respectively (equivalent to 0.14, 0.27 and 0.32 mg/kg bw per day). The alkaline-cooked-low, alkaline-cooked-mid and alkaline-cooked-high diets contained FB_1 at 0.08, 0.13 and 0.37 mg/kg diet, respectively. The control diet contained FB_1 at 0.22 mg/kg diet. None of the analyses were corrected for recoveries.

After 3 weeks, there were no significant effects on body weight, body weight gain, feed consumption, feed conversion or relative or absolute kidney weights in any of the groups consuming the alkaline-cooked or uncooked low, mid or high diets compared with the control group. The mean kidney pathology scores in the groups consuming the uncooked test diets (low, mid and high) followed a dose–response, although there were no statistically significant differences in the mean pathology scores in the uncooked test groups. Scoring criteria for nephrotoxicity were 0, no finding; 1, minimal change; 2, mild lesions, more obvious focal or multifocal apoptotic effect but with tubule structure intact; and 3, moderate lesions, widespread lesions involving most to all of the outer medulla. All the mean scores for the uncooked test groups were significantly higher than the pathology score of the control group and the alkaline-cooked-low and alkaline-cooked-mid test groups. Only the alkaline-cooked-high group had a pathology score significantly higher than the control group, and no lesions (all scores = 0) consistent with FB_1 exposure were found in rats fed the alkaline-cooked-low or alkaline-cooked-mid diets (FB_1 at 0.08 and 0.13 mg/kg feed, respectively). The changes in total kidney sphinganine (sphinganine plus sphinganine 1-phosphate) in the test groups and control mirrored the pathology scores as did the total kidney sphingosine (sphingosine plus sphingosine 1-phosphate) (Voss et al., 2013).

The findings provide evidence that alkaline cooking of whole kernel maize reduces the potential toxicity of fumonisin-contaminated maize in rat kidney. Based on the kidney pathology scores and analysis of FB_1 in the non-nixtamalized diets, the NOAEL was less than 1.8 mg/kg diet (equivalent to less than 0.14 mg/kg bw per day assuming 1 mg/kg diet = 0.075 mg/kg bw). The Committee concluded that uncertainty in characterizing the diets made the study unsuitable for dose–response assessment.

Poultry

Vizcarra-Olvera et al. (2011) reported a 21-day feeding study in which 20 groups of 10 layer chickens (Bovan) were used to test the ability of clays to ameliorate the effect of fumonisin exposure. A negative control group was fed the basal diet only and the positive control group was fed a diet containing culture material from *F. verticillioides* (strain not specified; only FB_1 data reported) to provide FB_1 at 59 mg/kg feed (equivalent to approximately 15 mg/kg bw per day). A statistically significant increase in serum aspartate aminotransferase (AST) was observed in the FB_1-only treated group compared with the other groups. Moderate fibrosis was reported in the livers in three of four animals in the group exposed to culture material only. Both clays appeared to offer some protection against the reported fumonisin effects.

Rauber et al. (2012) conducted a study to evaluate the individual and combined effects of fumonisin (*F. verticillioides* MRC 826; FB_1 only reported) and *Salmonella typhimurium* lipopolysaccharide (sLPS) in broiler chicks. The chicks were fed control diets (n = 6 replicate groups) or diets containing FB_1 at 100 or 200 mg/kg feed (calculated to be approximately 7.9 and 15.8 mg/kg bw per day, based on the overall mean feed intake of 2209 g over the length of the study) for 28 days (n = 6 replicate groups/dose). The sLPS was dosed every other day by oral gavage on days 15–27. Only the results of the fumonisin treatment are described in detail here. Body weight, feed intake and feed conversion were assessed. Relative liver weight, total plasma protein, serum albumin, calcium, phosphorus, uric acid, alanine aminotransferase (ALT), AST, γ-glutamyltransferase, alkaline phosphatase as well as total cholesterol, triglycerides, Sa/So ratio and C-reactive protein were measured. The lining of the small intestine was morphologically evaluated.

Both fumonisin doses significantly reduced body weight and feed intake and increased relative liver weight. Total plasma protein, calcium, ALT, AST, γ-glutamyltransferase, Sa/So ratio, cholesterol and triglycerides were increased at 28 days. Significant reductions were seen in uric acid levels. Changes in the structure of the lining of the ileum were observed. Some of the effects were greatest in the combined treatment (fumonisin plus sLPS).

This same research group published a report (Rauber et al., 2013) with the same study design as the one described here (Rauber et al., 2012). The only difference was that the broiler chickens were not challenged with sLPS. Also, values for the various end-points on day 14 were reported as were the 28-day data.

Poersch et al. (2014) divided 24 broiler chickens between a control group (n = 12) fed a basal diet and a fumonisin-treated group (n = 12) fed a

diet prepared using culture material from *F. verticillioides* (MRC 826; FB_1 only reported) with FB_1 at 100 mg/kg feed for 21 days.

After 21 days, the fumonisin-treated group had elevated serum Sa/So ratios and increased absolute and relative liver weights. Hepatic thiobarbituric acid reactive substances (indicating lipid peroxidation), vitamin C and catalase were increased. The authors suggested that acute exposure of broiler chicks to FB_1 induced liver oxidative stress concomitantly with Sa/So accumulation.

In a feeding study, 1-day-old broiler chickens ($n = 204$; males and females) divided between two groups were fed the control diet or a diet containing 18.6 mg of FB_1 + FB_2 per kg feed (equivalent to fumonisin at approximately 2.25 mg/kg bw per day). The source of the fumonisin was *F. verticillioides* culture material (strain not specified).

This dose resulted in a significant increase in the plasma Sa/So ratio compared with the birds on the control diet. Antonissen et al. (2015b) reported changes in the structure of the ileum lining associated with this dose of fumonisins. Extracted DNA from samples of the gut microbiome showed a shift in the microbiota with a relatively low resolution method. This was characterized as a reduced abundance of *Candidatus*, *Savagella* and *Lactobacillus* spp. Quantification of total *Clostridium perfringens* in the ileal samples conducted using the *cpa* gene (for the alpha toxin) by quantitative polymerase chain reaction (qPCR) showed an increase in *C. perfringens* in treated chickens compared with controls. When the birds were challenged with an oral bolus of *C. perfringens*, a higher percentage of treated birds than control birds developed subclinical necrotic enteritis.

Catfish

This dose–response study in catfish was published before the 2011 JECFA but was not reviewed at that time. Catfish fingerlings (~17 g each) were stocked in 16 tanks with 20 fish/tank. *F. verticillioides* maize culture material (MRC 826) was used to prepare three treatment diets containing FB_1 at 5, 10 or 15 mg/kg diet. The control diet was prepared using clean maize. Each treatment included four tanks of fish (n = 80 fish/treatment). The fish were fed the control and test diets for 6 weeks. Significant effects on growth and performance were reported for all treatment groups but were most marked in the mid-dose group. Water quality was reduced for all test groups as indicated by significantly reduced levels of dissolved oxygen and increased concentration (6–7-fold) of ammonia. Significant haematological changes were also seen at all dose levels. For example, the number of leukocytes increased and erythrocytes decreased in a dose-dependent fashion. Likewise, there were many significant changes in serum biochemistry with many significant differences seen at the lowest dose level. The high ammonia levels and

low dissolved oxygen in the experimental tanks for all treatment groups made interpretation of the effects problematic (Gbore et al., 2010).

The Committee noted that the use of FB_1 from culture material and uncertainty in characterizing the diets made the study unsuitable for dose–response assessment.

Pigs

Castrated male crossbred piglets (4 weeks old; 10–11 kg initial and 28–32 kg final body weights) were divided between four treatment groups (*n* = 6/group) and fed diets containing FB_1 at 0, 3, 6 or 9 mg/kg diet (equivalent to 0, 0.12, 0.24 and 0.36 mg/kg bw per day). The source of the fumonisin was *F. verticillioides* (M-1325) culture material, which was analysed by liquid chromatography–mass spectrometry (LC-MS) before and after preparing the diets. Concentrations were found to be as follows: control diet, total fumonisin at 3.7 mg/kg feed (FB_1 at 3.08 mg/kg + FB_2 at 0.66 mg/kg); total fumonisin at 8.1 mg/kg feed (FB_1 at 6.11 mg/kg + FB_2 at 1.96 mg/kg); and total fumonisin at 12.2 mg/kg diet (FB_1 at 9.01 mg/kg + FB_2 at 3.17 mg/kg). The diets were analysed using a multi-mycotoxin method with liquid chromatography–tandem mass spectrometry (LC-MS/MS). Deoxynivalenol (DON) and ochratoxin A (OTA) were present at 0.025 and 0.006 mg/kg feed, respectively. After 4 weeks on the diets, the spleen, liver, lung, kidney and heart were collected and examined using haematoxylin and eosin stain.

At the end of the 28-day feeding period, there were no significant differences in body weight gain, feed consumption, relative organ weights or macroscopic changes at necropsy. Histopathological examination found no lesions in spleen, liver, kidney or heart; however, dose-dependent moderate to high interstitial inflammation and alveolar oedema were seen in the lungs at all doses. Quantification or statistical analysis of the observed pathology was not conducted. The Committee concluded that use of culture material and lack of statistical analysis made the study unsuitable for dose–response assessment (Souto et al., 2015).

In a study not previously evaluated by JECFA, castrated male crossbred piglets (4 weeks old) were paired based on similar growth rate and body weights (*n* = 18 controls; *n* = 18 FB_1-treated) and fed, for 9 days, either basal diet (assumed to be fumonisin free) or basal diet plus a daily oral bolus of FB_1 extract diluted in a 20% solution of glucose. Controls received the 20% glucose solution only. The FB_1 dose (1.5 mg/kg bw per day) was prepared from a partially purified extract of *F. verticillioides* culture material that contained 2.3 mg/mL of FB_1, 0.34 mg/mL of FB_2 and 0.38 mg/mL of FB_3. DON, fusarochromanone and trichothecenes were not detected in the extract. After 9 days of treatment, the gastrointestinal tracts

were collected for morphological assessment of the small intestines and analysis of select stress proteins and β-actin in stomach, jejunum and colon.

Fumonisin treatment had no significant effects on final body weight or feed intake. The feed conversion ratio was significantly lower and liver weight significantly higher ($P < 0.05$) in the fumonisin-treated animals. There were no significant differences in the morphometric measurements of villi and crypts in the small intestines. Comparison of the relative expression of the stress proteins showed that cyclooxygenase-1 (COX-1) and neuronal nitric oxide synthase (nNOS) were significantly elevated in the stomach of the treated piglets; heat shock protein 70 (HSP70) was significantly elevated in the jejunum; and αB-crystallin, COX-1 and haem oxygenase-1 (HO-1) were significantly elevated in the colon. The most marked changes were in the colon of the fumonisin-treated animals, where there was an 8-fold increase in αB-crystallin and a 12-fold increase in COX-1 (Lalles et al., 2010).

The authors concluded that the pig colon is highly sensitive to the deleterious effects of fumonisin. Many of the observed effects were likely to have been a consequence of oxidative stress. Due to the single dose level of FB_1 in culture material, a NOAEL could not be determined.

2.2.3 Long-term studies of toxicity and carcinogenicity

Since the seventy-fourth JECFA evaluation (Annex 1, reference *205*), there have been no new long-term carcinogenicity bioassays (chronic exposure for most of the lifespan of an animal) to determine the potential carcinogenic hazard and dose–response relationships of fumonisins.

2.2.4 Genotoxicity

The ability of a carcinogenic mycotoxin such as FB_1 to directly bind to DNA or be metabolized into a DNA-reactive compound is of great importance to the risk assessment (IARC, 2012, Chapter 7). In all previous evaluations by authoritative groups (IPCS, 2000; IARC, 2002; Annex 1, references *152*, *153*, *205* and *206*), there were no studies showing that FB_1 can interact directly with DNA or be metabolized into a DNA-reactive compound. Fumonisin does cause DNA damage in liver and kidney from increased apoptotic and necrotic (oncotic) cell death. When DNA damage is observed in vivo or in vitro, it is likely to be secondary to fumonisin inhibition of ceramide synthase (Müller, Dekant & Mally, 2012). Many studies have demonstrated that fumonisin causes oxidative stress both in vitro and in vivo, although the cause of the oxidative stress is unknown (Gelderblom & Marasas, 2012). However, it is likely to be secondary to FB_1-induced ceramide synthase inhibition, disruption of lipid metabolism, altered membrane properties and altered lipid-signalling pathways. Although there is still no evidence that FB_1

or any of its metabolites react directly with DNA, several studies since 2011 have shown that oxidative stress plays an important role in indirectly causing DNA damage in cultured cells and in vivo.

In vivo studies conducted since the previous JECFA evaluation and one study not previously reviewed are summarized in Table 3.

In male and female BALB/c mice, a single intraperitoneal dose of FB_1 (pure) at 0.1, 1.0 or 10 mg/kg bw had no effect on micronuclei induction in polychromatic and normochromic erythrocytes harvested from the marrow 24 hours post-injection (Karuna & Rao, 2013). The study was repeated with three doses at 0, 24 and 48 hours, also with no evidence of micronuclei induction. In an earlier study by another group (reviewed in IARC, 2002), the results of the same test in CF1 mice were positive but the intraperitoneal doses were 25 and 100 mg/kg bw in the single dose study. For comparison, the lowest-observed-adverse-effect level (LOAEL) in female B6C3F1 mice was 7.5 mg of pure FB_1 per kg bw per day in a 28-day feeding study (Annex 1, reference *206*, "Fumonisins (addendum)", Table 2). Assuming 10% oral absorption, the intraperitoneal dose of 25 mg/kg bw in the study positive for micronuclei induction would be equivalent to an oral dose of 250 mg/kg bw.

Several recent studies have confirmed earlier reports that pure fumonisin or culture material extracts containing fumonisin are involved in closely coupled DNA damage and oxidative stress (Theumer et al., 2010; Bernabucci et al., 2011; Mary et al., 2012) and that antioxidants can reduce or prevent toxicity, lipid peroxidation and/or evidence of DNA damage in vitro (Domijan et al., 2015) and in vivo (Sozmen et al., 2014; Hassan et al., 2015).

The source of the ROS that could cause DNA damage is not certain, but one study in cultured rat primary astrocytes and human neuroblastoma cells found pure FB_1 inhibition of mitochondrial complex I, which resulted in decreased respiration, depolarization of the transmembrane potential difference and induction of ROS (Domijan & Abramov, 2011). The mitochondrial dysfunction was followed by deregulation of calcium homeostasis. The potential for damage to DNA was implied but not demonstrated.

In ceramide synthase 2–null mice, spontaneous hepatopathy is characterized by increased apoptosis and cell proliferation, similar to what is seen in fumonisin-treated mice, most recently in $p53^{+/-}$ transgenic mice (Bondy et al., 2012). In the ceramide synthase 2–null mice, there are significant increases in expression of antioxidant genes and increased ROS production associated with C-16 ceramide and sphinganine impairment of mitochondrial complex IV activity. ROS levels are reduced by treatment with antioxidants but without effect on mitochondrial impairment (Zigdon et al., 2013). The reduction in ROS without preventing mitochondrial impairment is reminiscent of the findings in a study by Abel & Gelderblom (1998) in primary rat (F344) hepatocytes in which

Table 3
In vivo genotoxicity assays, end-points related to genotoxicity or end-points likely to cause DNA damage

End-point	Test system	Concentration / dose	Results	Reference
Micronuclei induction	Male and female BALB/c mice	Single dose of pure FB_1 i.p. at 0, 0.1, 1 or 10 mg/kg bw	Negative	Karuna & Rao (2013)
		Three doses of pure FB_1 i.p. at 0, 0.1, 1 or 10 mg/kg bw at 0, 24 and 48 hours	Negative	
DNA damage, in spleen mononuclear cells based on alkaline comet assay and micronucleus assay. Increased lipid peroxidation and catalase and superoxide dismutase activities	Male Wistar rats	Culture material extract in diets at 10.9 mg/kg feed	Positive	Theumer et al. (2010)
Increased apoptosis and mediators of apoptosis. Protection by an antioxidant	Female BALB/c mice	Partially purified FB_1 i.p. at 1.5 or 4.5 mg/kg bw	Positive	Sozmen et al. (2014)
Increased DNA damage (micronuclei), lipid peroxidation, decreased glutathione in liver and kidney and protection by an antioxidant	Female Sprague Dawley rats	Partially purified FB_1 in diet at 20 mg/kg feed	Positive	Hassan et al. (2015)

bw: body weight; FB_1: fumonisin B_1; i.p.: intraperitoneal

α-tocopherol totally prevented the FB_1-induced lipid peroxidation but only reduced lactate dehydrogenase release by approximately 30%.

While there is little or no convincing evidence for fumonisin being metabolized to a DNA-reactive metabolite, there is increasing evidence for the potential of epigenetic effects and subsequent effects on gene expression that might alter risk of disease. For example, Chuturgoon, Phulukdaree & Moodley (2014b) found that global DNA methylation and expression of DNA methyltransferases were modulated (increased or decreased) in human HepG2 hepatoma cells treated with cytotoxic doses of FB_1 for 24 hours. The comet assay found DNA hypomethylation associated with DNA migration patterns indicative of structural alterations. Global histone modifications have also been shown in rat kidney epithelial (NRK-52E) cells exposed to FB_1 (5–100 μmol/L) for up to 96 hours (Sancak & Ozden, 2015). These findings in cultured cells suggest hypomethylation of DNA and histone modifications as possible contributors to fumonisin toxicity and carcinogenicity.

Other recent studies suggest that FB_1-induced epigenetic changes can be mediated by disrupted sphingolipid metabolism. Specifically, FB_1 and FTY720 (see section 2.2.5(b)) both decreased histone deacetylase activity and increased acetylation of histones in the nuclear fractions of cultured mouse embryonic fibroblasts (Gardner et al., 2016a,b). These effects were associated with accumulation of sphinganine 1-phosphate or FTY720 1-phosphate in the nuclear

fractions, suggesting that nuclear accumulation of sphinganine 1-phosphate plays a role in histone modification.

2.2.5 Reproductive and developmental toxicity

Since the previous evaluation (Annex 1, reference *205*), there has been continuing interest in fumonisin exposure as a contributing factor in the high incidence of neural tube defects (NTDs) in humans in areas where maize is consumed in large amounts and fumonisin contamination is likely. The ability of fumonisin to interfere with folate transport by disrupting sphingolipid metabolism provides the mechanistic basis for concern. It should be noted that neural tube closure occurs early in pregnancy and in a very narrow window of time. Thus, while induction of NTDs in animal models probably requires dosages that also can induce maternal toxicity, prolonged exposure is not necessary and evidence of maternal toxicity can be lacking later in the pregnancy if exposure only occurred during the window of neural tube closure. (For a recent in-depth review of the reproductive and developmental toxicity of fumonisins, see Voss et al., 2017b.)

In vivo studies conducted since the previous evaluation (Annex 1, reference *205*) that add mechanistic information are summarized in Table 4. In vitro studies are summarized in the text (section 2.2.5(c)).

(a) In vivo studies using purified FB_1 dosed orally

Mice

Riley et al. (2015a) conducted a study in support of developing methods for validating the potential usefulness of sphingoid base 1-phosphates in blood spots for developing biomarkers of effect with UFB_1 as a biomarker of exposure in human studies. Pregnant LM/Bc dams were administered pure FB_1 by oral gavage on embryonic days 6.5, 7.5 and 8.5 at 0 (n = 4), 5 (n = 2), 10 (n = 2), 15 (n = 3), 25 (n = 3) or 50 (n = 2) mg/kg bw per day.

The frequency of exencephaly in the LM/Bc fetuses increased in a dose-dependent manner, although the sample size was very small (n = 2–4/treatment). No NTDs were observed in the controls or the 5 mg/kg bw per day group. Exencephalic fetuses were detected in litters of the three groups at 10 mg/kg bw per day and above (8/11 litters). The half-life of FB_1 in the urine was short (<24 hours), and the elevation in sphingoid base 1-phosphates in blood was also short although more persistent than the UFB_1. The peak levels (24–48 hours) of sphinganine, sphinganine 1-phosphate and sphingosine 1-phosphate in blood occurred after the peak level for FB_1 in urine (<4 hours). The results of the sphingolipid analysis showed dose–response relationships for the levels of (1) elevated sphinganine in liver, kidney, blood spots and embryos; (2) elevated 1-deoxysphinganine in liver and kidney; (3) sphinganine 1-phosphate in kidney

Table 4
Overview of studies on reproduction and developmental toxicity of fumonisins

Species / description	Length of study	No. per group	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female LM/Bc mice, 60–90 days old, weight not stated	Pure FB_1 treatment on ED 6.5, 7.5 and 8.5 with embryos examined on ED 9.5	2–4	0, 5, 10, 15, 25 or 50	Oral gavage	No. of embryos with NTDs increased in a dose-dependent manner (≥10 mg/kg bw per day). Dose-dependent increased maternal UFB_1 and sphingoid base 1-phosphates in maternal blood and tissues	10	5	Riley et al. (2015a)
Female CD1 mice, 35–40 g	Pure FB_1 treatment on ED 7.5 and 8.5 and collect embryos on ED 10.5 and 17.5	3–7	12.5	Oral intubation	On ED 17.5 there were 4 NTD-positive litters ($n = 7$ dams) in the FB_1-only treatment group. Treatment with an FB_1 binder reduced FB_1 by 86% in the plasma, and 1/5 dams treated with FB_1-plus-binder had a positive litter (1 exencephalic embryo in 57 fetuses collected from 5 litters)	12.5	<1.75[a]	Liao et al. (2014)

bw: body weight; ED: embryonic day; FB_1: fumonisin B_1; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; NTD: neural tube defect; No.: number; UFB_1: urinary FB_1

[a] An 86% reduction in dose would equate to FB_1 at 1.75 mg/kg bw per day, which could arguably be taken as the LOAEL or very close to the NOAEL because out of five litters with a total of 57 fetuses, only one was a positive NTD.

and blood spots; and (4) sphingosine 1-phosphate in blood spots. In blood spots from the dams, FB_1-induced changes in sphinganine, sphinganine 1-phosphate and sphingosine 1-phosphate were significantly positively correlated with UFB_1 (Riley et al., 2015a).

The results support the utility of using both UFB_1 and blood spot sphingoid base 1-phosphates as measures for assessing potential biological effects in human populations consuming large quantities of fumonisin-contaminated maize-based foods. The Committee concluded that the small number of mice per dose group makes this dose–response study unsuitable for modelling.

As part of a larger study on the safety of nanosilicate platelets (NSP) and their efficacy in preventing fumonisin-induced toxicity, Liao et al. (2014) divided pregnant CD1 mice (35–40 g) between four groups: a control group gastric-intubated with water; an NSP-only group gastric-intubated with 100 µg NSP; an FB_1-only group gastric-intubated with 500 µg pure FB_1 (12.5 mg/kg bw per day); and an NSP-plus-FB_1 group gastric-intubated with NSP and FB_1. All four groups were dosed on embryonic days 7.5 and 8.5. After the last dose, a blood sample was collected. Mice were killed on embryonic day 10.5 or 17.5, and fetuses examined for NTDs. Total RNA was extracted from tissues of a subset

of mice from each treatment group killed on embryonic day 10.5; this RNA was examined for expression of proteins involved in sphingolipid biosynthesis and turnover.

Examination of fetuses collected on embryonic day 10.5 found one fetus with a mild NTD and one with a brain haemorrhage (out of a total of 40 fetuses collected from four pregnant mice). No abnormalities were observed in the 35 fetuses collected from the NSP-plus-FB_1 group (n = 3). In the fetuses collected on embryonic day 17.5, there were no exencephalic fetuses in the control group (n = 0/6) or NSP-only group (n = 0/5). In the FB_1-only group (n = 7 dams), there were four positive litters (with at least one exencephalic fetus). In the NSP-plus-FB_1 group (n = 5 dams), there was one positive litter. The gene expression results provide evidence that, in the FB_1-only group, sphingosine 1-phosphate phosphatase was upregulated in maternal liver and sphingosine 1-phosphate phosphatase and sphingosine 1-phosphate lyase were upregulated in the uterus, suggesting that maternal tissues had accumulated sphingoid base 1-phosphates and were working to eliminate them. The fetuses and placenta from the FB_1-only group showed downregulation of sphingosine kinase 1 and sphingosine 1-phosphate lyase, and the fetuses, downregulation of ceramide synthase. There were no changes in expression in any of the other treatment groups, confirming that NSP treatment was protective.

Analysis of the blood collected from the dams on embryonic day 8.5 showed an approximate 86% reduction in plasma concentration of FB_1 in the NSP-plus-FB_1 group compared with the FB_1-only group. As the FB_1 dose was 12.5 mg/kg bw per day, and the measured internal dose was reduced by 86% in the NSP-plus-FB_1 group, in which 1/57 fetuses collected on embryonic day 17.5 was exencephalic, an 86% reduction in dose would equate to FB_1 at 1.75 mg/kg bw per day, which could arguably be taken as the LOAEL (Liao et al., 2014).

Rats

Pellanda et al. (2012) fed male and female Wistar rats (3.5 months old) for 30 days either a control (folate, vitamin B_{12} and choline–sufficient) or a methyl-deficient (folate, vitamin B_{12} and choline–deficient) diet with or without daily oral gavage of pure FB_1 at 4 µg/kg bw. The FB_1 dose was chosen to be twice the JECFA PMTDI of 2 µg/kg bw. After 30 days on the control or methyl-deficient diet (MDD), the rats were mated and pregnant dams divided between four treatment groups: (1) control (n = 13); (2) MDD-only (n = 15); (3) FB_1-only (n = 2); and (4) MDD-plus-FB_1 (n = 3). The animals were kept on the control or MDD-only diets during mating and gestation. The dams were killed on gestation day 20, and blood samples and livers were collected from all dams to measure vitamin B_{12}, folate and homocysteine concentrations. It should be noted that only

two dams from the control group and three dams from the MDD-only treatment group were included in the following statistical summary (see Pellanda et al., 2012, Tables 1 and 2).

The MDD-only group had significantly decreased maternal plasma folate and vitamin B_{12} and increased homocysteine compared with the control group. The FB_1-only group had decreased maternal folate and vitamin B_{12}. The MDD-plus-FB_1 group had significantly increased homocysteine levels compared with the MDD-only group. In the MDD-only group, pericentrilobular steatosis (fatty liver) was rated as grade 1, whereas in the MDD-plus-FB_1 group it was rated as grade 2 (33–66% parenchymal involvement). Fetal body weights and lengths of the MDD-only group (n = 3) and the MDD-plus-FB_1 group (n = 3), but not the FB_1-only group (n = 2), were significantly decreased. Fetal liver tissues from the FB_1-only group (n = 2), the MDD-plus-FB_1 group (n = 3), subsets of the control group (n = 2) and MDD-only group (n = 3) were analysed. MDD-only and MDD-plus-FB_1 fetuses had significantly less folate and vitamin B_{12} than the controls. Livers from FB_1-only fetuses had *S*-adenosylmethionine/*S*-adenosylhomocysteine ratios decreased to the same extent as the MDD-only or the MDD-plus-FB_1 fetuses. The MDD-only fetuses showed upregulation of the folate receptor and reduced folate carrier (*Slc19al*) transcripts compared with controls. *Slc19a1* was elevated in the MDD-only fetuses compared with controls. Folate receptor transcripts were lower in the MDD-plus-FB_1 fetuses than the MDD-only fetuses. When fetal liver histone modifications were compared, H4K20me3 was significantly decreased and H3K9me3 significantly increased in the MDD-only and MDD-plus-FB_1 fetal livers relative to the controls. MDD-plus-FB_1 treatment significantly elevated H3R2me2 compared with MDD-only treatment (Pellanda et al., 2012).

In light of the very small number of fumonisin-treated dams (n = 2 or 3) and the selection of the subset of embryos for the analysis of fetal livers, any implications for the risk of fumonisin are problematic.

(b) In vivo studies using intraperitoneal dosing

Female LM/Bc mice were fed folate and vitamin B_{12}–sufficient (control) or folate and vitamin B_{12}–deficient diets from 5 weeks before mating. After mating, confirmed pregnant dams continued on the assigned diets and were given intraperitoneal injections of pure FB_1 at 0, 2.5 or 10 mg/kg bw on embryonic days 7 and 8. The dams and litters were necropsied on embryonic day 16 (n = 9–13/treatment). Dose-dependent induction of NTDs (exencephaly) was found in groups fed the folate/vitamin B_{12}–sufficient control diet. In the high-dose group, 10 of 11 litters were positive for NTDs. In the groups fed the folate-deficient diet, NTDs were found only in 4 of 11 high-dose litters (Voss, Riley & Gelineau-van Waes, 2014).

In the second trial (n = 5–8/treatment), consumption of a folate-deficient diet also resulted in fewer NTDs and reduced maternal RBC folate levels by 80%. While some reproductive findings were statistically significantly different between treatment groups, for the most part effects on implantations, resorptions, viable fetus weight, late fetal death and placenta weight were all minimal.

It was concluded that in utero death did not fully account for the differences in NTD rates and that folate deficiency did not exacerbate NTD induction by FB_1 in LM/Bc mice. A NOAEL could not be determined (Voss, Riley & Gelineau-van Waes, 2014).

In vivo experiments examined the potential role of sphingosine 1-phosphate receptor activation during neurulation as a contributing factor in the NTD phenotype observed after FB_1 exposure. Inbred female SWV and LM/Bc mice were mated, and pregnant mice injected intraperitoneally with pure FB_1 at 20 mg/kg bw on embryonic days 7.5 and 8.5 or gavaged orally with 10 mg/kg bw of pure FTY720 on embryonic days 6.5, 7.5 and 8.5. FTY720 is a water-soluble synthetic sphingoid base analogue that can be phosphorylated by sphingosine kinase, the same enzyme that phosphorylates sphinganine.

FTY720 treatment resulted in exencephaly in both the SWV and LM/Bc strains of mice. An oral FTY720 dose of 5 mg/kg bw per day also induced exencephaly in the LM/Bc mouse using the same protocol. In both SWV and LM/Bc mouse strains, FB_1 treatment significantly elevated sphinganine, sphingosine 1-phosphate and sphinganine 1-phosphate in maternal blood spots. Plasma levels of sphinganine and sphinganine 1-phosphate were significantly elevated by FB_1 treatment, with the increase much greater in the LM/Bc strain than the SWV strain. Levels of FTY720-1-phosphate were greater than the parent FTY720 in blood spots and in plasma; the level of FTY720-1-phosphate was significantly greater in LM/Bc mice than in SWV mice (in both blood spots and plasma). In agreement with the trend observed in FB_1-treated mice, the concentration of the phosphorylated metabolite was significantly greater in the blood spots than in plasma. Blood spots taken from LM/Bc and SWV mice on embryonic days 9.0, 10.5, 11.5 and 12.5 showed that the half-life of these compounds in the blood was very short. Sphinganine was significantly elevated in the embryos of FB_1-treated dams, in agreement with previously reported results. The concentration of sphinganine in the embryos of FB_1-treated LM/Bc dams was 5-fold and 10-fold higher than their respective controls. However, the difference between the SWV and LM/Bc FB_1-treated embryos was not significant, even though the mean concentration in the LM/Bc embryos was 2.7-fold greater than that in the SWV embryos. The results support the hypothesized role of altered sphingosine 1-phosphate receptor signalling in fumonisin-induced NTDs in mice.

A NOAEL could not be determined (Gelineau-van Waes et al., 2012).

(c) In vitro studies using purified FB_1

Cultured serum free mouse neural progenitor cells exposed to pure FB_1 accumulated both sphingosine 1-phosphate and sphinganine 1-phosphate (Gelineau-van Waes et al., 2012). Accumulation of sphinganine and sphinganine 1-phosphate was significantly higher in mouse embryonic fibroblasts prepared from the fumonisin-induced NTD-susceptible LM/Bc mouse strain than in the fumonisin-induced NTD-resistant SWV mouse strain. This was the same relative strain sensitivity as seen in vivo.

This same strain-specific response (LM/Bc > SWV) in sphinganine and sphinganine 1-phosphate levels was seen in a subsequent study with mouse embryonic fibroblasts in both the nuclear and cytoplasmic compartments (Gardner et al., 2016a). The levels of accumulation of sphinganine and sphinganine 1-phosphate were significantly higher in the nuclear fractions of both strains than in the cytoplasmic fraction. In the nuclear fraction, there was a concurrent strain-dependent decrease in histone deacetylase activity in the fumonisin-treated cells accompanied by increased histone acetylation (Gardner et al., 2016a).

In a companion study with the sphingoid base analogue FTY720 and LM/Bc mouse embryonic fibroblasts, FTY720 was phosphorylated to FTY720 1-phosphate in both the cytoplasmic and nuclear fraction (Gardner et al., 2016b). Histone deacetylase activity was significantly decreased and histone acetylation significantly increased. It was concluded that because other histone deacetylase inhibitors are known to cause NTDs, elevated nuclear sphingoid base 1-phosphates, histone deacetylase inhibition and histone hyperacetylation may have a potential role in the failure of neural tube closure after FB_1 or FTY720 treatment.

In human embryonic stem cell–derived neural epithelial progenitor cells, pure FB_1 induced the accumulation of sphinganine and sphinganine 1-phosphate (Callihan et al., 2012). Thus, normal human embryonic cells responded to ceramide synthase inhibition in a similar way to what was seen in mouse neural progenitor cells.

Several in vitro studies have used pure FB_1 to demonstrate the deleterious effects of de novo ceramide production on developmental aspects of oocyte complexes. For example, Lolicato et al. (2015) exposed bovine cumulus–oocyte complexes to FB_1 at 50 μmol/L for 23 hours in order to confirm the involvement of de novo ceramide production in the lipotoxic effects of supplementation with saturated fatty acids. Fumonisin treatment protected the cumulus complexes from damage (apoptosis) and suppressed ROS resulting from mitochondrial deterioration.

In another study with bovine cumulus–oocyte complexes, Kalo & Roth (2011) showed that treatment with 5 or 25 μmol/L FB_1 partially protected against

the effects of 22 hours of heat shock treatment, suggesting de novo ceramide production involvement in heat shock–induced apoptosis. Studies such as these underscore the complexity of fumonisin-induced toxicity, with numerous bioactive lipid pools altered as a consequence of ceramide synthase inhibition (Fig. 1 and Fig. 2). In this study and the previously summarized study (Lolicato et al., 2015), the focus was de novo ceramide-induced apoptosis and ROS, but the fact is that 22–23 hours of exposure of cells to micromole per litre concentrations of FB_1 is highly likely to have resulted in much more than decreased ceramide production de novo but also elevated sphinganine and sphinganine 1-phosphate, which are ligands for sphingosine 1-phosphate receptors (Annex 1, reference *206,* "Fumonisins (addendum)", Fig. 2).

Increased levels of ceramide promote insulin resistance whereas sphingosine 1-phosphate is associated with increased insulin sensitivity (Chavez & Summers, 2012).

Adiponectin is a hormone that inhibits insulin signalling in the placenta. Aye et al. (2014) hypothesized that adiponectin inhibition in primary human trophoblasts was due to peroxisome proliferator–activated receptor-α (PPARα)-mediated de novo ceramide biosynthesis. Primary human trophoblasts isolated from human placenta were cultured for 65.5 hours and then treated with pure FB_1 at 50 µmol/L followed by adiponectin treatment at 66 hours. The trophoblasts were harvested at 90 hours.

The results obtained with FB_1 provided additional evidence for adiponectin-mediated trophoblast insulin resistance being dependent on ceramide biosynthesis. Inhibition of ceramide synthase with FB_1 reversed the inhibitory effects of adiponectin on insulin signalling and amino acid transport. The effects of FB_1 on other sphingolipid pools were not examined, but other studies, including one in insulin-resistant hepatocytes from "old" rats, have shown that fumonisin inhibition of ceramide synthase improves insulin regulation of glucose metabolism (Babenko & Kharchenko, 2015).

The enzyme 10-formyltetrahydrofolate dehydrogenase plays a key role in regulating folate metabolism. Rapidly proliferating cancer cells often lack this enzyme, and reconstitution of 10-formyltetrahydrofolate dehydrogenase in these cells has strong antiproliferative effects (cell cycle arrest, inhibition of motility, apoptosis), referred to as the folate stress response. Hoeferlin et al. (2013) used pure FB_1 to confirm that the folate-induced stress response is mediated by upregulation of de novo ceramide biosynthesis. In addition, FB_1-treated cancer cells reconstituted with 10-formyltetrahydrofolate dehydrogenase grew normally, similarly to cancer cells not transfected with the enzyme, and were resistant to apoptosis. Interestingly, folate withdrawal results in elevated ceramide, a process that would be inhibited by FB_1 and might have implications in humans consuming fumonisin-contaminated maize as a dietary staple.

2.2.6 Special studies

(a) Immunotoxicity

In the 2011 evaluation, in vivo studies in mice, rats and pigs documented the immunotoxic effects of purified FB_1 and effects associated with consumption of diets containing fumonisins from culture material (Annex 1, reference *206*, "Fumonisins (addendum)", Table 7). Since 2011, additional in vivo and in vitro studies have documented the immunotoxic effects of fumonisin and, in particular, the ability to alter the response to infectious agents. In vitro studies are summarized in the text. All new in vivo studies that add new mechanistic information are summarized in Table 5.

In vivo studies using pure FB_1

An in vivo study was designed to confirm the findings from in vitro studies (summarized below under "In vitro studies using pure FB_1"), using cell lines, that showed that de novo sphingolipid biosynthesis was required for phagocytosis of *Candida albicans*. C57BL/6 mice (sex and age not specified) were divided between five treatment groups (n = 10/group) as follows: control plus ultraviolet (UV)-irradiated *C. albicans*; control plus live *C. albicans*; FB_1 plus UV-irradiated *C. albicans*; FB_1 plus live *C. albicans*; and FB_1-only (Tafesse et al., 2015). FB_1 was administered subcutaneously by injection at 2 mg/kg bw per day for 14 days. The dose was chosen based on a preliminary study that showed that 2 mg/kg bw per day for 5 days elevated sphingoid bases and depleted complex sphingolipids in peritoneal macrophages and liver but caused no gross differences in health or behaviour compared with untreated mice. After five daily injections of FB_1, mice received a single inoculation via the tail vein of either live or UV-irradiated (killed) *C. albicans* and continued to receive daily injections of FB_1 for 9 more days.

At 9 days post-injection, no animals survived in the FB_1-plus-live-*C. albicans* group, whereas survival was 100% (i.e. no mice died) in all other groups. Both kidney and brain were heavily colonized with *C. albicans* in the FB_1-plus-live-*C. albicans* group. It was concluded that the cause of death was systemic candidiasis. The results were consistent with the findings from the in vitro studies showing that phagocytosis requires sphingolipid biosynthesis.

A NOAEL for FB_1 could not be determined.

In vivo studies using *F. verticillioides* culture material containing fumonisins

Weaned Duroc × Landrace cross or Kahyb pigs (mixed sex; 4 weeks old; weight unknown) were used in four experiments (n = 5–11/experiment), one of which is described here because it involved fumonisin treatment. The purpose was to see if fumonisin predisposed pigs to enteropathogenic *E. coli* (EPEC) attachment and

Table 5
Overview of new in vivo studies on immunotoxicity of fumonisins

Species / description	Length of study	No. per group	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
C57BL/6 mice, age, sex and weight unknown	14 days dosing with pure FB_1 and day 5 single inoculation of *Candida albicans*	10	2	Subcutaneous	Depletion of complex sphingolipids in peritoneal macrophages and liver. No animals survived beyond 9 days post-injection in the FB_1-plus-live *C. albicans* group. Kidney and brain were heavily colonized. Death was due to systemic candidiasis.	2	–	Tafesse et al. (2015)
Male and female crossbred pigs, 4 weeks old, weight unknown	15 or 16 days total with first 12 days dosing with *F. verticillioides* culture material extracts followed by inoculation with EPEC	5–11	0.5	Intragastric gavage	Fumonisin did not predispose pigs to EPEC attachment and induction of ileal-attaching effacing lesions.	–	0.5	Malik, Toth & Nagy (2012)
Female piglets (breed not specified), 3 days old, 2 kg at start and 9 kg at end of study	39 days Inoculation with *Bordetella bronchiseptica* on day 4 and *Pasteurella multocida* on day 16. Dosing with *F. verticillioides* culture material on days 16–39	7	0.5 (FB_1 at 10 mg/kg diet)	Oral	Elevated Sa/So ratio and increased risk of pneumonia and extent and severity of the lung lesions.	0.5	–	Pósa et al. (2011)
Female piglets, Seghers hybrid herd, 3 days old	58 days[a] Dosing with *F. verticillioides* culture material extracts on days 16–58. Inoculation with *Mycoplasma hyopneumoniae* on day 30	7	1 (20 mg/kg diet)	Oral	Elevated Sa/So ratio and exacerbation of *M. hyopneumoniae* infection based on mild to "strong" interstitial oedema at necropsy and increased permeability of blood vessels.	1	–	Pósa et al. (2009, 2013, 2016)
Male and female Large White pigs, 11 weeks old, 42 kg	63 days fed diets made with maize naturally contaminated with FB_1 and FB_2. On day 7 inoculated with *Salmonella enterica*	12	0.63 mg/kg bw per day ($FB_1 + FB_2$) (12 mg/kg diet)	Oral	Increased Sa/So ratios in serum, liver and kidney. In the words of the authors, the potential of FB as a predisposing factor to disease at the dose used in this study is "questionable".	–	0.63 ($FB_1 + FB_2$)	Burel et al. (2013)

bw: body weight; FB_1: fumonisin B_1; EPEC: enteropathogenic *E. coli*; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; Sa/So: sphinganine/sphingosine
[a] First 15 days on milk replacement with no other treatment.

induction of ileal-attaching effacing lesions. The negative control group received only nonpathogenic commensal porcine *E. coli*. The EPEC and control bacteria were inoculated intragastrically for 2–3 days as monocultures, and the piglets were killed 3–4 days after inoculation. Crude extracts of *F. verticillioides* culture material containing FB_1 at 0.5 mg/kg bw per day were administered by intragastric gavage for 12 days prior to inoculation with the bacteria. A therapeutic dose of dexamethasone (0.9 mg/kg bw per day) was injected for 3 days preceding the bacterial inoculation to 50% of the piglets (1 = control and 2 = EPEC treated).

None of the piglets in the experimental groups treated with fumonisin and fumonisin-plus-dexamethasone showed any EPEC attachment or induction of ileal-attaching effacing lesions (Malik, Toth & Nagy, 2012).

The authors concluded that in order to produce colonization, the dose of fumonisin applied must be extreme. No statistical analysis was conducted. The NOAEL for the parameters measured was 0.5 mg/kg bw per day.

Healthy female piglets (breed not specified; n = 28; 3 days old) were selected from sows free from *Pasteurella multocida* and with a low prevalence of *Bordetella bronchiseptica* infection and divided between four treatment groups (n = 7/group): control; fumonisin only; *P. multocida*/*B. bronchiseptica*; and *P. multocida*/*B. bronchiseptica* plus fumonisin. The *P. multocida*/*B. bronchiseptica* and the *P. multocida*/*B. bronchiseptica*-plus-fumonisin groups were inoculated intratracheally with *B. bronchiseptica and P. multocida* on days 4 and 16, respectively. From days 16 to 39, the fumonisin-only and the *P. multocida*/*B. bronchiseptica*-plus-fumonisin groups were fed diets prepared with extracts of *F. verticillioides* culture material containing fumonisins with FB_1 at 10 mg/kg diet (equivalent to approximately 0.5 mg/kg bw per day).

Both groups treated with *P. multocida*/*B. bronchiseptica* (with and without fumonisin treatment) showed clinical signs of respiratory infection from day 4 onward. Computed tomography showed lung lesions on day 16 onward with increasing severity with time in these groups, while no lung lesions were detected in the control and fumonisin-only groups. Elevated blood Sa/So ratios were detected in both fumonisin treatment groups on day 39. The gross pathological findings confirmed the computed tomography findings. Necropsy on day 39 found the incidence of lung lesions to be greatest in the *P. multocida*/*B. bronchiseptica*-plus-fumonisin group. This was consistent with the conclusion that dietary fumonisin can increase the risk of pneumonia and the extent and severity of the lung lesions (Pósa et al., 2011).

A NOAEL for FB_1 could not be determined because only one dose level was used.

Three studies conducted using computed tomography evaluated the interaction between fumonisin exposure and the extent and severity of lung

lesions in piglets experimentally infected with *Mycoplasma hyopneumoniae* (Pósa et al., 2009, 2013, 2016). Each study used 3-day-old female piglets from a Seghers hybrid herd. The piglets were selected from sows that were serologically negative for *M. hyopneumoniae*. The 28 piglets were divided between four treatment groups (n = 7/group): control; fumonisin only; *M. hyopneumoniae* only; and *M. hyopneumoniae* plus fumonisin. The *M. hyopneumoniae*-only and *M. hyopneumoniae*-plus-fumonisin groups were inoculated intratracheally with *M. hyopneumoniae* on day 30. From days 16 to 58, the fumonisin-only and *M. hyopneumoniae*-plus-fumonisin groups were fed diets prepared with extracts of *F. verticillioides* culture material containing fumonisins to contain FB_1 at 20 mg/kg diet (equivalent to approximately 1.0 mg/kg bw per day).

The results of the first study (Pósa et al., 2009) showed elevated body temperature from day 31 onward and clinical signs of respiratory infection from day 37 onward in the two groups inoculated with *M. hyopneumoniae* on day 30. Computed tomography showed lung lesions from day 44 onward, with increasing severity with time, in the two groups inoculated with *M. hyopneumoniae*. No lung lesions were detected in the control or fumonisin-only groups using computed tomography. No lung lesions were found at necropsy in the control group but four of the fumonisin-only treated piglets showed mild interstitial oedema. All *M. hyopneumoniae*-only and *M. hyopneumoniae*-plus-fumonisin treated animals exhibited catarrhal pneumonia. The ratios of the non-air-containing lung area to air-containing lung area in both *M. hyopneumoniae* inoculated groups were not significantly different, but both were significantly higher than the control and fumonisin-only groups. The ratios of the non-air-containing lung area to air-containing lung area in the control and fumonisin-only groups were not significantly different. The authors also stated that the macroscopic lung lesions were greatest in the *M. hyopneumoniae*-plus-fumonisin group, but the data presented do not support that statement.

The results of the second study (Pósa et al., 2013) showed that the average pulmonary density of the lungs of the two groups inoculated with *M. hyopneumoniae* were significantly higher than either the control or fumonisin-only groups. On day 58, elevated blood Sa/So ratios were detected in both the fumonisin groups. As in the first study, no lung lesions were found at necropsy in the control group, but all of the fumonisin-only piglets showed mild interstitial oedema and all the animals in both the *M. hyopneumoniae*-only and the *M. hyopneumoniae*-plus-fumonisin groups exhibited catarrhal pneumonia. The *M. hyopneumoniae*-plus-fumonisin groups also exhibited pulmonary oedema at necropsy. The histological alterations were greatest in the *M. hyopneumoniae*-plus-fumonisin group.

In the third study (Pósa et al., 2016), the treatment-related effects in the lung were similar to what was reported in the previous two studies, although

in the fumonisin-only piglets interstitial oedema in the lungs was characterized as "strong", whereas in the previous studies it was reported as "mild". Likewise, the pathomorphological changes reported in the lungs of the piglets in the *M. hyopneumoniae*-plus-fumonisin group were more pronounced than in either the *M. hyopneumoniae*-only or fumonisin-only group. Pósa et al. (2016) also reported perivascular and especially pericapillary oedema in the lungs, brain, cerebellum and kidneys in both the fumonisin groups, which was attributed to fumonisin-induced increased permeability of blood vessels.

The authors concluded that consumption of fumonisin-contaminated feed may exacerbate *M. hyopneumoniae* infection. A NOAEL for FB_1 interaction with *M. hyopneumoniae* could not be determined because only one dose level of culture material containing FB_1 was used in these studies.

In a study measuring the effects of chronic exposure to fumonisins from naturally contaminated feeds on growing pigs infected with *Salmonella* spp., pathogen-free Large White piglets (n = 48, one third females and two thirds castrated males; 4 weeks old) were divided between four groups (Burel et al., 2013). At 11 weeks of age (41.6 kg), the pigs were fed the following diets for 1 week: the control group (n = 24) was fed a diet formulated with fumonisin-free maize, and the fumonisin-treated (n = 24) group was fed a diet formulated with maize naturally contaminated with fumonisins so as to contain FB_1 at 8.6 mg/kg diet and FB_2 at 3.2 mg/kg diet. Both diets were analysed by LC-MS for a wide range of mycotoxins and were positive for only aflatoxin B_1 (<0.24 µg/kg) and DON (<131 µg/kg). After 1 week on the diets, half of the pigs (n = 12 control/*Salmonella*-treated and n = 12 fumonisin/*Salmonella*-treated) from each group were orally inoculated with *Salmonella enterica* Typhimurium in order to provoke asymptomatic carriage and excretion. The remaining 24 pigs received placebo inoculation (n = 12 control/placebo-treated and n = 12 fumonisin/placebo-treated). Two days after the inoculation, four pigs from each group were killed and necropsied, and tissues were collected. The rest of the pigs remained on their respective diets for 8 more weeks, at which time they were also killed and necropsied, and their tissues collected. Faecal samples were collected weekly, and blood samples were collected weekly or every other week.

There was a significant increase in the Sa/So ratio in serum, liver and kidney at days 9 and 63 post-inoculation. There were no effects on growth performance or feed intake in any of the treatment groups nor were there any clinical signs associated with *Salmonella* inoculation. The measured FB_1 and total fumonisin intakes (FB_1 + FB_2) were 0.46 mg/kg bw per day and 0.63 mg/kg bw per day, respectively. The number of seropositive pigs was consistently lower in the fumonisin/*Salmonella*-treated group but the difference was only significant at 28 days post-inoculation compared with the control/*Salmonella*-treated group. Fumonisin treatment had no effect on the intensity of seroconversion

or on mitogen-stimulated lymphocyte proliferation. There was little evidence (significant at one sampling time) of any effect of fumonisin treatment on *Salmonella*-specific antigen-stimulated lymphocyte proliferation. There was some evidence for a transient change in the faecal microbiota profile in the fumonisin/*Salmonella*-treated group compared to the other treatment groups (Burel et al., 2013).

In the words of the authors, the potential of FB_1 as a predisposing factor to disease at the dose (0.46 mg FB_1/kg bw per day) used in this study is "questionable". The NOAEL for the measured toxicological end-points, excluding the increased Sa/So ratio, was on average close to 0.46 mg/kg bw per day (0.63 mg total fumonisin/kg bw per day). The Committee concluded that this study was not suitable for dose–response modelling.

In vitro studies using pure FB_1

Mahmoodi et al. (2012) used human peripheral lymphocytes, gastric epithelial cell line (AGS) and the human adenocarcinoma cell line (SWV742) to assess the ability of fumonisin B_1 to affect lipopolysaccharide-induced selected cytokine production and secretion into culture medium. The results showed that fumonisin treatment induced dose-dependent decreased cell viability, increased levels of tumour necrosis factor-α and interleukin-1β and decreased levels of interleukin-8 in the supernatant of the culture medium from all three cell types after stimulation with lipopolysaccharide (Mahmoodi et al., 2012).

The macrophage cell line RAW264.7 and dendritic cell line DC2.4 dosed with or without FB_1 or myriocin (inhibitor of serine palmitoyltransferase) were used to determine whether de novo sphingolipid biosynthesis was required for phagocytosis of *C. albicans*. The depletion of complex sphingolipids by FB_1 or myriocin was confirmed by LC-MS. In both cell lines the biosynthesis of sphingolipids was necessary for phagocytosis of *C. albicans*. To further confirm the in vitro findings, serine palmitoyltransferase–knockout DC2.4 cells were created; these did not produce complex sphingolipids and were defective in their ability to phagocytose *C. albicans* compared to wild-type DC2.4 cells. Phagocytic activity was restored by supplementing the growth medium with ganglioside GM1 (Tafesse et al., 2015).

Confirmatory in vivo studies in mice are described above under "In vivo studies using pure FB_1".

Flaviviruses such as West Nile virus and dengue virus alter cellular lipid pools during infection, which appears to have a role in virus replication. Changes in sphingolipid pools associated with West Nile virus, dengue virus and hepatitis C virus (HCV) infection have been shown. Aktepe, Pham & Mackenzie (2015) used FB_1 to explore how altering endogenous ceramide levels can affect

viral replication. Vero cells were treated with myriocin or FB_1 and subsequently infected with West Nile or dengue virus. Prolonged treatment showed that ceramide biosynthesis is necessary for West Nile virus replication and secretion, but the opposite is true for dengue virus where ceramide depletion increased replication.

The inhibitory activity of FB_1 against HCV replication has been patented in the USA as a pharmaceutical agent for treating HCV infections (Sudo & Sakamoto, 2012). The inventors claim that the agent inhibits the replication of RNAs and the expression of HCV proteins. They also claim to have produced serine palmitoyltransferase knockouts and that the activities of the HCV replicon and HCV-protein expression were inhibited significantly in cells lacking serine palmitoyltransferase. Sphingolipid biosynthesis was shown to be involved in infection.

FB_1 inhibition of sphingomyelin biosynthesis in cultured HeLa and human lymphoid Jurkat cells reduced the number of infectious *Chlamydia trachomatis* progeny (Kubo et al., 2012). This study shows that eukaryotic host sphingolipids are important for maintaining the intracellular growth of *C. trachomatis*.

Human gastric epithelial mucosa cells (GES-1) were used to study whether FB_1 could affect expression of membrane human leukocyte antigen (HLA) class 1 (Yao et al., 2011). HLA are membrane proteins that regulate the human immune system and provide for effective immunosurveillance. Fumonisin treatment (24 hours; 5–20 μmol/L) significantly decreased HLA-A and HLA-B mRNA compared to control. HLA-A, B, C proteins were also significantly reduced. The inhibition was time- and dose-dependent. Expression of the *LMP2* and *TAP1* genes was also affected.

(b) Neurotoxicity

In previous evaluations, the most notable neurological effect of fumonisin was the induction of ELEM. It is now believed that ELEM is a result of vascular deregulation because of fumonisin-induced disruption of sphingolipid metabolism, most likely elevation in sphingoid base 1-phosphates and alterations in sphingoid base 1-phosphate signalling pathways (see Fig. 2 above). In mice, fumonisin treatment causes high levels of 1-deoxysphingoid bases to accumulate (Bondy et al., 2012), and although there is no evidence that this can also occur in humans exposed to fumonisin, there is considerable evidence that 1-deoxysphingoid base accumulation due to a mutation in serine palmitoyltransferase and the production of 1-deoxysphingolipids is the cause of the disease known as human sensory neuropathy type 1 (Penno et al., 2010).

Since the previous evaluation there have only been a few studies investigating the potential neurotoxicity of fumonisin. In vivo studies conducted

since the 2011 evaluation (Annex 1, references *205* and *206*) that add new mechanistic information are summarized in Table 6. In vitro studies are summarized in the text.

In vivo studies

Mice

A single intraperitoneal injection of pure FB_1 at 8 mg/kg bw sensitized male C57BL/6 mice to pentylenetetrazol-induced seizures (decreased latency and increased frequency of myoclonic jerks) (Poersch et al., 2015). Changes in mitochondrial function were also observed. The authors noted that the levels of fumonisin contamination in human food needed to attain an oral dose equivalent to the intraperitoneal dose used in this mouse study would be rare.

Rats

Wistar rats (male; 21 days old; 50 g) were divided between three groups (n = 10/group) and fed diets containing FB_1 plus FB_2 at 0, 1 or 3 mg/kg diet. The diets were prepared using *F. verticillioides* culture material (analysis determined the FB_1 plus FB_2 doses to be 0.16, 1.00 and 2.82 mg/kg diet, respectively, reported as 17, 103 and 311 µg/kg bw per day). Approximately 70% of the total fumonisin was FB_1. Animals were fed the diets for 15 and 45 days. At each time point, five rats were sampled from each group. At 15 days, blood was analysed for ALT and AST; at 45 days, blood was analysed for ALT and AST and the small intestines and proximal portion of the jejunum were collected. The area of the small intestines was measured, and the jejunum samples were analysed by immunostaining for HuC/D protein and neuronal nitric oxide synthase (nNOS).

There were no significant differences in growth, feed intake, ALT or AST activity, or area of the small intestines. No significant differences in neuronal density were found for either the HuC/D protein or nNOS. However, morphometric analysis showed a decrease in the average area of the cellular profile for the general population of immunoreactive myenteric neurons (HuC/D protein positive) and the immunoreactive nNOS-positive subpopulation within the myenteric neurons (Sousa et al., 2014).

The Committee concluded that the statistical analysis of the comparison of the areas of the cellular profile of myenteric neurons of the jejunum was not clear. A NOAEL could not be determined for FB_1 using diets prepared with *F. verticillioides*-containing culture material.

Pigs

Male Large White weanling pigs (n = 24; 8–9 weeks old; 7 kg) divided between four groups were fed age-appropriate diets for 6 months (Gbore, 2013). *F.*

Table 6
Overview of in vivo studies on neurotoxicity of fumonisins

Species / description	Length of study	No. per group	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Male C57BL/6 mice, age unknown, 22–25 g	Single dose pure FB_1	7–10	8	i.p.	Sensitized to seizures	8	–	Poersch et al. (2015)
Male Wistar rats, 21 days old, 50 g	15 and 45 days *F. verticillioides* culture material	10	0.017 (control), 0.103 and 0.311 $FB_1 + FB_2$[a]	0.16 (control), 1.0 or 2.8 mg/kg diet	Morphometric changes in immunoreactive myenteric neurons and nNOS-positive subpopulation	0.1	–	Sousa et al. (2014)
Male Large White pigs, 8–9 weeks old, 7 kg	6 months *F. verticillioides* culture material	6	Equivalent to 0, 0.2, 0.4 and 0.6 µg/kg bw per day, but Gbore (2009) reported this to be 0, 6.0, 11.5 or 17.0[a]	0, 5, 10 or 15 mg/kg diet	Decreased serum protein, albumin and globulin; decreased protein in hypothalamus, cerebellum and medulla oblongata; increased protein in the cerebral cortex	0.2 or 6.0 based on calculated "equivalent" dose or the dose reported by Gbore (2009)	–	Gbore (2013)

bw: body weight; FB_1: fumonisin B_1; i.p.: intraperitoneal; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; nNOS: neuronal nitric oxide synthase
[a] Control diet also contained fumonisin.

verticillioides (MRC 826) maize culture material was used to prepare three treatment diets containing FB_1 at 0, 5, 10 or 15 mg/kg diet (with FB_1 intake reported to be 0, 6.0, 11.5 and 17.0 mg/kg bw per day, according to Gbore, 2009; equivalent to 0, 0.2, 0.4 and 0.6 µg/kg bw per day based on JECFA conversion tables). Diets were analysed for FB_1 and other mycotoxins (zearalenone [ZEA], DON, T-2 toxin) by enzyme-linked immunosorbent assay (ELISA). The control diet containing FB_1 at 0.2 mg/kg diet was prepared using maize grits. After 6 months on the diets, blood samples were collected, the pigs killed, and brains and hypophyses collected and the pons, cerebellum, amygdala, hippocampus, hypothalamus, cerebral cortex, mid-brain, medulla oblongata, adenohypophysis and neurohypophysis dissected. The blood sera were analysed for serum protein, albumin and globulin. The brains and hypophyses were analysed for total protein.

The total serum protein, albumin and globulin were significantly decreased in the pigs consuming the diets containing FB_1 at 10 and 15 mg/kg diet. There were significant changes in the total protein in some brain regions. The most pronounced decrease was seen in the hypothalamus where total protein was

reduced by over 50% in the pigs at 5 mg/kg diet. The total protein was significantly increased in the cerebral cortex at 5 mg/kg diet but not at the higher doses. The cerebellum and medulla oblongata had significantly reduced total protein at both 10 and 15 mg/kg diet. Many of the changes in the brain regions did not show dose-dependence (Gbore, 2013).

The authors concluded that feed contamination with FB_1 at greater than 5 mg/kg diet for 6 months poses a health risk for pigs. The Committee noted that uncertainty in the analysis of the diets precludes the use of this study for the risk assessment.

In vitro studies

Co-cultures of cortical neurons and glial cells were treated with 0.5 µmol/L pure FB_1 and 5 µmol/L glutamate under low magnesium conditions (Domijan, Kovac & Abramov, 2012). Co-exposure significantly increased the calcium rise when compared to the glutamate-only treatment group. The rise in calcium coincided with a decrease in the transmitochondrial membrane potential difference. Fumonisin treatment made the cells more sensitive to glutamate-induced toxicity.

ScGT1 cells (immortalized CNS neurons) chronically infected with Rocky Mountain Lab prion strain were treated with 25 µmol/L pure FB_1 for 2 or 7 days in order to deplete membrane sphingolipids (Agostini et al., 2013). After 7 days the FB_1-treated cells had 50% less protease K-resistant cellular prion protein compared with the controls, suggesting that the lipid environment in lipid rafts plays a role in development of sporadic forms of prion diseases.

(c) Combined toxicity of fumonisins with other mycotoxins

At the 2011 JECFA evaluation, the Committee was asked to consider the toxicology associated with concurrent exposure to fumonisin and other mycotoxins and agents. Numerous in vivo and in vitro studies have shown a wide range of responses suggesting antagonistic, additive (no interaction) and more-than-additive (synergy) responses. However, many of the studies involve only a single dose level of individual mycotoxins, and the Committee concluded that none of the studies were adequate for quantitative assessment of interactions.

Of special interest, however, were studies in previous evaluations that documented the ability of dietary FB_1 to promote aflatoxin B_1 hepatocarcinogenicity in trout (Carlson et al., 2001) and orally dosed pure FB_1 to induce precancerous lesions in rats (Gelderblom et al., 2002). Fumonisins were discovered because of their ability to promote *N*-nitrosodiethylamine preneoplastic foci in rat liver (Gelderblom et al., 1988). In 2011, it was noted that the interaction between DNA-reactive aflatoxin B_1 and FB_1, with its potential to induce regenerative proliferation, was a concern. The combined toxicity of fumonisins and aflatoxins

is summarized in a separate monograph ("Co-exposure of fumonisins with aflatoxins", pages 879–960). All the studies describing the toxicity of fumonisins in combination with mycotoxins other than aflatoxins are briefly described in this section.

All of the available in vivo studies of combined effects were evaluated qualitatively for evidence of interactions. Where possible, interactions were classified as suggesting or appearing to be less than additive, additive or more than additive based solely on the arithmetic sum of the observed individual and combined response. The Committee was aware that such an approach has many potential pitfalls (for example, see Chou, 2010). Combined cytotoxicity (in vitro) studies are also summarized and, as with in vivo studies, there are many pitfalls in reaching conclusions about the quantitative interactions among mycotoxins during co-exposure (Smith et al., 2016; Alassane-Kpembi et al., 2016).

Pure fumonisin and pure DON in vivo

Male and female Swiss mice (7–8 weeks old; 20–25 g) were divided into four groups (n = 5 males and 5 females/group): control group; DON-only group dosed by oral gavage with 45 µg/kg bw per day for 7 days; FB_1-only group dosed by oral gavage with 110 µg/kg bw per day for 7 days; and DON-plus-FB_1 group dosed by oral gavage with both mycotoxins (Kouadio et al., 2013). Urine samples were collected (days not given), and on day 7 the animals were killed and serum and tissues were collected.

At day 3 and day 7, the animals consuming the diets containing both mycotoxins had significantly lower weight gains than the mice consuming only one of the mycotoxins. The male mice fed the fumonisin-only diets had significantly reduced weight gain between days 3 and 7 of the study. There were several statistically significant differences in blood parameters, most notably serum creatinine in males and triglycerides in males and females, which were elevated in what appeared to be an additive manner. DNA methylation in kidney of both males and females was increased in a manner suggesting a more-than-additive effect and urinary creatinine was decreased in what appeared to be due to a more-than-additive effect.

The Committee concluded that co-exposure suggested additivity, and the effects on growth, clinical chemistry and biochemical parameters were possibly more than additive. It was noted that the reduced weight gain was seen at a very low dose of FB_1 compared to other studies, for example, Bondy et al. (2012).

Culture material or partially purified fumonisin and DON in vivo

Chickens

Ross 308 broiler chickens (1 day old; males and females) were divided between four groups and fed the following diets for 15 days: the control group was fed a basal diet; the DON-only group was fed the basal diet containing DON at 4.6 mg/kg diet; the fumonisin-only group was fed the basal diet containing FB_1, FB_2 and FB_3 at 18.4, 7.0 and 1.7 mg/kg diet, respectively; the DON-plus-fumonisin-group was fed the basal diet with DON at 4.3 mg/kg diet and FB_1, FB_2 and FB_3 at 16.8, 6.1 and 1.5 mg/kg diet, respectively. The DON- and fumonisin-containing diets appear to have been prepared using, respectively, *Fusarium graminearum* and *F. verticillioides* culture material; the actual purity of the DON and fumonisins was unclear. Trace amounts of nivalenol, DON and enniatin were present in the control diet. After 15 days the animals were killed and blood, mid-duodenum, mid-jejunum and mid-ileum were collected.

There were no effects on growth. Sphinganine and the Sa/So ratio were significantly elevated in the fumonisin-only and DON-plus-fumonisin groups. The combined treatment appeared to show less-than-additive decreased intestinal mucin 2 expression in duodenum and decreased xanthine oxidoreductase in jejunum. Some changes in the intestinal mucin monosaccharide composition were also seen and appeared to be additive or less than additive. Many significant changes in mRNA expression of several membrane transporters appeared to be either less than additive or antagonistic. For example, the four amino acid transporters/exchangers analysed in the jejunum from the fumonisin-only groups with FB_1, FB_2 and FB_3 at 18.4, 7.0 and 1.7 mg/kg diet showed a 22.4-, 23.3-, 64.2- and 19.1-fold increase, respectively, in relative gene expression compared with the control group. However, there was no evidence of increased expression in either the DON-only or DON-plus-fumonisin groups for the same four amino acid transporters/exchangers (Antonissen et al., 2015c).

The Committee concluded that some of the effects of co-exposure appeared to be antagonistic, less than additive or additive.

Pigs

Crossbred castrated male pigs (n = 24; 10.2 kg) divided between four groups were fed for 35 days with the following diets: the control group was fed a diet containing DON at 0.5 mg/kg diet; the DON-only group was fed a diet containing DON at 2.8 mg/kg diet; the fumonisin-only group was fed a diet containing fumonisin at 5.9 mg/kg diet (4.1 mg FB_1 plus 1.8 mg FB_2); and the DON-plus-fumonisin group was fed a diet containing 3.1 mg DON and 6.5 mg fumonisin (4.5 mg FB_1 plus 2 mg FB_2). The DON for preparing the diets was from *Fusarium graminearum* culture material and the fumonisin from *F. verticillioides* culture material (strains

not specified). The stated doses were 130 and 260 µg/kg bw per day for DON and fumonisin, respectively (Bracarense et al., 2012). The fumonisin-only diet probably also contained DON at 0.4 mg/kg diet. The cereals used to prepare the diets were naturally contaminated with DON, enniatin and ZEA. After 35 days the animals were killed, and the mid-jejunum and proximal ileum collected for measurement of intestinal parameters including morphology, histology, expression of cytokines and junctional proteins.

The results showed numerous significant treatment-related changes in morphology, histology, atrophy, fusion of villi and decreased height and cell proliferation as well as other changes. The expression of cytokines was upregulated and junctional E-cadherin and occludin expression were reduced. The authors reported that the changes induced by co-exposure were in some cases synergistic, additive, less than additive or antagonistic. A role of other biologically active agents in the diets is possible but was not discussed. Effects related to the co-exposure were suggestive of additive and possibly more-than-additive effects (Bracarense et al., 2012).

The Committee concluded that the design of the study made it unsuitable for drawing conclusions on the combined effects of fumonisin and DON.

Grenier et al. (2013) conducted a study following the same experimental protocol as used in the Bracarense et al. (2012) study but with four additional experimental groups fed diets containing a feed additive hypothesized to reduce the adverse effects of DON or fumonisin alone or in combination (n = 6 pigs/group). The diets amended with the feed additive had similar levels of DON, fumonisins and other mycotoxins as reported in the summary above.

As in the earlier Bracarense et al. (2012) study, Grenier et al. (2013) found numerous significant treatment-related changes, many of which were suggestive of less-than-additive, additive and, in some cases, more-than-additive effects that were related to the co-exposure. For example, lesion scores in the jejunum were 0.67, 6.17, 4.17 and 6.17, respectively, for the control group, DON-only group, fumonisin-only group and DON-plus-fumonisin group, a result that is suggestive of a less-than-additive effect. An effect suggestive of additivity was the effect on the immunoglobulin G anti-ovalbumin activities, which were 1545, 1333, 1101 and 789 [arbitrary units], respectively, for the control group, DON-only group, fumonisin-only group and DON-plus-fumonisin group. The expression of the cytokine interleukin-12p40 is another example suggestive of additivity. There were also effects (e.g. on interleukin-6 in ileum) that appeared to be antagonistic. A role of other biologically active agents in the diets is possible because these were prepared using fungal culture material; however, the levels of the other mycotoxins in the diets were not likely to have much effect on the reported outcome.

The Committee concluded that some of the effects from co-exposure were suggestive of being additive or more than additive. However, it should be noted that this study and many of the others described in the in vivo section show that declaring a mycotoxin combination as suggesting additive, antagonistic, less-than-additive, more-than-additive, multiplicative or synergistic effects depends on the end-points measured. That is to say, agent A plus agent B in combination at particular doses may appear to be much more than additive (synergistic) for a specific end-point under specific experimental conditions, but not others.

Culture material or partially purified fumonisin and OTA in vivo

Quail

Japanese quail chicks (*n* = 300; 1 day old) were divided between four groups and fed the following diets: the control group (*n* = 75) was fed chick mash; the fumonisin-only group (*n* = 75) was fed the chick mash with FB_1 at 200 mg/kg diet; the OTA-only group (*n* = 75) was fed chick mash with OTA at 2 mg/kg diet; the fumonisin-plus-OTA group (*n* = 105) was fed chick mash containing fumonisin at 200 mg/kg diet and OTA at 2 mg/kg diet. The fumonisin was from *F. verticillioides* (M-1325) culture material and the OTA from *Aspergillus ochraceus* (NRRL 3174) culture material. Analysis of the feed samples detected aflatoxin B_1 at 12 µg/kg but no OTA, citrinin, ZEA, T-2 toxin or aflatoxins B_2, G_1 and G_2. The levels of other fumonisins in the culture material/diets were not stated. The quail were killed and the kidneys collected on days 7, 14, 21 and 28 for gross pathological and histopathological examination and scoring of lesions (parameters included interstitial congestion, degeneration, tubular necrosis and eosinophilic luminal hyaline bodies).

The total lesion scores did not show a clear time-dependent treatment-related trend. Significantly, elevated scores were observed for all parameters at various sampling times but overall, for each parameter, the mean treatment effect showed significantly higher scores for the combined (fumonisin-plus-OTA) treatment group. The interactions based on the mean overall scores appeared to be less than additive or additive for the measured parameters. The effects of co-exposure in this study were mostly suggestive of less-than-additive or additive renal effects (Khan, Iqbal & Rajesh, 2012).

Pigs

In a study assessing the possible role of combined OTA and FB_1 in the pathology of spontaneous mycotic porcine nephropathy, Landrace × Bulgarian white pigs (*n* = 24; 8 weeks old; 12–14 kg) were divided between four groups (*n* = 6 pigs/group; males and females) and fed the following diets: the control group was

fed a basal diet; the OTA-only group was fed the basal diet with *A. ochraceus* (isolate D2306) culture material to have OTA at 0.5 mg/kg diet; the fumonisin-only group was fed the basal diet amended with *F. verticillioides* (isolate MRC 826) culture material to have FB_1 at 10 mg/kg diet; and the OTA-plus-fumonisin group was fed the basal diet amended with the culture material to have OTA and FB_1 at 0.5 and 10 mg/kg diet, respectively (equivalent to 0.02 and 0.4 mg/kg bw per day, respectively). After 14 days, all the pigs were immunized against Morbus Aujeszky disease; 21 and 49 days later, blood samples were collected; on day 90, all the pigs were slaughtered and tissues collected for gross and histopathological examination.

At 21 and 49 days post-immunization, there were numerous significant changes (for example, increased creatinine, urea and ALT and decreased antibody titre) in the measured serum biochemical parameters, and in all cases these changes, and especially the nephrotoxic effects, were more pronounced in the OTA-plus-fumonisin group (Stoev et al., 2012). Changes suggestive of less-than-additive, additive and more-than-additive effects were observed, but given the study design, the Committee could not draw any firm conclusions. For example, lack of quantification made conclusions about histopathological changes highly subjective. The Committee concluded that given that both fumonisin and OTA are nephrotoxic, more carefully designed studies in animals are needed.

Culture material or partially purified fumonisin and T-2 toxin in vivo

Male Pannon white rabbits ($n = 40$; 35 days old) were divided between four groups and fed the following diets for 4 weeks: the control group was fed a basal diet; the T-2 toxin–only group was fed the basal diet with T-2 toxin at 2 mg/kg diet; the fumonisin-only group was fed the basal diet with FB_1 at 10 mg/kg diet; and the T-2 toxin-plus-fumonisin group was fed the basal diet with T-2 toxin at 2 mg/kg diet and FB_1 at 10 mg/kg diet. The T-2 toxin was from *Fusarium sporotrichioides* (NRRL 3299) culture material and the fumonisin from *F. verticillioides* (MRC 826) culture material. The T-2 toxin culture material also contained HT-2 toxin (at 0.4 mg/kg diet). The levels of other fumonisins in the culture material were not stated. Blood samples were collected after 2 weeks and then again at 4 weeks.

The final body weights of the groups exposed to T-2 toxin did not differ significantly from each other but were significantly lower than either the control group or fumonisin-only group (which also did not significantly differ from each other). The liver weights of the T-2 toxin–only group were significantly higher (>2 times) than those of any of the other treatment groups; the liver weights in the rabbits in the other treatment groups were not significantly different from the control group or each other, suggesting an antagonistic effect of fumonisin.

At 4 weeks, the Na^+/K^+ adenosine triphosphatase activity in the RBCs was significantly elevated in the fumonisin-only group and significantly decreased in the T-2 toxin–only group compared with all of the other groups; the Na^+/K^+ adenosine triphosphatase activity in the T-2 toxin-plus-fumonisin group was not significantly different from the control group, suggesting an antagonistic effect. Differences in RBC fatty acid profiles were much less pronounced in the T-2 toxin-plus-fumonisin group, the group that was most similar to the control group. The apparent antagonistic effects were attributed to the differing mechanisms of action. The effects of co-exposure in this study were mostly suggestive of antagonism (Szabó et al., 2014).

Culture material or partially purified fumonisin and moniliformin in vivo

Japanese quail chicks (n = 390; 1 day old) were divided between four groups and fed the following diets: the control group (n = 75) was fed a chick mash diet; the fumonisin-only group (n = 105) was fed chick mash with FB_1 at 200 mg/kg diet; the moniliformin-only group (n = 105) was fed chick mash containing moniliformin at 100 mg/kg diet; and the fumonisin-plus-moniliformin group (n = 105) was fed chick mash containing fumonisin at 200 mg/kg diet and moniliformin at 100 mg/kg diet. The fumonisin was from *F. verticillioides* (M-1325) culture material and the moniliformin from *F. fujikuroi* (M-1214) culture material. Analysis of the feed samples detected aflatoxins at 6 µg/kg. The levels of other fumonisins in the culture material/diets were not stated.

The quail were killed on days 7, 14, 21 and 28 and their hearts collected for evaluation of gross pathology, histopathology, scoring of lesions and transmission electron microscopy. In the words of the authors, the effect of moniliformin was "exaggerated" in the fumonisin-plus-moniliformin group. The interaction appeared to be more pronounced at days 7 and 14 such that the observed effects were more than additive. Based on the scoring of gross lesions, the interaction appeared to be less than additive or antagonistic. Scoring of the microscopic lesions showed what appeared to be less-than-additive scores at 7 and 28 days, additive scores at 21 days and more-than-additive scores at 14 days. The mean microscopic lesion scores were also highly variable with scores ranging from no interaction (additive) to antagonistic or more than additive based on the type of lesion (congestion, haemorrhages, necrosis, degenerative changes, hypertrophy and atrophy). Overall, the quantifiable effects of co-exposure in this study were highly variable but mostly suggestive of less-than-additive effects (Sharma et al. 2012).

Interpreting the results of in vivo studies in terms of their relevance to human exposure is highly problematic due to the species-, strain- and sex-specificity of the fumonisin-induced diseases seen in domestic and experimental

animals. For example, FB_1 causes brain disease in horses and pulmonary disease in pigs; it is a complete renal carcinogen in male F344/N rats and a complete liver carcinogen in male BDIX rats and female B6C3F1 mice. Thus, the experimental design of studies intended to reveal in vivo interaction between mycotoxins should be mechanism-based, hypothesis driven and carefully designed. In addition, in order to be of value for risk assessment purposes and determining interactions, the studies must include multiple dose levels so as to include intake at levels without adverse effects.

In vitro studies investigating cytotoxicity using pure FB_1 and co-treatment with other mycotoxins/fungal metabolites other than aflatoxin B_1

In vitro studies, even more than in vivo studies, are fraught with potential for problems in revealing information useful for understanding the role of co-exposure as a contributing factor in human diseases. The in vitro cytotoxicity of FB_1 is very much dependent on cell type, and in many cell lines the levels of FB_1 necessary to cause cell death can be very high (Chuturgoon, Phulukdaree & Moodley, 2015; Riedel et al., 2016). Thus, the choice of cell type for conducting in vitro studies needs to be carefully considered. For example, cultured cells derived from human tumours, such as HeLa cells, are likely to be highly resistant to fumonisin-induced cytotoxicity compared to primary rat hepatocytes. This was shown in the recent study by Riedel et al. (2016), in which the resistances of Chang cells (presumably HeLa cells) and susceptibility of primary hepatocytes to FB_1 cytotoxicity could be attributed to differences in lipid metabolism. An additional concern is interpreting the relationship between the dosages used in vitro and in vivo exposure.

A large number of in vitro studies have investigated the effects of FB_1 on its own and in combination with mycotoxins other than aflatoxins. Studies comparing effects of fumonisins and fumonisins in combination with one other mycotoxin (other than aflatoxins) include (1) the effects of FB_1 and DON using jejunal explants from piglets (Basso, Gomes & Bracarense, 2013); (2) fumonisin and DON myelotoxicity using human granulo-monocytic haematopoietic progenitor cells (Ficheux, Sibiril & Parent-Massin, 2012); (3) FB_1 and DON or zearalenol (α or β) or ZEA on cell proliferation and steroid production of bovine or porcine granulosa cells (Cortinovis et al., 2014; Albonico et al., 2016); (4) FB_1 and beauvericin cytotoxicity and modulation of nuclear receptor transcriptional activity (Fernández-Blanco et al., 2016).

There has also been a series of studies investigating the individual and combined effects of FB_1 plus up to three other mycotoxins simultaneously (nivalenol, DON, ZEA). These include studies using swine jejunal epithelial cells (IPEC-J2) to investigate cytotoxicity, modulation of β-defensins and effects on

expression of pro-inflammatory cytokines (Wan, Turner & El-Nezami, 2013; Wan et al., 2013a,b). The same four mycotoxins were also studied using mixed cultures of Caco-2 and HT29 MTX cells to determine the individual and combined effects on mucin mRNA expression, protein production and secretion (Wan et al., 2014).

The Committee concluded that the in vitro combined mycotoxin toxicity studies available since 2011 provided some insights into the potential for interactions. However, with a few exceptions, the relevance of the interactions to the current JECFA risk assessment was not apparent. Given that combined effects are likely dose-dependent, the single dose levels used in the reviewed in vivo studies were considered not relevant to human exposure.

2.3 Observations in domestic animals/veterinary toxicology

Fumonisins are a proven cause of field outbreaks of ELEM and porcine pulmonary oedema and of performance problems in poultry (see IARC, 2002, for a recent detailed review of the effects of mycotoxins on farm and domestic animals). The relative species sensitivity to fumonisin toxicity in farm animals is horses and rabbits > pigs and catfish > ruminants and poultry. Breeding animals are more sensitive than animals being raised for slaughter (IARC, 2012). Just as turkeys played an important role in the discovery of aflatoxins, ELEM associated with consumption of mouldy maize played an important role in the discovery of the fumonisins.

The first animal toxicosis to be associated with the consumption of maize affected by *F. verticillioides* and related species was ELEM. First reported in 1891, this syndrome is characterized by the presence of liquefactive necrotic lesions, mainly in the white matter of the cerebrum (Mayo, 1891; IPCS, 2000). Wilson & Maronpot (1971) succeeded in establishing the causative agent when they isolated *F. verticillioides* as the main contaminant of mouldy maize that had caused many cases of ELEM. In addition, these authors reproduced ELEM by feeding maize culture material. ELEM was reproduced by the intravenous administration of FB_1 in 1988 (Marasas et al., 1988). Horses dosed with FB_1 at 0.2 mg/kg bw per day developed neurological signs consistent with ELEM 7–9 days after dosing began. Horses dosed with FB_1 at 0.01 mg/kg bw per day did not develop neurological signs.

Cultures containing primarily FB_2 are also capable of inducing ELEM at a dose approximately 10 times higher. The lowest FB_1 dose that has resulted in ELEM, in a controlled experiment, is 22 mg/kg in diets formulated with naturally contaminated maize screenings. The minimum toxic oral dose of pure FB_1 is unknown.

In addition to the brain lesions, histopathological abnormalities in liver and kidney have been reported in horses orally dosed with pure fumonisins, maize screenings naturally contaminated with fumonisins or culture material containing known amounts of fumonisins (IPCS, 2000; Annex 1, references *152*, *153*, *205* and *206*).

The following is a brief summary of the few available in vivo toxicological observations in domestic and farm animals published since 2011.

2.3.1 Pigs

Since the previous evaluation, there have been no new published reports of fumonisin-associated field disease outbreaks in pigs. A single report (summarized in section 2.2.6(c)) purported to provide experimental evidence supporting the possible involvement of OTA and fumonisin in mycotic nephropathy in pigs in Bulgaria and South Africa (Stoev et al., 2012).

2.3.2 Horses

A case report of ELEM in Argentina consistent with the literature was associated with concentrations of FB_1 and FB_2 at 12.5 and 5.2 mg/kg feed in the feed supplement sample obtained (Giannitti et al., 2011).

2.3.3 Other farm animal species

A recent case report suggested that fumonisin was involved in optic neuropathy in a herd of cattle in Canada (Sandmeyer et al., 2015). However, no data were presented showing fumonisin exposure in the cattle and the connection was circumstantial at best and less than compelling.

In another study in dairy calves exhibiting haemorrhagic enteritis associated with Shiga toxin–producing *E. coli* in three production sites, ELISA determined low levels of aflatoxin (1–3 parts per billion [ppb]) and fumonisin (50–350 ppb) in samples of the haemorrhaged jejuna mucosa (Baines et al., 2013). It was stated that exposure to aflatoxin and fumonisin in the calf ration promoted these Shiga toxin–producing *E. coli*–associated haemorrhagic enteritis outbreaks. The levels of aflatoxin and fumonisin in the feed were not presented but the levels in the digesta were reported to be the same as those in the mucosa.

2.4 Observations in humans

2.4.1 Biomarkers of exposure

Since the Committee's previous evaluation (Annex 1, reference *205*), fumonisin biomarkers have been increasingly used to estimate human exposure (reviewed

in Turner et al., 2012a). Because there is no evidence for metabolism of fumonisins in humans, analytical methods for the parent compounds alone have been developed to represent exposure. Direct measurements of fumonisins in biological samples such as urine, faeces, hair and breast milk have been reported in different geographical areas worldwide.

(a) Urinary fumonisin B_1 (UFB_1)

UFB_1 is the most commonly used biomarker of exposure for fumonisins in humans. UFB_1 as a biomarker was first validated and shown to reflect the human fumonisin exposure levels in a South African study (van der Westhuizen et al., 2011; Annex 1, reference *205*). More recently, a human kinetic study in the USA detected UFB_1, but not UFB_2 or UFB_3, in volunteers (n = 8) consuming maize-based foods under controlled exposure conditions. The FB_1 excretion was 0.5 ± 0.24% of the total FB_1 intake on a daily basis (Riley et al., 2012). In spite of a large interindividual variability, the results of this study further validated UFB_1 as a useful biomarker of exposure. This study also showed that FB_1 was undetectable in the urine 5 days after the consumption stopped, indicating that UFB_1 is only a biomarker of recent fumonisin exposure.

New analytical approaches have been developed to simultaneously detect many mycotoxin biomarkers in urine samples. However, according to a comparison study the results from multi-mycotoxin analysis methods did not agree well with those from single-mycotoxin analysis methods. The reasons for this discrepancy require further investigation (Solfrizzo et al., 2013). Nevertheless, since the last JECFA evaluation UFB_1 has been increasingly used to estimate exposure to fumonisin.

The co-occurrence of urinary fumonisin and aflatoxin biomarkers is discussed in a separate monograph below ("Co-exposure of fumonisins with aflatoxins", pages 879–960).

Fumonisins were measured in 177 spot urine samples collected from Guatemalan residents. The mean UFB_1 level was 0.3 ng/mL, while FB_2 and FB_3 were not detectable in these samples. Assuming a daily urine output of 1000 mL, the authors estimated a daily average FB_1 excretion of 0.3 µg per day. Compared to the estimated FB_1 intake of 30 µg per day for this population, the urinary excretion appears to account for 1.0% of daily FB_1 intake. Thus, the percentage of FB_1 intake excreted in the urine from natural exposure in this Guatemalan population was similar to that seen in the USA volunteers with controlled exposure (Riley et al., 2012).

UFB_1 was determined in 1240 maize-consuming Guatemalan women from three communities representing either high (Jutiapa) or low (Chimaltenango and Escuintla) fumonisin contamination in maize. The mean ±

standard deviation (SD) level of UFB_1 in women from Jutiapa was 2.27 ± 4.08 ng/mL, which was significantly higher than the UFB_1 levels in women from Chimaltenango (0.38 ± 0.87 ng/mL) and Escuintla (0.26 ± 0.49 ng/mL). UFB_1 was present at much higher levels than UFB_2 or UFB_3, which were rarely detected in the urine samples. The fumonisin intake for each individual was estimated using the mean total FB ($FB_1 + FB_2 + FB_3$) levels in local maize samples, self-reported tortilla consumption from a food questionnaire and the individual's body weight. UFB_1 was associated with fumonisin intake in a dose-dependent manner, with a statistically significant correlation between UFB_1 and the estimated total fumonisin intake ($r = 0.26$; $P < 0.001$). Using the measured UFB_1 data and assuming that 0.5% of the fumonisin intake was excreted, the authors estimated that a considerable proportion of the Guatemalan population had fumonisin intake greater than the JECFA PMTDI. Using the UFB_1–dietary intake correlation, the authors further estimated that individuals with UFB_1 levels greater than 0.5 ng/mL were at significantly higher risk of fumonisin intake greater than the JECFA PMTDI (Torres et al., 2014).

Mycotoxin biomarkers, including UFB_1, were measured in 53 urine samples collected from female residents of a region of South Africa formerly known as Transkei with high rates of oesophageal cancer. Participants completed a 24-hour dietary recall questionnaire to determine their maize intake and were requested to cook their traditional evening meal porridge using home-grown maize. A representative portion of the maize was analysed for mycotoxin contamination. Their first void urine samples were collected the next morning for UFB_1 analysis using single-mycotoxin and multi-mycotoxin methods in two independent laboratories. The single-mycotoxin biomarker method (LOD: 0.01 ng/mL: limit of quantification [LOQ]: 0.02 ng/mL) detected UFB_1 in 87% of the samples at a mean concentration of 0.342 ± 0.466 ng/mg creatinine (corresponding to 0.185 ± 0.236 ng/mL urine). In comparison, the multi-mycotoxin analysis method (LOD: 0.04 ng/mL; LOQ: 0.12 ng/mL) detected UFB_1 in 96% of the samples at a mean concentration of 1.52 ± 2.17 ng/mg creatinine (corresponding to 0.841 ± 1.06 ng/mL urine). The difference in the reported values between the two analytical methods (both LC-MS/MS-based) may be due to the specific sample clean-up procedures used; the difference also indicated potentially significant interlaboratory variation in the analysis. Of note, a statistically significant correlation was found between the UFB_1 measured by the multi-mycotoxin analysis method and the FB_1 probable daily intake ($r = 0.302$; $P = 0.031$) (Shephard et al., 2013). This well-designed study provided strong evidence for UFB_1 validation as a biomarker of exposure.

In Cameroon, urinary mycotoxin biomarkers were measured in 220 children (aged 1.5–4.5 years) from high mycotoxin contamination regions. Using a multi-mycotoxin analysis method, the study found that 11% of the urine samples

were positive for UFB_1, with a geometric mean of 2.96 ng/mL (95% confidence interval [CI]: 0.06–48 ng/mL). Other mycotoxins, such as OTA, DON and aflatoxin M_1, were also detected in the urine samples. However, no information about the co-occurrence of these mycotoxins was provided. The study found no association between urinary mycotoxin concentration and stunted, wasting and underweight conditions in children, and there was no statistical difference in the mean concentration of UFB_1 across the different age groups. The results showed that the geometric mean UFB_1 level was significantly higher in boys (0.59 ng/mL, n = 126, range: 0.06–50 ng/mL) than in girls (0.02 ng/mL, n = 94; range: 0.02–5.7 ng/mL). Although the reason for the sex difference was not clear, the authors speculated that it might be due to chance or boys' overall higher food consumption. UFB_1 was detected at levels ranging from 0.73 to 1.3 ng/mL urine in four children exclusively fed breast milk, suggesting that FB_1 could be carried-over in human breast milk (Njumbe Ediage et al., 2013).

In another study in Cameroon, 175 urine samples from adults resident in regions with high prevalence of human immunodeficiency virus (HIV) were analysed using a multi-mycotoxin detection method. Of the 145 samples that tested positive for HIV, only 3% (5/145) had detectable UFB_1 (mean concentration: 0.63 ng/mL). Only one HIV-negative individual had detectable UFB_1, and the concentration was below its LOQ of 0.5 ng/mL. Of the six UFB_1-positive samples, four showed co-occurrence with DON, a mycotoxin commonly detected in these urine samples (62% positive). FB_2 was detected in only one sample, which belonged to an HIV-positive individual, and the level was below its LOQ of 1.7 ng/mL. With the low frequency of positive UFB_1 in this population, no conclusion could be drawn regarding the effect of HIV infection on fumonisin exposure or excretion (Abia et al., 2013a).

In northern Nigeria, 120 urine samples were collected from five families from Nasarawa State (representing high mycotoxin exposure) and five families from Kaduna State (representing low mycotoxin exposure). A 25 g random portion of the meal consumed by the members in each family was taken for mycotoxin analysis on the day before urine collection. The urine samples were analysed using a multi-mycotoxin method; 61 (50%) contained at least one mycotoxin biomarker, of which 16 were positive for UFB_1 (mean: 4.6 ± 2.8 ng/mL). The mean UFB_1 level for children (n = 4), adolescents (n = 3) and adults (n = 9) was 3.7, 6.9 and 4.2 ng/mL, respectively. Although 15 samples contained more than one mycotoxin biomarker, the authors did not specify which biomarker(s) co-occurred with UFB_1. Overall, mycotoxins were more frequently observed in urine samples from Nasarawa State than Kaduna State; however, the authors did not report the individual mycotoxin levels by region. A correlation analysis found that UFB_1 was modestly associated with FB_1 levels in food (r = 0.28; P = 0.02). Using the UFB_1 data, the authors estimated the mean FB_1 intake in adults with detectable

UFB_1 to be 35 µg/kg bw per day (assuming a 1.5 L daily urine production, 60 kg body weight and 0.3% FB_1 excretion), a level significantly greater than the recommended JECFA PMTDI of 2 µg/kg bw (Ezekiel et al., 2014).

Urine samples collected from 252 adults participating in the Swedish National Dietary Survey (Riksmaten) from 2010 to 2011 were analysed for mycotoxin biomarkers. UFB_1 and UFB_2 were found in 6% and 23% of the samples, respectively, and the mean UFB_1 concentration (0.004 ng/mL) was lower than the mean UFB_2 concentration (0.01 ng/mL) (Wallin et al., 2015). In contrast, in other studies UFB_1 is the dominant biomarker and UFB_2 is rarely detected (Riley et al., 2012; Abia et al., 2013a; Ezekiel et al., 2014). The reasons for this discrepancy are not clear, but the authors mentioned the fact that FB_2 alone had been previously found in food samples contaminated with *A. niger*, a fungus that produces only FB_2 but not FB_1. Unfortunately, FB_1 and FB_2 were not analysed in the food items so as to be able to examine this hypothesis.

Co-occurrence of more than one mycotoxin biomarker was found in 69% of the samples, with most samples containing 1–3 biomarkers. Co-exposure of fumonisins and other mycotoxins was found, with the most common combination being ZEA + FB_2 (n = 39) and ZEA + FB_1 + FB_2 (n = 4). The authors analysed the relationship between dietary characteristics (including nutrient intake and food components) and the number of mycotoxins detected in the urine samples. The study subjects were divided between three multi-mycotoxin groups based on the number of mycotoxins detected in the urine: individuals in the low, medium and high multi-mycotoxin groups had 0–1, 2 and 3–5 different mycotoxin(s) detected in their urine samples, regardless of the type and the level of each mycotoxin. No difference in food intake pattern was observed between the three multi-mycotoxin groups, besides slight differences in the intake of saturated fat, pork, dried fruits and coffee (Wallin et al., 2015).

The Committee noted that the way of grouping the study subjects did not reflect level of exposure. Therefore, the information provided by this study about the association between food intake patterns and mycotoxin co-exposure was very limited.

Urine samples from 52 individuals in Apulia region in southern Italy were analysed using a multi-mycotoxin biomarker method. FB_1 was detected in 56% of the samples, with a mean ± SD concentration of 0.055 ± 0.073 ng/mL. Mycotoxin co-exposure was common in this population. The co-occurrence of UFB_1 with biomarkers of DON, ZEA and OTA together was found in 52% of the samples. Based on the measured UFB_1 levels, the authors estimated the probable daily intake by these volunteers using a default daily urine volume of 1.5 L. Assuming a per cent FB_1 excretion rate of 2.6% (using piglet data) or 0.5% (using human data), the estimated probable daily intake of FB_1 in this Italian population was 0.053 µg/kg bw and 0.274 µg/kg bw, respectively. These exposure estimates were

largely below the PMTDI of 2 μg/kg bw, indicating a low risk of FB_1 exposure in this sample population (Solfrizzo, Gambacorta & Visconti, 2014).

In addition to the above-mentioned studies, several publications reported undetectable levels of UFB_1 in samples from different geographical regions, including Belgium, the Islamic Republic of Iran, Portugal, Republic of Korea, Spain and Bangkok, Thailand (Ahn et al., 2010; Silva et al., 2010; Rubert et al., 2011; Turner et al., 2012b; Heyndrickx et al., 2014; Warth et al., 2014). These publications were generated from attempts to measure FB_1 or other concurrent mycotoxins in human urine, mostly aiming at analytical method development. The sample sizes were typically small and the corresponding dietary intake information was unknown. Therefore, the Committee decided that these studies only provide limited value in the validation of urinary fumonisin biomarkers and the estimate of human exposure. Therefore, a detailed description for each of these studies is not given in this report.

UFB_1 has been used as a biomarker to evaluate the effectiveness of dietary interventions designed to help decrease the fumonisin exposure in humans. In a double-blind, placebo-controlled trial in Ghana, 177 volunteers were recruited in a 3-month intervention study of calcium montmorillonite, a type of clay that might decrease the bioavailability of fumonisin. At week 10, a significant reduction in UFB_1 was observed in the treatment groups. The median UFB_1 levels in the low (calcium montmorillonite at 1.5 g/day) and high (3.0 g/day) treatment groups were 0.21 ng/mL and 0.44 ng/mL, respectively; the median UFB_1 level in the placebo group was 5.45 ng/mL (Robinson et al., 2012).

In a more recent study, UFB_1 was used to investigate the chemopreventive effect of green tea polyphenols (GTP) in a Chinese population residing in an area with high incidence of and mortality from hepatocellular carcinoma. The results indicated a time- and dose-dependent reduction in UFB_1 levels by GTP: after 3 months of intervention, the median UFB_1 levels in the low (GTP at 500 mg/day) and high (1000 mg/day) treatment groups were 319.45 and 215.83 pg/mg creatinine, respectively, both significantly lower than the median UFB_1 level in the placebo group (591.24 pg/mg creatinine) (Xue et al., 2015).

(b) FB_1 in breast milk

It was formerly believed that the feed-to-milk carry-over of FB_1 was very low, reportedly 0–0.05% (Fink-Gremmels, 2008), unlike with aflatoxins. However, after UFB_1 was found in the urine of exclusively breastfed Cameroonian children (Njumbe Ediage et al., 2013), it has been suggested that FB_1 in human breast milk could be an important source of exposure in infants. Magoha et al. (2014a) reported detecting considerable levels of FB_1 in breast milk samples collected from 131 women in Rombo, a northern region of the United Republic of Tanzania,

during their first month of lactation. FB_1 extracted from breast milk underwent clean-up using strong anion exchange columns and was then quantified by HPLC (LOD: 5.5 ng/mL; LOQ: 19.5 ng/mL). The authors found that 58 (44%) samples contained FB_1 at 6.57–471.05 ng/mL, with a median of 26.23 ng/mL. Using a point estimate of 510 mL daily milk intake, the exposure to FB_1 in the infants of these lactating women ranged from 0.78 to 64.93 µg/kg bw per day (median: 3 µg/kg bw per day).

This estimation indicated that 29% of these infants exceeded the PMTDI of 2 µg/kg bw. While the likely presence of FB_1 (based on exact molecular mass) in a few samples was confirmed by time-of-flight mass spectrometry, no supporting documentation was provided. The Committee noticed the comparatively high LOD and LOQ for this detection method and the fact that no LC-MS/MS confirmation was provided. In a recent review of biomonitoring of mycotoxins in human breast milk, Warth et al. (2016) proposed using mass spectrometry to improve analytical performance and accuracy.

(c) FB_1 in hair

In the previous JECFA evaluation, fumonisin in hair was not regarded as a validated biomarker of exposure due to the lack of comparison to the actual dietary exposure, even though it could represent long-term exposure to fumonisin (Annex 1, references *205* and *206*).

Recently, Bordin et al. (2015) measured FB_1 in hair samples and maize products consumed by 56 volunteers in Brazil. The authors reported an LOD and LOQ of 3.3 ng/g and 5.5 ng/g, respectively, for LC-MS quantification of fumonisin in hair. Using this method, FB_1 was detected in four hair samples (7.2%) with a mean level of 21.3 ng/g. To estimate the corresponding FB_1 intake, FB_1 was measured in the maize products consumed by each volunteer and a corresponding food frequency questionnaire was collected. The mean probable daily intake for FB_1 in this population was estimated to be 159 ± 47 ng/kg bw. A nonlinear positive correlation between hair FB_1 and probable daily intake was found, with a polynomial correlation coefficient (r^2) of 0.885 (Bordin et al., 2015).

While the authors suggested that hair fumonisin could be a possible exposure biomarker, the Committee noted the limitation of validation with a small number of positive samples (n = 4). To establish hair fumonisin as a useful and practical biomarker for exposure, further validation is needed from populations with different age distribution, living in different geographical regions and using different analytical laboratories.

2.4.2 Biomarkers of effects

Since the interruption of sphingolipid metabolism through ceramide synthase inhibition is a plausible mode of action of fumonisin in animals, the levels of sphinganine, sphingosine and their ratio (Sa/So) have been studied as potential biomarkers in human samples, including whole blood, urine, serum, plasma and buccal cells. Due to inconsistent results in these studies, the Committee did not consider sphingolipids valid human biomarkers in its previous evaluation (Annex 1, reference *205*). Because sphingoid bases are natural metabolites, it has been suggested that their levels in biofluids can be influenced by dietary factors other than fumonisin. This would explain why the correlation between fumonisin exposure and sphingoid base biomarkers has been found to be good in experimental animals (with high exposure) but poor in humans owing to the less controlled and much lower exposure levels (Shephard et al., 2007).

Riley et al. (2015a) developed and validated a method to quantify fumonisin-induced changes in sphingosine 1-phosphate and sphinganine 1-phosphate in blood spots collected from mice and humans. RBCs are the main source of sphingoid base 1-phosphates in the blood. These cells are only capable of phosphorylating sphingoid bases as they do not synthesize sphingolipids de novo or dephosphorylate sphingosine 1-phosphate or sphinganine 1-phosphate. This means that the levels of phosphorylated sphingoid bases in blood spots are a result of accumulated free sphingoid bases produced in non-RBC tissues such as liver and kidney, and the levels in blood spots represent fumonisin-induced changes better than those measured in serum or plasma. In addition, collecting blood spots rather than serum or plasma has the advantage of being less invasive and less expensive. The aim of the method developed by Riley et al. (2015a) was to estimate the volume of blood collected on absorbent paper, which is critical to normalizing the level of sphingolipid biomarkers measured in blood spots, and to validate the use of the Sa 1-P/So 1-P ratio as a biomarker for de novo ceramide synthase inhibition where the increase in the ratio is due to much greater production of the ceramide precursor sphinganine than the turnover product sphingosine.

This method was validated in humans consuming maize-based foods in Guatemala. In this study, blood spots and urine samples were collected every 3 months for a year from women living in low (Chimaltenango and Escuintla) and high (Jutiapa) fumonisin exposure communities (n = 1240). The blood spot samples were analysed for sphinganine 1-phosphate, sphingosine 1-phosphate, and Sa 1-P/So 1-P ratio and the urine samples for UFB_1 (Torres et al., 2014). The sphinganine 1-phosphate and Sa 1-P/So 1-P ratio in blood spots and UFB_1 level were significantly greater in the community with high FB_1 exposure than in the communities with low FB_1 exposure. The increases in Sa 1-P/So 1-P ratio were

due primarily to increased sphinganine 1-phosphate. Sa 1-P/So 1-P ratio and sphinganine 1-phosphate levels were positively correlated with UFB_1 level. The results were further confirmed in 299 women living in different low-exposure (Sacatepéquez) and high-exposure (Santa Rosa and Chiquimula) communities (Riley et al., 2015b).

These results support the hypothesis that fumonisin disrupts sphingolipid metabolism through the inhibition of ceramide synthase in humans.

In a 1-year human biomarker study (n = 1239), in comparison with volunteers with no detectable UFB_1, statistically significant increases in the Sa 1-P/So 1-P ratio and the sphinganine 1-phosphate concentration were first observed in the window of UFB_1 that was greater than 0.5 but less than 1.0 ng/mL. This was confirmed in a biomarker response validation study (n = 299): the first statistically significant increases in both the Sa 1-P/So 1-P ratio and sphinganine 1-phosphate concentration were also observed in the window of UFB_1 that was greater than 0.5 but less than 1.0 ng/mL. A UFB_1 concentration of 0.5 ng/mL was estimated to reflect an intake of FB_1 of 1.67 µg/kg bw per day (Riley et al., 2015b).

The Committee noted that because of the noninvasive nature of blood spot collection, sphinganine 1-phosphate and Sa 1-P/So 1-P ratio in blood spots could be potential biomarkers of biological effects, which would be valuable in human health risk assessment when used in combination with a validated exposure biomarker such as UFB_1.

2.4.3 Clinical observations in humans

Although clinical symptoms of fumonisin exposure have been reported in animal species such as pigs, horses and rodents, none have been reported in humans.

2.4.4 Epidemiological studies

The seventy-fourth JECFA reviewed studies on four health end-points for fumonisin exposure: oesophageal cancer, NTDs, HIV infection and childhood growth stunting. This section includes epidemiological studies published since the last Committee evaluation on the association of fumonisin exposure with oesophageal cancer and hepatocellular carcinoma. No new epidemiological studies on the associations between fumonisin exposure and the risk of NTDs or HIV infection were identified in the literature search. No new studies have been published on the association between growth stunting and fumonisin exposure alone, although the impairment of childhood growth including growth stunting from fumonisin and aflatoxin co-exposure was investigated in the United Republic of Tanzania.

(a) Oesophageal cancer

Previous ecological studies evaluated at the fifty-sixth and seventy-fourth meetings were suggestive of an association between fumonisin exposure and oesophageal cancer, although a causative relationship was not established. The 2011 Committee called for case–control or cohort studies, with valid biomarkers and controlled for confounders and known risk factors.

Since the previous JECFA evaluation, one epidemiological study conducted in the northeast part of the Islamic Republic of Iran investigated the relationship between FB_1 contamination in foods and oesophageal cancer; however, this study is also an ecological study. In Golestan province, rice and maize samples were randomly collected from silos located in 22 geographical subdivisions, each in an area of either high or low oesophageal cancer risk based on the age-standardized incidence rates. The FB_1 levels in these samples were measured and compared between the areas with high and low risk of oesophageal cancer. FB_1 was found in 75% of rice samples from high-risk areas and 21% of samples from low-risk areas, with the mean FB_1 occurrence of 43.75 μg/g and 8.93 μg/g, respectively (U test, $P = 0.01$). The frequency of FB_1 contamination in the maize samples from the areas with high (57%) and low (47%) risk of oesophageal cancer was not significantly different ($P = 0.79$), and the difference in FB_1 levels was not statistically significant (167.14 versus 150 μg/g in the high- and low-risk areas; chi-squared test, $P = 0.79$). The authors concluded that fumonisin contamination in commonly used staple foods, especially rice, might be a potential risk factor for oesophageal cancer in this high-risk region (Alizadeh et al., 2012).

The Committee noted that there are many known risk factors of oesophageal cancer in Golestan province, including opium consumption, drinking hot tea, poor oral hygiene, obesity, exposure to polycyclic aromatic hydrocarbons and genetic factors. The ecological setting in this study does not allow for control of these factors. To establish dietary fumonisin exposure as a causative factor for oesophageal cancer, studies determining exposure and outcome on an individual level (case–control and cohort studies) are needed.

(b) Hepatocellular cancer (HCC)

Although FB_1 has been shown to induce hepatocellular carcinomas in rodents, it has not been demonstrated to cause hepatocellular cancer in humans. Hepatocellular cancer was not evaluated by the previous Committee as a separate health end-point of fumonisin.

Persson et al. (2012) investigated the risk of hepatocellular carcinoma from fumonisin exposure using nested case–control studies from two prospective cohorts in China. The Haimen City cohort (hepatocellular cancer case $n = 271$;

control n = 280) represents an adult population in an area with high risk for liver cancer, while the Linxian cohort (hepatocellular cancer case n = 72; control n = 147) represents an adult population in an area with high risk for gastric and oesophageal cancers. In this study, FB_1 exposure was measured by quantification of FB_1 in toenail samples. In the Haimen City cohort, there was no statistical difference between the toenail FB_1 levels in the cases (mean: 0.375 ng/mg) and the controls (mean: 0.143 ng/mg; P = 0.16). Although the FB_1 levels measured in the toenail samples from the Linxian cohort were higher, there also was no statistical difference between the cases and the controls (mean: 1.96 versus 2.27 ng/mg; P = 0.50). Due to the low frequency of toenail samples with detectable FB_1 (19–24% in the cases and controls of both cohorts), the exposure to FB_1 was described as a categorical variable in the disease regression models. The analysis found no statistically significant association between toenail FB_1 and hepatocellular cancer in either Haimen City (OR = 1.10; 95% CI: 0.64–1.89) or Linxian (OR = 1.47; 95% CI: 0.70–3.07), after adjusting for sex, age, area of residence, alcohol consumption and hepatitis B virus surface antigen (HBsAg) status. A pooled meta-analysis was conducted using data from both cohorts, and the result also showed no statistically significant association between FB_1 exposure and hepatocellular cancer (OR = 1.22; 95% CI: 0.79–1.89) (Persson et al., 2012).

The Committee noted that despite a previous report that the maize harvested for consumption by the Haimen City cohort (Jiangsu Province) was highly contaminated with fumonisin (Ueno et al., 1997), toenail FB_1 was much lower than in samples from Linxian. Since a deterministic method was not used to estimate food exposure in either of these populations, the validity of nail FB_1 as an exposure biomarker is unknown. Although the results of this study do not support an association between FB_1 and hepatocellular cancer, more studies are necessary to confirm this conclusion.

Although there is evidence in laboratory animals suggesting that co-exposure of fumonisins and aflatoxins may act additively or synergistically in the development of hepatocellular carcinoma (Torres et al., 2015), currently no epidemiological data are available on such an association in humans. The evidence documenting effects from aflatoxin and fumonisin co-exposure are presented in detail in a separate monograph below, "Co-exposure of fumonisins with aflatoxins", (pages 879–960).

(c) Childhood growth impairment

A study conducted in the United Republic of Tanzania (and reviewed by the seventy-fourth Committee) found that infants with fumonisin exposure greater than the PMTDI were shorter and lighter than those with fumonisin exposure

below the PMTDI. The proposed mechanism of action of this effect is reduced food consumption and inhibited sphingolipid metabolism (Smith, Stoltzfus & Prendergast, 2012). Infants and children are especially vulnerable to mycotoxin exposure, mostly because of a lower detoxification capacity, rapid growth and higher intake of food and water per unit body weight (Lombard, 2014).

Since the last JECFA evaluation, no new studies have been published on the association between childhood growth and fumonisin exposure alone. However, two epidemiological studies conducted in the United Republic of Tanzania investigated the association of fumonisin–aflatoxin co-exposure and childhood growth. One study was conducted in infants up to 5 months of age (Magoha et al., 2016) and the other in children aged 6–14 months (Shirima et al., 2015).

In the Magoha et al. (2016) study, 143 infants were progressively recruited after birth and followed up at 1, 3 and 5 months of age. At each follow-up visit, the infants' weight and length were measured and the weight-for-age *z*-score (WAZ) and length-for-age *z*-score (LAZ) computed according to the WHO child growth standards. Exclusive breastfeeding is rarely practised in the United Republic of Tanzania; 80% of the infants had started receiving complementary food at 3 months of age and 97% at 5 months. The majority of the complementary food was prepared from maize flour or mixed cereal flours with maize as the primary constituent. For infants who had been introduced to maize foods (in the form of maize flour), a 24-hour dietary recall was used to estimate the flour intake and samples of maize flour were collected from families for mycotoxin analysis. Of these flour samples (n = 67), 39 had detectable aflatoxins (median: 6 µg/kg diet), 21 had detectable fumonisins (median: 124 µg/kg diet) and 15 contained both aflatoxins and fumonisins.

The infants' growth status at 5 months of age was compared with that at 3 months of age. A slightly higher weight and length gain was found in exclusively breastfed infants (n = 23) than in those fed complementary foods (n = 92), regardless of mycotoxin contamination. Of the infants who had been introduced to maize-based food (n = 67), 6% were underweight and 18% were stunted. Of these underweight and stunted infants, 39 had been exposed to aflatoxins (3% underweight and 15% stunted); 21 had been exposed to fumonisins alone (0 underweight and 5% stunted); and 15 had been exposed to both aflatoxins and fumonisins (0 underweight and 7% stunted). The likelihood of an association between level of exposure to fumonisins or aflatoxins (alone or combined) and underweight or stunting was analysed using logistic regression. No statistically significant associations were observed (Magoha et al., 2016).

Although the results of the study did not show a significant association between mycotoxin exposure and impairment of growth in this infant population, the Committee acknowledged two limitations in the study that may compromise

the value of the results. First, growth impairment in infants is influenced by multiple factors, which were not controlled for in the study analysis. Second, previous studies conducted in the same region of the United Republic of Tanzania indicated that breast milk was also an important source of exposure to mycotoxins. Magoha et al. (2014a,b) reported that 100% of the breast milk samples collected from local lactating women were contaminated with aflatoxin M_1 and 44% with FB_1. Unfortunately, without taking into account this exposure from breast milk, the total exposure to these mycotoxins was not accurately estimated.

The Committee noted that the total exposure measured by validated biomarkers would be useful in determining the association between mycotoxin exposure and child growth.

The other study conducted in the United Republic of Tanzania (Shirima et al., 2015) used biomarkers to determine the total mycotoxin exposure in 166 seemingly healthy children, aged 6–14 months, randomly recruited from three villages and subsequently followed up after 6 and 12 months. At the recruitment and each of the two follow-up visits, the height and the weight of each child were measured and a dietary recall was performed. Blood and urine samples were collected for aflatoxin and fumonisin biomarker analysis. The results showed that mean LAZ and WAZ declined with increased age, indicating growth impairment in this population. Geometric mean UFB_1 concentrations at the three sampling times were 313.9 pg/mL at recruitment, 167.3 pg/mL at 6 months after recruitment and 569.5 pg/mL at 12 months after recruitment. The prevalence of stunted children was 44%, 55% and 56% at the three time points, respectively. The authors reported that the mean body length gain from recruitment to 12 months was 1.8 cm lower in children with mean UFB_1 concentrations (all three samples) in the highest (>935 pg/mL) versus lowest (<224 pg/mL) quartile (P = 0.028).

Using individual data, multiple regression analysis was performed to determine the association between UFB_1 and childhood growth. All models were adjusted for village, breastfeeding, maternal education, socioeconomic status and protein/energy intakes. The results showed that UFB_1 levels were not negatively associated with WAZ and WLZ (weight-for-length *z*-score); the negative association between aflatoxin–albumin (AF–alb) and child growth did not reach statistical significance; and the statistical analysis of the joint fumonisin–aflatoxin effect did not produce interpretable results. On the other hand, UFB_1 concentrations measured at recruitment were negatively associated with LAZ at 6 months (P = 0.016) and at 12 months (P = 0.014) from recruitment. The mean UFB_1 concentration of all three sampling times was negatively associated with LAZ (P < 0.001) and length velocity (P = 0.004) at 12 months from recruitment.

The Committee noted that these results indicated that fumonisin exposure could be a risk factor for stunting in children (Shirima et al., 2015).

3. Levels and patterns of contamination in food commodities

3.1 Surveillance data

Information on the natural occurrence of fumonisins for this report was collated from two sources. First, the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database was screened for fumonisin data (FB_1, FB_2, FB_3 and total fumonisins) submitted from January 2011 until August 2016; second, a literature search for fumonisin occurrence data was conducted for the same time period. An initial literature search conducted by the Food and Agriculture Organization of the United Nations (FAO) Secretariat was extended to match the time period of the dataset from the GEMS/Food contaminants database. A selection of keywords was prepared and the terms were researched using Scopus, PubMed and Ovid (which makes use of AGRICOLA, AGRIS and CAB abstracts). The literature for fumonisin occurrence was collated from January 2011 to August 2016. For this report, total fumonisins refers to the sum total of FB_1, FB_2 and FB_3.

The dataset from the GEMS/Food contaminants database was screened to remove aggregated data that were also entered as individual sample data, feed samples and samples with extremely high reported LOD or LOQ. An analysis of the distribution of the LOD and LOQ showed these to be highly skewed and best fitted a log-normal distribution with some high reported LOD and LOQ that would render non-detected results of little value. A cut-off of greater than two SDs from the mean of $\log_{10}$ transformed values was set. Consequently, samples of FB_1, FB_2 or FB_3 with an LOD or LOQ of greater than 250 and 750 µg/kg, respectively, were removed from the dataset. For total fumonisins, samples with an LOD above 550 µg/kg or LOQ above 2000 µg/kg were removed. The screened dataset contained 56 702 records. Most of the records were for FB_1 and FB_2 (25 143 and 19 990, respectively) with a smaller dataset for FB_3 and total fumonisins (8164 and 3405, respectively). In total, 96% of records had unique serial numbers for individual samples, which allowed calculation of the combined concentration of fumonisins when more than one fumonisin was analysed. Some samples had records for FB_1, FB_2 and total fumonisins, whereas others had records for FB_1, FB_2, FB_3 and total fumonisins, indicating that total fumonisins was reported for some samples as $FB_1 + FB_2$ and for others as $FB_1 + FB_2 + FB_3$. As such, records of total fumonisins were not included but data for total fumonisins were calculated based on the sum of FB_1, FB_2 and FB_3 (7580 records).

Fumonisins were analysed as FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$). Summary statistics from the GEMS/Food contaminants database are presented

in tables and include number of samples; per cent occurrence (percentage of positive samples, i.e. above the LOQ); LOD and LOQ range; lower bound (LB) mean (LB mean samples below LOQ entered as zero values); upper bound (UB) mean (UB mean samples below LOQ entered as LOQ value); and the maximum value (LOQ value used for samples below the LOQ). The sensitivity of the assays used varied widely, as evidenced in the range of reported LOD and LOQ values. Several of the summary statistics depend on the sensitivity of the assays used (e.g. percentage occurrence). The summing of FB_1 + FB_2 + FB_3 values resulted in an additive effect on the LOD and LOQ values for total fumonisins, which resulted in higher estimates of the UB mean.

Table 7 shows the FB_1 concentration for each food category. As is typical for mycotoxins, the concentration of fumonisins in any foodstuff, where they are detected, is not normally distributed with a proportion of samples undetected (left tail censorship) and a highly skewed right hand tail with maximum values often 2–3 orders of magnitude above the mean value. Most of the samples were cereals and cereal products (77%), and the highest occurrences and concentrations were detected in samples of cereals, cereal-based products and the other food categories that may contain cereals.

Products for special nutritional use had a high occurrence and concentration (occurrence: 43%; LB mean: 118 µg/kg; maximum: 2809 µg/kg). This category primarily contained medical food (specially formulated and intended for the dietary management of a disease with distinct nutritional needs that cannot be met by a normal diet; intended to be used under medical supervision). The cereal content of these products is not stated, but maize-based gluten-free products are likely the samples with high concentrations. Previously, Dall'Asta et al. (2009) identified high concentrations of fumonisins in some maize-based gluten-free products in Italy, with the highest median and maximum total fumonisin concentrations in flour samples (1020 and 3310 µg/kg food, respectively). The food category composite dishes had a moderate fumonisin occurrence and mean concentration. This was primarily due to higher concentrations in cereal-based dishes (n = 40; LB mean: 109 µg/kg; maximum: 727 µg/kg).

Of the cereals and cereal-based products, maize and maize-based products had the highest concentrations of FB_1 in the GEMS/Food contaminants database (Table 8). This was mirrored in the published literature (Table 9). For maize samples, the occurrence above the LOQ was just over 50% with an LB mean of 310 µg/kg, a UB mean of 392 µg/kg and a maximum of 23 800 µg/kg. The FB_1 maize results identified were mainly from Europe (n = 3757), followed by Canada (n = 898) and Japan (n = 103). No results were reported from Africa, South America or China, despite that these are major maize-producing areas

Table 7
Summary of FB_1 global occurrence data in food categories from the GEMS/Food contaminants database[a]

Food category[b]	No. of samples	% positive[c]	LOD range (µg/kg)	LOQ range (µg/kg)	LB mean (µg/kg)[d]	UB mean (µg/kg)[e]	Max. (µg/kg)
Cereals and cereal-based products	19 345	29.2	0.2–222	0.5–740	129	194	35 400
Snacks and desserts	1 078	45.5	0.2–222	0.5–740	60	107	4 824
Composite food	121	15.7	2.5–50	5–250	42	84	727
Food for infants and small children	1 710	7.3	0.2–200	0.5–667	3.6	83	1 110
Products for special nutritional use	245	43.3	4–100	13–333	118	158	2 809
Fats and oils (excluding butter)	134	3.7	0.5–25	1.7–83	0.7	32	83
Fruit and fruit products	126	6.3	2.5–100	5–333	5.3	68	333
Fruit and vegetable juices	28	10.7	2–10	5–35	0.5	19	35
Herbs, spices and condiments	226	4.4	2.5–25	5–83	7.0	40	435
Legumes and pulses	375	1.9	0.2–60	0.5–200	0.5	32	200
Meat and meat products	39	2.6	2.5–10	5–33	3.1	9.3	120
Milk and dairy products	32	0.0	10–20	30–67	0.0	34	67
Nuts and oilseeds	320	0.3	0.8–40	2.7–133	0.1	50	133
Starchy roots and tubers	68	0.0	2.5–25	5–83	0.0	40	83
Sugar and confectionery	216	1.9	0.8–222	2.7–740	0.5	54	740
Vegetables and vegetable products	147	0.0	10–220	30–733	0.0	53	733
Other foods	53	7.5	2.5–25	5–83	2.8	55	83
Alcoholic beverages	579	0.9	0.1–20	0.4–67	0.0	26	67
Nonalcoholic beverages	97	1.0	2–20	5–40	11	35	1 025
Stimulant beverages	204	2.5	2.5–20	5–67	1.0	33	67
Overall	**25 143**	**30.7**	**0.1–220**	**0.4–733**	**47**	**93**	**35 400**

FB_1: fumonisin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; No.: number; UB: upper bound
[a] Data submitted from January 2011 to August 2016.
[b] Food categories with less than 10 samples were not included.
[c] Percentage of samples above the LOD/LOQ.
[d] LB mean, samples below LOQ entered as zero values.
[e] UB mean, samples below LOQ entered as LOQ value.

and retrieving relevant published information for these areas was considered important.

The literature search retrieved nine studies with data for maize in Africa (not including two studies with only 1 or 2 samples). Occurrence and concentration of FB_1 were generally high in Africa, with reported means ranging from 226 to 1552 µg/kg and maximums from 1106 to 53 863 µg/kg (Kimanya et al., 2008; Phoku et al., 2012; Warth et al., 2012; Abia et al., 2013b; Adetunji et al., 2014; Kouadi et al., 2014; Kamala et al., 2015; Hove et al., 2016; Mngqawa et al., 2016). It is important to note that Phoku et al. (2012) compared FB_1 in maize and prepared maize porridge and showed a 90% reduction that was attributed to

Table 8
Summary of global occurrence of FB_1 in cereals and cereal-based products from the GEMS/Food contaminants database[a]

Cereals and cereal-based products	No. of samples	% positive[c]	LOD range (µg/kg)	LOQ range (µg/kg)	LB mean (µg/kg)[d]	UB mean (µg/kg)[e]	Max.
Cereal grains (not defined)	866	11	0.2–200	0.7–667	52	107	35 400
Barley	258	0.4	4–222	17–740	0.6	136	740
Buckwheat	109	1.8	4–25	17–83	0.8	42	83
Maize	4 854	52	0.2–222	0.5–740	310	392	23 800
Millet	130	1.5	4–100	17–333	0.4	46	333
Oats	465	0.6	2.5–220	5–733	0.9	61	733
Rice	703	1.3	2.5–200	5–667	0.8	62	667
Rye	205	4.4	4–100	167–333	1.8	57	333
Sorghum	1 569	12	13–222	25–740	31	64	3 419
Triticale	2	0.0	20–20	25–25	0.0	25	25
Wheat	1 049	1.6	2–222	5–740	2.5	152	750
Popcorn	223	29	4–133	25–400	108	189	6 601
Sweet corn (corn on the cob)	266	9.8	0.5–100	1.7–333	12	86	793
Sweet corn (kernels)	32	0	10–50	30–250	0	71	250
Bran, unprocessed of cereal grain	81	11	4–120	30–400	26	79	655
Bread & other cooked cereal products	3 511	27	0.5–200	1.7–667	46	88	2 270
White bread	116	0.9	2–200	5–667	0.3	143	667
Wholemeal bread	8	0.0	2–50	10–167	0.0	45	167
Cereal-based food for infants and babies	1 224	5.2	0.8–200	2.7–667	3.9	89	1 110
Other cereals and cereal-based products	4 930	36	0.2–222	0.7–740	141	198	11 963
Overall	**20 601**	**27.8**	**0.2–222**	**0.5–740**	**121**	**188**	**35 400**

FB_1: fumonisin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; No.: number; UB: upper bound
[a] Data submitted from January 2011 to August 2016.
[b] Percentage of samples above the LOD/LOQ.
[c] LB mean, samples below LOQ entered as zero values.
[d] UB mean, samples below LOQ entered as LOQ value.

overnight soaking of the maize meal and subsequent draining of the steep water prior to cooking.

In South America, two studies were conducted on maize. The first was across four regions of Brazil showing close to 100% occurrence (LOQ: 15 µg/kg) and a range of concentrations; the region with the highest contamination had a mean of 2810 µg/kg and a maximum of 9670 µg/kg (Rocha et al., 2009). The second study in southern Brazil had a lower occurrence (47%; LOQ: 21 µg/kg) and a mean of positives of 1114 µg/kg (Scussel et al., 2014). Several studies have reported fumonisin concentrations in maize in China. Fu et al. (2015) reported a

Table 9
Summary of occurrence of FB_1 in cereals and their ingredients from the published literature[a]

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Barley												
Barley	Brazil	HPLC-UV	50	10	0.2	1	–	6	–	–	13	Piacentini et al. (2015)
Barley	Malaysia	UHPLC-MS/MS	7	43	20	40	–	–	–	–	31	Soleimany et al. (2012)
Barley and malt	Czech Republic	UHPLC-MS/MS	52	5	3	10	–	–	–	<10	–	Bolechová et al. (2015)
Barley balls with wheat syrup (organic)	France	HPLC-MS	27	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Barley flakes (organic)	Germany	HPLC-MS	43	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Beer	Brazil	HPLC-UV	53	15	7	21	–	105	–	90	285	Piacentini et al. (2015)
Beer	Brazil	HPLC-UV	53	15	–	21	–	105	–	90	285	Rubert et al. (2013b)
Beer	Europe	HPLC-MS	106	97	0.1	0.3	–	6	–	–	30	Bertuzzi et al. (2011)
Beer	Japan	LC-MS/MS	70	–	–	2	–	–	5	–	77	Sugita-Konishi et al. (2013)
Buckwheat												
Buckwheat flakes (organic)	France	HPLC-MS	30	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Buckwheat flakes (organic)	Germany	HPLC-MS	28	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Cereal grains												
Barley, oat and wheat flakes (organic)	France	HPLC-MS	15	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Barley, oat, rice, rye and wheat flakes (organic)	France	HPLC-MS	33	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Bran, corn, oat, rice and wheat biscuits (organic)	France	HPLC-MS	41	0	3	10	–	–	ND	–	–	Rubert et al. (2013b)
Ethnic foods	Spain	HPLC-FD	35	51	5	15	–	–	–	–	547	Cano-Sancho et al. (2012a)
Gluten-free foods	Spain	HPLC-FD	18	28	5	15	–	–	–	–	19	Cano-Sancho et al. (2012a)
Rice and wheat flakes	France	HPLC-MS	48	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Corn and rice bars	France	HPLC-MS	69	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)

Table 9 (continued)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Corn and wheat squares	France	HPLC-MS	54	17	3	10	–	–	–	–	49	Rubert et al. (2013a)
Corn, oats, rice and wheat bars with fruits	France	HPLC-MS	58	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Corn, rice and wheat bars	France	HPLC-MS	27	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Muesli (organic)	France	HPLC-MS	19	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Oat, rye and wheat flakes (organic)	France	HPLC-MS	27	11	3	10	–	–	–	–	98	Rubert et al. (2013a)
Maize												
Beer (maize)	Cameroon	LC-MS/MS	14	100	0.14	0.50	–	334	–	–	741	Abia et al. (2013b)
Beer (maize)	Malawi	LC-MS/MS	9	100	11	22	–	1 522	–	1 721	–	Matumba et al. (2014)
Corn balls	France	HPLC-MS	59	0	3	10	–	–	–	–	–	Rubert et al. (2013a)
Corn flour	Brazil	HPLC-FD	15	53	2.5	13	–	–	–	–	1 542	Savi et al. (2016)
Corn flour	Brazil	HPLC	25	76	30	100	247	–	–	–	559	Bordin et al. (2014)
Corn flour	Brazil	HPLC-FD	15	73	–	78	254	–	–	–	555	Martins et al. (2012)
Corn flour	Cameroon	LC-MS/MS	8	100	0.05	–	–	90	–	–	132	Abia et al. (2013b)
Corn flour	United Republic of Tanzania	HPLC	67	30	53	–	–	–	–	140	974	Magoha et al. (2016)
Corn grits	Brazil	HPLC-FD	15	100	2.5	13	–	719	–	–	2 727	Savi et al. (2016)
Corn grits	Brazil	HPLC-FD	28	68	–	78	414	–	–	–	3 462	Martins et al. (2012)
Corn grits	Japan	LC-MS/MS	63	–	–	2	–	–	197	–	1 929	Sugita-Konishi et al. (2013)
Corn meal	Brazil	HPLC-FD	15	100	2.5	13	–	1 305	–	–	5 439	Savi et al. (2016)
Corn meal	Brazil	HPLC	32	78	30	100	475	–	–	–	1 209	Bordin et al. (2014)
Corn meal	Brazil	HPLC	18	72	30	100	423	–	–	–	–	Bordin et al. (2014)
Corn meal	Brazil	HPLC-FD	29	97	–	78	494	–	–	–	2 322	Martins et al. (2012)
Corn puffs	Romania	ELISA	4	25	–	–	92	–	–	92	92	Galbenu, Damiescu & Trif (2011)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Corn snacks	Japan	LC-MS/MS	120	–	–	2	–	–	87	–	1 673	Sugita-Konishi et al. (2013)
Corn snacks	Romania	ELISA	1	0	–	–	–	–	ND	–	–	Galbenu, Damiescu & Trif (2011)
Cornflakes	Brazil	HPLC-FD	11	82	–	78	341	–	–	–	840	Martins et al. (2012)
Cornflakes	Brazil	HPLC-FD	25	56	2.5	13	–	78	–	–	317	Savi et al. (2016)
Cornflakes	Brazil	HPLC	14	50	30	100	104	–	–	–	–	Bordin et al. (2014)
Cornflakes	Brazil	HPLC	6	50	30	100	171	–	–	–	–	Bordin et al. (2014)
Cornflakes	France	HPLC-MS	96	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Cornflakes	Japan	LC-MS/MS	121	–	–	10	–	–	6	–	103	Sugita-Konishi et al. (2013)
Corn flakes	Romania	ELISA	4	50	–	–	31	–	–	31	31	Galbenu, Damiescu & Trif (2011)
Corn flakes	Spain	HPLC-MS	85	12	3	10	–	–	–	–	205	Rubert et al. (2013a)
Corn flakes (organic)	France	HPLC-MS	37	11	3	10	–	–	–	–	190	Rubert et al. (2013a)
Corn soups (powder)	Japan	LC-MS/MS	59	–	–	10	–	–	1	–	27	Sugita-Konishi et al. (2013)
Corn starch	Japan	LC-MS/MS	45	–	–	2	–	–	2	–	63	Sugita-Konishi et al. (2013)
Gofio of corn (organic)	Spain	HPLC-MS	34	97	3	10	–	–	–	–	1 202	Rubert et al. (2013a)
Kunu (maize drink)	Nigeria	LC-MS/MS	1	100	0.14	0.50	123	–	–	–	–	Ezekiel et al. (2015)
Maize	Brazil	HPLC-FD	15	80	2.5	13	–	289	–	–	1 732	Savi et al. (2016)
Maize	Brazil	HPLC	232	47	7	21	–	1 114	–	–	7 832	Scussel et al. (2014)
Maize	Brazil	HPLC	50	100	–	15	2 810	–	–	–	9 670	Rocha et al. (2009)
Maize	Brazil	HPLC	50	100	–	15	2 750	–	–	–	9 420	Rocha et al. (2009)
Maize	Brazil	HPLC	50	100	–	15	720	–	–	–	6 270	Rocha et al. (2009)
Maize	Brazil	HPLC	50	92		15	730	–	–	–	8 440	Rocha et al. (2009)
Maize	Burkina Faso	LC-MS/MS	26	81	20	–	–	–	–	269	1 343	Warth et al. (2012)
Maize	Cameroon	LC-MS/MS	37	100	0.05	–	–	508	–	–	2 313	Abia et al. (2013b)
Maize	China	UHPLC	255	52	10	65	1 318	–	–	–	–	Feng YZ et al. (2011a)
Maize	China	HPLC-FD	36	23	9	62	–	1 124	–	–	3 480	Feng Y et al. (2011b)
Maize	China	HPLC-FD	–	25	–	–	48	–	–	–	–	Fu et al. (2015)

Table 9 (continued)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Maize	China	HPLC-FD	–	78	–	–	1 885	–	–	–	–	Fu et al. (2015)
Maize	China	HPLC-FD	–	42	–	–	3 993	–	–	–	–	Fu et al. (2015)
Maize	China	HPLC-FD	–	10	–	–	271	–	–	–	–	Fu et al. (2015)
Maize	China	HPLC-FD	–	81	–	–	922	–	–	–	–	Fu et al. (2015)
Maize	China	HPLC-FD	–	15	–	–	85	–	–	–	–	Fu et al. (2015)
Maize	China	HPLC-FD	225	–	4	13	1 878	–	–	–	28 285	Guo et al. (2016)
Maize	China	HPLC-FD	125	46	4	13	–	–	344	–	4 933	Li et al. (2015b)
Maize	China	HPLC-FD	146	38	4	13	–	–	340	–	15 221	Li et al. (2015a)
Maize	China	HPLC-FD	31	90	–	–	300	–	–	–	12 500	Sun et al. (2011)
Maize	China	HPLC-FD	34	85	–	–	400	–	–	–	37 000	Sun et al. (2011)
Maize	China	HPLC-FD	43	100	–	–	2 600	–	–	–	5 900	Sun et al. (2011)
Maize	China	HPLC	307	36	11	36	522	–	–	–	9 975	Wei et al. (2013)
Maize	India	HPLC	1	86	–	–	–	–	1 575	–	6 540	Sreenivasa et al. (2013)
Maize	Cote d'Ivoire	LC-MS/MS	51	100	1.1	–	–	–	226	176	1 464	Kouadio et al. (2014)
Maize	Mozambique	LC-MS/MS	13	92	20	–	–	–	–	869	7 615	Warth et al. (2012)
Maize	Nigeria	LC-MS/MS	65	93	7	–	1 552	–	–	1 064	10 447	Adetunji et al. (2014)
Maize	Romania	ELISA	11	36	–	–	75	–	–	78	88	Galbenu, Damiescu & Trif (2011)
Maize	Russian Federation	HPLC	16	88	10	40	1 440	–	–	780	5 130	Tutelyan et al. (2013)
Maize	Russian Federation	HPLC	5	60	10	40	217	–	–	80	56	Tutelyan et al. (2013)
Maize	Russian Federation	HPLC	18	83	10	40	184	–	–	170	840	Tutelyan et al. (2013)
Maize	Russian Federation	HPLC	2	100	10	40	85	–	–	85	110	Tutelyan et al. (2013)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Maize	Russian Federation	HPLC	8	63	10	40	862	–	–	880	1 940	Tutelyan et al. (2013)
Maize	Russian Federation	HPLC	2	50	10	40	1 880	–	–	1 880	–	Tutelyan et al. (2013)
Maize	South Africa	LC-MS/MS	29	86	–	10	720	–	–	–	8 514	Mngqawa et al. (2016)
Maize	South Africa	LC-MS/MS	23	100	–	10	279	–	–	–	1 584	Mngqawa et al. (2016)
Maize	South Africa	LC-MS/MS	31	35	–	10	545	–	–	–	2 732	Mngqawa et al. (2016)
Maize	South Africa	LC-MS/MS	31	58	–	10	1 088	–	–	–	18 924	Mngqawa et al. (2016)
Maize	South Africa	HPLC-FD	54	91	–	–	–	8 189	–	–	53 863	Phoku et al. (2012)
Maize	United Republic of Tanzania	HPLC	120	52	53	–	–	–	–	206	6 125	Kimanya et al. (2008)
Maize	United Republic of Tanzania	UHPLC-MS	60	73	4	8	–	1 361	–	–	18 184	Kamala et al. (2015)
Maize	Viet Nam	HPLC	47	55	–	–	740	–	–	391	2 747	Phuong et al. (2015)
Maize	Viet Nam	HPLC	50	78	–	–	1 757	–	–	871	10 799	Phuong et al. (2015)
Maize	Zimbabwe	LC-MS/MS	95	95	12.5	25	–	242	–	146	1 106	Hove et al. (2016)
Maize-based products	Brazil	HPLC	50	60	30	100	360	–	–	–	–	Bordin et al. (2015)
Maize-based products	China	UHPLC-MS/MS	103	98	0.08	0.3	268	–	–	–	5 046	Li et al. (2015)
Maize germ (dry-milled)	Spain	HPLC-FD	12	100	50	100	563	–	–	–	1 553	Escobar et al. (2013)
Maize germ (wet-milled)	Spain	HPLC-FD	12	100	50	100	847	–	–	–	1 544	Escobar et al. (2013)
Maize porridge	South Africa	HPLC-FD	47	87	–	–	–	6	–	–	20	Phoku et al. (2012)
Maize porridge	United Republic of Tanzania	UHPLC-MS/MS	34	100	0.5	1	–	96	–	60	–	Geary et al. (2016)
Maize porridge	United Republic of Tanzania	UHPLC-MS/MS	34	100	0.5	1	–	391	–	290	–	Geary et al. (2016)

Table 9 (continued)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Maize porridge	United Republic of Tanzania	UHPLC-MS/MS	33	100	0.5	1	–	408	–	384	–	Geary et al. (2016)
Maize porridge (organic)	France	HPLC-MS	36	0	3	10	–	–	–	–	–	Rubert et al. (2013a)
Polenta	Brazil	HPLC	2	100	30	100	182	–	–	–	214	Bordin et al. (2014)
Polenta	Brazil	HPLC	7	43	30	100	214	–	–	–		Bordin et al. (2014)
Popcorn	Brazil	HPLC	39	82	30	100	278	–	–	–	1 127	Bordin et al. (2014)
Popcorn	Brazil	HPLC	15	73	30	100	537	–	–	–		Bordin et al. (2014)
Popcorn	Brazil	HPLC-FD	17	88		78	338	–	–	–	1 770	Martins et al. (2012)
Popcorn	Brazil	HPLC-FD	10	40	2.5	13	–	86	–	–	180	Savi et al. (2016)
Popcorn	Japan	LC-MS/MS	79	–	–	2	–	–	43	–	354	Sugita-Konishi et al. (2013)
Sweet corn	Brazil	HPLC	8	25	30	100	112	–	–	–	–	Bordin et al. (2014)
Sweet corn	Brazil	HPLC	4	0	30	100	ND	–	–	–	–	Bordin et al. (2014)
Sweet corn	Japan	LC-MS/MS	126	0	–	10	–	–	ND	–	36	Sugita-Konishi et al. (2013)
Sweet corn	Romania	ELISA	6	17	–	–	–	–	–	91	91	Galbenu, Damiescu & Trif (2011)
Millet												
Millet	Japan	LC-MS/MS	62	–	–	2	–	–	3	–	32	Sugita-Konishi et al. (2013)
Oats												
Oat flakes	Germany	HPLC-MS	38	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Oat flakes (organic)	Germany	HPLC-MS	41	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Rice												
Rice	Canada	HPLC-FD	199	8	0.7	–	–	5	0.4	–	–	Bansal et al. (2011)
Rice	China	HPLC-FD	9	89	–	–	300	–	–	–	400	Sun et al. (2011)
Rice	China	HPLC-FD	10	90	–	–	200	–	–	–	300	Sun et al. (2011)
Rice	China	HPLC-FD	10	90	–	–	200	–	–	–	500	Sun et al. (2011)
Rice	Ecuador	UHPLC	23	30	16	–	277	–	–	–	1 146	Ortiz et al. (2013)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Rice	Ecuador	UHPLC	20	15	16	–	40	–	–	–	54	Ortiz et al. (2013)
Rice	Iran (Islamic Republic of)	LC-MS/MS	65	9	–	7	–	111	–	–	177	Nazari et al. (2014)
Rice	Malaysia	UHPLC-MS/MS	40	8	20	40	–	–	–	–	33	Soleimany et al. (2012)
Rice	Nigeria	HPLC-FD	21	14	0.01		–	–	0.2	–	4	Makun et al. (2011)
Rye												
Rye flakes (organic)	Germany	HPLC-MS	28	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Sorghum												
Pito (sorghum drink)	Nigeria	LC-MS/MS	1	0	0.14	0.50	ND	–	–	–	–	Ezekiel et al. (2015)
Sorghum	India	HPLC	14	100	–	–	–	–	2 429	–	14 510	Sreenivasa et al. (2013)
Sorghum	Tunisia	HPLC-MS/MS	60	0	5	18	–	–	–	–	–	Oueslati et al. (2014)
Spelt												
Spelt flakes (organic)	France	HPLC-MS	42	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Spelt flakes (organic)	Germany	HPLC-MS	61	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Wheat												
Gofio of wheat (organic)	Spain	HPLC-MS	35	77	3	10	–	–	–	–	1 001	Rubert et al. (2013a)
Wheat	Argentina	LC-MS/MS	135	97	0.01	–	–	–	30	–	680	Cendoya et al. (2014)
Wheat	Brazil	HPLC-FD	11	55	200	680	–	2 814	–	–	5 319	Mendes et al. (2015)
Wheat	Malaysia	UHPLC-MS/MS	20	15	20	40	–	–	–	–	29	Soleimany et al. (2012)
Wheat	Serbia	ELISA	28	89	–	750	–	–	–	2 079	5 400	Stankovic et al. (2012)
Wheat	Serbia	ELISA	75	92	–	750	–	–	–	919	4 900	Stankovic et al. (2012)
Wheat	Syrian Arab Republic	HPLC-MS/MS	40	10	5	15	–	–	1	–	6	Alkadri et al. (2014)
Wheat (durum)	Argentina	LC-MS/MS	40	78	0.01	–	–	–	66	–	1 304	Cendoya et al. (2014)
Wheat (durum)	Argentina	LC-MS/MS	29	97	10	–	–	–	194	–	–	Palacios et al. (2011)
Wheat-based products	Multiple	LC-MS/MS	65	3		83	–	–	–	–	184	Serrano et al. (2012)

Table 9 (continued)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Wheat bran (organic)	Germany	HPLC-MS	32	0	3	10	–	–	ND	–	–	Rubert et al. (2013a)
Wheat flakes (organic)	France	HPLC-MS	63	14	3	10	–	–	–	–	126	Rubert et al. (2013a)
Wheat flakes (organic)	Germany	HPLC-MS	44	14	3	10	12	–	–	–	60	Rubert et al. (2013a)
Wheat flour	China	UHPLC-MS/MS	369	6	0.08	0.27	–	–	–	–	35	Li et al. (2015)
Wheat flour	China	HPLC-FD	7	71	–	–	200	–	–	–	400	Sun et al. (2011)
Wheat flour	China	HPLC-FD	9	89	–	–	200	–	–	–	400	Sun et al. (2011)

ELISA: enzyme-linked immunosorbent assay; FB_1: fumonisin B_1; FD: fluorescence detection; HPLC: high-performance liquid chromatography; LC: liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS: mass spectrometry; MS/MS: tandem mass spectrometry; ND: not detected; No.: number; Max.: maximum; Med., median; UHPLC: ultra-high-performance liquid chromatography; UV: ultraviolet detection

[a] Literature search collated from January 2011 to August 2016.

[b] Refers to the source of the samples. Most samples were locally produced; however, some were imported goods.

[c] Percentage of samples above the LOD/LOQ.

[d] Mean calculation not defined.

[e] Mean of positive samples.

[f] Mean of all samples.

wide range of mean concentrations for FB_1 across six provinces from 48 to 3993 µg/kg. Similar results have been reported by Sun et al. (2011), Wei et al. (2013), Li et al. (2015a) and Guo et al. (2016) with maximum concentrations of 37 000, 9975, 15 221 and 28 285 µg/kg, respectively. Phuong et al. (2015) reported similar high concentrations in two regions of Viet Nam with means of 740 and 1757 µg/kg and maximum concentrations of 2747 and 10 799 µg/kg for FB_1 within each region.

Sorghum had intermediate levels of FB_1 with an occurrence of 12%, an LB mean of 31 µg/kg and a maximum of 3419 µg/kg (Table 8). The literature search identified two studies with large differences between geographical locations, with no detectable FB_1 (LOQ: 18 µg/kg) found in 60 samples of sorghum in Tunisia (Oueslati et al., 2014) compared to a mean of 2429 µg/kg and a maximum of 14 510 µg/kg in sorghum samples in India (Sreenivasa et al., 2013).

All other cereal grains had a much lower occurrence (<5%) and LB means below 3 µg/kg (Table 8). However, wheat, barley and oats all had maximum concentrations between 500 and 1000 µg/kg, indicating that higher concentrations can occasionally occur in these cereals. This was similarly described in the reviewed literature except in two reports of high fumonisins in wheat, in Serbia and Brazil. The study in Serbia reported a per cent occurrence of above 80% (LOQ: 750 µg/kg) in two separate study years, means of positive samples of 2079 and 919 µg/kg in the two separate years and a maximum of 5400 µg/kg for FB_1 (Stankovic et al., 2012). The study in southern Brazil reported a per cent occurrence of 55% (LOQ: 680 µg/kg), a mean of positive samples of 2814 µg/kg and a maximum of 5319 µg/kg from 11 wheat samples (Mendes et al., 2015).

A review of all cereal products in the GEMS/Food contaminants database containing FB_1 at more than 1000 µg/kg indicated that the majority were maize-based products whereas the cereal content of the remainder was undefined (Table 8). Compared to maize grains, there was a lower occurrence and concentration in popcorn (LB mean: 108 µg/kg) and an even lower occurrence and concentration in the two forms of sweet corn (corn on the cob and kernels). The apparent difference between the two forms of sweet corn may well be because of the differences in the sensitivity of assays used (up to 20-fold), because although the occurrence and LB means are markedly different, the UB means are similar.

The non-cereal-based category samples in the GEMS/Food contaminants database had predominantly undetectable or low concentrations of FB_1 (Table 7). Corresponding results were reported in the published literature (Table 10). In the herbs, spices and condiments section of the GEMS/Food contaminants database, two herb samples, turmeric and white pepper, both had FB_1 concentrations of approximately 400 µg/kg. The literature search found three studies that analysed a wide range of herbs and spices, all with low or undetectable levels found (Waśkiewicz et al., 2013; Soyogul et al., 2016; Reinholds et al., 2017). The

Table 10
Occurrence of FB_1 in non-cereal food categories from the published literature[a]

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Fruits and fruit products												
Raspberry	Turkey	HPLC-FD	2	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Figs (dried)	Japan	LC-MS/MS	10	–	–	2	–	–	4	–	27	Sugita-Konishi et al. (2013)
Figs (dried)	Turkey	HPLC	125	51	10	30	52	–	–	–	163	Kosoglu et al. (2011)
Figs (dried)	Turkey	HPLC	137	81	10	30	75	–	–	–	332	Kosoglu et al. (2011)
Herbs, spices and condiments												
Allspice	Poland	UHPLC-MS/MS	5	–	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Basil	Poland	UHPLC-MS/MS	3	–	1	3	8	–	–	–	–	Waśkiewicz et al. (2013)
Basil	Turkey	HPLC-FD	5	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Bay leaf	Poland	UHPLC-MS/MS	3	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Black pepper	Pakistan	UHPLC-MS/MS	82	–	–	–	–	123	–	130	135	Yogendrarajah et al. (2014)
Black pepper	Poland	UHPLC-MS/MS	4	–	1	3	11	–	–	–	–	Waśkiewicz et al. (2013)
Black pepper	Poland	UHPLC-MS/MS	5	–	1	3	10	–	–	–	–	Waśkiewicz et al. (2013)
Cayenne pepper	Poland	UHPLC-MS/MS	8	–	1	3	33	–	–	–	–	Waśkiewicz et al. (2013)
Cinnamon	Poland	UHPLC-MS/MS	4	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Cloves	Poland	UHPLC-MS/MS	2	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Coriander	Turkey	HPLC-FD	8	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Curry	Poland	UHPLC-MS/MS	5	–	1	3	–	–		–	–	Waśkiewicz et al. (2013)
Horsetail	Turkey	HPLC-FD	1	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Laurel	Turkey	HPLC-FD	1	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Lime	Turkey	HPLC-FD	8	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Marjoram	Poland	UHPLC-MS/MS	5	–	1	3	6	–		–	–	Waśkiewicz et al. (2013)
Marshmallow	Turkey	HPLC-FD	3	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Mint	Turkey	HPLC-FD	15	13	23	78	–	–	25	–	256	Soyogul et al. (2016)
Nettle	Turkey	HPLC-FD	7	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Nutmeg	Latvia	HPLC-MS/MS	50	2	3	9	25	–	–	–	–	Reinholds et al. (2017)
Nutmeg	Poland	UHPLC-MS/MS	5	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Oregano	Poland	UHPLC-MS/MS	4	–	1	3	7	–	–	–	–	Waśkiewicz et al. (2013)
Oregano	Poland	UHPLC-MS/MS	2	–	1	3	8	–	–	–	–	Waśkiewicz et al. (2013)
Paprika – hot	Latvia	UHPLC-MS	14	71	0.8	2.7	105	–	–	–	140	Reinholds, Pugajeva & Bartkevics (2016)
Paprika – sweet	Latvia	UHPLC-MS	36	11	0.8	2.7	22	–	–	–	33	Reinholds, Pugajeva & Bartkevics (2016)
Poppy	Turkey	HPLC-FD	3	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Rosehip	Turkey	HPLC-FD	9	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Rosemary	Poland	UHPLC-MS/MS	2	–	1	3	8	–	–	–	–	Waśkiewicz et al. (2013)
St. John's wort	Turkey	HPLC-FD	1	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
Tarragon	Poland	UHPLC-MS/MS	1	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)

Table 10 (continued)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Thyme	Poland	UHPLC-MS/MS	3	–	1	3	5	–	–	–	–	Waśkiewicz et al. (2013)
Thyme	Turkey	HPLC-FD	14	7	23	78	–	–	9	–	125	Soyogul et al. (2016)
Turmeric	Poland	UHPLC-MS/MS	1	100	1	3	5	–	–	–	–	Waśkiewicz et al. (2013)
Walnut leaves	Turkey	HPLC-FD	1	0	23	78	–	–	ND	–	–	Soyogul et al. (2016)
White mustard	Poland	UHPLC-MS/MS	4	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
White pepper	Poland	UHPLC-MS/MS	7	–	1	3	7	–	–	–	–	Waśkiewicz et al. (2013)
Plant oils												
Corn oil	China	UHPLC-MS/MS	50	0	0.08	0.27	ND	–	–	–	–	Li et al. (2015)
Corn oil	Spain	HPLC-FD	25	4	50	100	27	–	–	–	77	Escobar et al. (2013)
Margarine (corn oil)	Spain	HPLC-FD	25	8	50	100	31	–	–	–	109	Escobar et al. (2013)
Plant oil	China	HPLC-FD	39	0	–	–	ND	–	–	–	–	Sun et al. (2011)
Pulses, nuts and oilseeds												
Groundnut	Burkina Faso	LC-MS/MS	9	0	20	–	ND	–	–	–	–	Warth et al. (2012)
Groundnut	Cameroon	LC-MS/MS	35	51	0.14	0.50	–	5	–	–	10	Abia et al. (2013b)
Groundnut	Cameroon	LC-MS/MS	15	73	0.14	0.50	–	4	–	–	17	Abia et al. (2013b)
Groundnut	China	HPLC-FD	17	0	–	–	ND	–	–	–	–	Sun et al. (2011)
Groundnut	Mozambique	LC-MS/MS	23	0	20	–	ND	–	–	–	–	Warth et al. (2012)
Kuru-Kuru	Cameroon	LC-MS/MS	6	100	0.14	0.50	–	3	–	–	4	Abia et al. (2013b)
Soybean	Cameroon	LC-MS/MS	10	100	0.14	0.50	–	49	–	–	68	Abia et al. (2013b)
Soybean	Japan	LC-MS/MS	84	–	–	2	–	–	1	–	9	Sugita-Konishi et al. (2013)
Soybean products	Japan	LC-MS/MS	18	–	–	2	–	–	1	–	8	Sugita-Konishi et al. (2013)

Cereal / Food name	Country[b]	Analytical method	No. of samples	% positive[c]	LOD (µg/kg)	LOQ (µg/kg)	Mean? (µg/kg)[d]	Mean +ve (µg/kg)[e]	Mean total (µg/kg)[f]	Med. (µg/kg)	Max. (µg/kg)	Reference
Sunflower	Poland	UHPLC-MS/MS	3	0	1	3	ND	–	–	–	–	Waśkiewicz et al. (2013)
Starchy roots and tubers												
Water yam	Nigeria	HPLC-MS	15	13	0.5	1	ND	–	–	–	2	Somorin et al. (2012)
White yam	Nigeria	HPLC-MS	58	19	0.5	1	5	–	–	–	91	Somorin et al. (2012)
Stimulant beverages												
Coffee (caffeinated)	Spain	LC-MS/MS	52	87	16	53	130	–	–	–	537	García-Moraleja et al. (2015a)
Coffee (decaffeinated)	Spain	LC-MS/MS	40	55	16	53	75	–	–	–	183	García-Moraleja et al. (2015a)
Coffee	Spain	LC-MS/MS	169	4	3	3	–	16	0.6	–	18	García-Moraleja et al. (2015b)
Vegetables and vegetable products												
Boiled asparagus	Japan	LC-MS/MS	10	0	–	2	–	–	ND	–	–	Sugita-Konishi et al. (2013)
Canned asparagus	Japan	LC-MS/MS	40	–	–	2	–	–	0.1	–	3	Sugita-Konishi et al. (2013)
Garlic	Poland	UHPLC-MS/MS	3	–	1	3	11	–	–	–	–	Waśkiewicz et al. (2013)

HPLC: high-performance liquid chromatography; FB_1: fumonisin B_1; FD: fluorescence detection; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; Med.: median; MS: mass spectrometry; MS/MS: tandem mass spectrometry; No.: number; UHPLC: ultra-high-performance liquid chromatography

[a] Literature search collated from January 2011 to August 2016.

[b] Refers to the source of the samples. Most samples were locally produced; however, some samples, particularly in the herb, spices and condiments section, were imported goods.

[c] Percentage of samples above the LOD/LOQ.

[d] Calculation not clearly defined.

[e] Mean of positive samples.

[f] Mean of all samples.

maximum for FB_1 was 135 µg/kg in a sample of black pepper (Yogendrarajah et al., 2014).

Fumonisins were absent from all alcoholic beverages other than beer, which had a very low occurrence (1% FB_1). The main cereal ingredient in beer is not identified in the GEMS/Food contaminants database, but the literature describes a distinct difference in the fumonisin levels between barley-based and maize-based beers. Low levels were detected in barley-based beers (maximum 285 µg/kg; Bertuzzi et al., 2011; Rubert et al., 2013b; Sugita-Konishi et al., 2013) with higher concentrations in maize-based beers from Cameroon (mean 334 µg/kg; Abia et al., 2013b) and Malawi (mean 1522 µg/kg; Matumba et al., 2014).

All samples of the nonalcoholic beverages had undetectable levels of FB_1 (LOQ: 5–40 µg/kg) except a single sample of soy-based beverage with a concentration of 1025 µg/kg (Table 7). Of the fermented cereal beverages from Nigeria, the sorghum-based drink (pito) had no detectable fumonisins, whereas the maize-based drink (kunu) had FB_1 at 123 µg/kg although this was much lower than the maize used in its production (Ezekiel et al., 2015).

For stimulant beverages, fumonisins were absent or at low concentrations (percentage positive 2.5%; FB_1 LB mean: 1 µg/kg) (Table 7). Higher concentrations were detected in coffee products (García-Moraleja et al., 2015a) but not in the prepared beverage (García-Moraleja et al., 2015b).

FB_1 was undetectable (LOQ: 30–67 µg/kg) in milk and dairy products and rarely detected in meat and meat products (occurrence 2.6% [LOQ: 5–33 µg/kg] and LB mean 3.1 µg/kg), indicating that carry-over from animal products is negligible. The presence of fumonisins in bovine milk has only been conducted in two previous studies performed in the USA and in Italy. Both these studies identified low or undetectable concentrations with a maximum of 1.3 and 0.4 µg/kg, respectively (Maragos & Richard, 1984; Gazzotti et al., 2009). The absence or low level of fumonisins in dairy and meat products indicates negligible transfer of these mycotoxins. This is in agreement with the previous JECFA assessment (Annex 1, reference *205*). Consequently, the current Committee meeting did not conduct an evaluation of the occurrence of fumonisins in feed.

A recent study of human breast milk in the United Republic of Tanzania reported high concentrations of fumonisins with 44% of samples with detectable FB_1 (LOQ: 19.5 ng/mL) and a maximum of 471 ng/mL (Magoha et al., 2014a). The reported high concentrations in this study may be due to (1) very high transfer rates in humans that would be counter to the known physicochemical properties of FB_1 (low absorption by humans and very low transfer rates from feed to milk in dairy cows; Fink-Gremmels, 2008; Annex 1, reference *206*); (2) extraordinarily high FB_1 exposure in the mothers' diet; and/or (3) matrix issues with the HPLC coupled with fluorescence detection (HPLC-FD) methodology.

Further studies are required to elucidate the cause of these reported high values in human breast milk.

For fruit in the GEMS/Food contaminants database, the only examples above negligible occurrence/concentrations were figs, with an occurrence of 26% (LOQ: 30–133 µg/kg) and LB mean for FB_1 of 22 µg/kg. In the literature, dried figs from Turkey had an overall mean of 64 µg/kg (Kosoglu et al., 2011).

All other food categories in the GEMS/Food contaminants database (vegetables, pulses, oilseeds, nuts, starchy roots and tubers) had undetectable or negligible levels of FB_1; this was also the case with the literature search.

Table 11 shows that 7580 samples had results reported for FB_1, FB_2 and FB_3 allowing the calculation of total fumonisins in these samples. The distribution across the food categories is similar to the FB_1 dataset with 80% of samples being cereals and cereal-based products. As with FB_1, the higher occurrences were in categories (Table 11) and sub-categories (Table 12) containing cereals and cereal-based products. On a global scale, reported concentrations of total fumonisins were lower compared to FB_1 alone. This is because most of the samples submitted to the GEMS/Food contaminants database with results reported for all three fumonisins (FB_1, FB_2 and FB_3) were from countries/regions with lower fumonisin concentrations (Canada, the European Union, Japan). Analysis of the ratios of the individual fumonisins was conducted for all samples with quantifiable FB_1, FB_2 and FB_3 (n = 963). The overall ratio was consistently 68:20:12 across WHO regions and across most food categories, with the exceptions of food for infants in Japan (49:38:13) and snack food in Japan (62:25:13). This does not appear to be a regional difference of the raw ingredient as maize samples in Japan had a similar ratio to the global one.

Overall the estimated global means from the GEMS/Food contaminants database reported in this current monograph are lower than in the Committee's previous assessment in 2011 (Annex 1, reference *205*). For example, the LB mean for global FB_1 in maize is reported here as 310 µg/kg and in 2011 as 1237 µg/kg. It is not possible to directly compare the databases as different GEMS/Food clusters were used in the two studies and mean values for WHO regions were not reported in 2011. However, there is evidence that the differences observed are largely due to a major shift in the geographical distribution of reported samples. In 2011, data on maize were submitted from a broader global distribution with the greatest contribution from GEMS/Food cluster K (some South American and Caribbean countries). This corresponded to 30% of the data submitted on maize; the LB mean was 1675 µg/kg for FB_1. In this current evaluation, data submitted to the GEMS/Food contaminants database were primarily from countries with lower fumonisin contamination (Table 13): 77% (3757/4854) of the data on maize were submitted from the WHO European Region and had an LB mean of 341 µg/kg for FB_1. In 2016, the countries from the WHO European Region were closely

Table 11
Summary of global occurrence of total fumonisins ($FB_1 + FB_2 + FB_3$) in food categories from the GEMS/Food contaminants database[a]

Food category[b]	No. of samples	% positive[c]	LOD range (µg/kg)	LOQ range (µg/kg)	LB mean (µg/kg)[d]	UB mean (µg/kg)[e]	Max. (µg/kg)
Cereals and cereal-based products	6 087	32	0.2–220	0.5–733	56	100	9 091
Snacks and desserts	426	59	0.2–50	0.5–167	13	80	445
Composite food	24	75.0	10–100	33–333	68	149	336
Food for infants and small children	738	9.5	0.1–100	0.5–333	0.9	57	256
Products for special nutritional use	89	45	5–100	15–333	38	134	571
Fruit and fruit products	20	0.0	10–10	33–33	0.0	33	33
Legumes and pulses	49	8.2	0.2–10	0.5–50	1.0	13	50
Milk and dairy products	16	0.0	10–10	33–33	0.0	33	33
Nuts and oilseeds	17	0.0	10–50	33–167	0.0	41	139
Starchy roots and tubers	11	0.0	10–25	50–83	0.0	46	50
Other foods	11	36	50–50	167–167	9.2	128	139
Alcoholic beverages	60	6.7	0.1–10	0.4–33	0.0	6.0	33
Overall	**7 580**	**31**	**0.1–220**	**0.4–733**	**46.8**	**93**	**9 091**

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; No.: number; UB: upper bound
[a] Data submitted from January 2011 to August 2016.
[b] Food categories with less than 10 samples were not included.
[c] Percentage of samples above the LOD/LOQ.
[d] LB mean, samples below LOQ entered as zero values.
[e] UB mean, samples below LOQ entered as LOQ value.

correlated to the GEMS/Food cluster E in the 2011 assessment which, in 2011, had an LB mean for FB_1 of 278 µg/kg. The difference observed between the 2011 and 2016 dataset for this region/cluster could be explained by seasonal variations in fumonisin concentrations.

As is typical of mycotoxins in the field, all studies reviewed that reported results for samples from different climatic regions showed that there can be very large variations in fumonisin concentration across these regions. Data from different seasons were very limited in the published literature reviewed. The first fumonisin assessment at the fifty-sixth Committee meeting (Annex 1, reference *152*) included several studies conducted over many years that reported a large variation in the fumonisin contamination over time.

3.2 Modified and bound fumonisins

There are various terms for mycotoxins that are not readily detected by standard analytical techniques: "modified", "masked", "hidden" and/or "bound" (Rychlik et al., 2014). For the fumonisins, there are limited data on the occurrence of these

Table 12
Summary of global occurrence of total fumonisins ($FB_1 + FB_2 + FB_3$) in cereals and cereal-based products from the GEMS/Food contaminants database[a]

Cereals and cereal-based products	No. of samples	% positive[b]	LOD range (µg/kg)	LOQ range (µg/kg)	LB mean (µg/kg)[c]	UB mean (µg/kg)[d]	Max. (µg/kg)
Cereal grains (not defined)	76	40	7–50	23–167	19	77	231
Barley	39	2.6	10–50	50–167	1.2	96	167
Buckwheat	29	3.4	10–25	50–83	0.1	51	83
Maize	1 089	73	0.2–100	0.5–333	149	185	9 091
Millet	15	6.7	7–10	23–50	0.1	45	50
Oats	198	2.0	10–50	45–167	0.7	54	167
Rice	271	3.0	7–50	23–167	0.4	56	143
Rye	30	30	7–25	23–83	4.5	42	50
Sorghum	1 533	12	20–20	40–40	14	46	1 807
Wheat	494	2.2	7–50	23–167	0.5	53	167
Popcorn	10	30	10–10	50–50	18	56	100
Sweet corn (corn on the cob)	13	0.0	7–25	23–83	0.0	26	43
Bran, unprocessed of cereal grain	69	10	10–15	45–50	8.8	55	276
Bread & other cooked cereal products	1 176	27	7–100	23–333	20	75	1 005
White bread	30	0.0	20–20	67–67	0.0	67	67
Cereal-based food for infants and babies	544	2.6	7–100	23–333	0.6	66	256
Other cereals and cereal-based products	1 015	55	1–220	4–733	132	172	5 476
Overall	**6 631**	**29**	**0.2–220**	**0.5–333**	**52**	**97**	**9 091**

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; No.: number; UB: upper bound
[a] Data submitted from January 2011 to August 2016.
[b] Percentage of samples above the LOD/LOQ.
[c] LB mean, samples below LOQ entered as zero values.
[d] UB mean, samples below LOQ entered as LOQ value.

various forms of undetected mycotoxins. Partially and fully hydrolysed forms of fumonisins are usually present as a low proportion of the parent fumonisins, but these increase in proportion to the parent fumonisins and in absolute values after nixtamalization (cooking maize under alkaline conditions to produce a dough called masa that is used to make tortillas and other maize products) (De Girolamo et al., 2016).

Fumonisins are also non-covalently bound with proteins and complex carbohydrates. The use of alkaline hydrolysis when analysing for fumonisins results in the release of the bound fumonisins and allows the quantification of the combined concentration of both free and bound fumonisins as the hydrolysed form. Previous studies have shown that the level of bound fumonisins is generally higher than that of the free forms and the ratio is highly variable (Dall'Asta et al.,

Table 13
Occurrence of FB_1 in maize by WHO region and country/region from the GEMS/Food contaminants database[a]

WHO region	Country	No. of samples	% positive[b]	LOD range (µg/kg)	LOQ range (µg/kg)	LB mean (µg/kg)[c]	UB mean (µg/kg)[d]	Max. (µg/kg)
Americas	Canada	898	64	4–20	25–50	199	211	6 139
	USA	51	71	47–133	140–400	588	647	5 480
	Overall	**949**	**64**	**4–133**	**25–400**	**220**	**235**	**6 139**
European	Austria	35	23	20–100	67–333	41	191	333
	Belgium	132	46	25–50	83–167	52	98	1 039
	Cyprus	18	17	3–6	10–20	14	30	194
	Czech Republic	102	51	50–170	167–567	1 139	1 239	12 202
	Finland	20	60	10–30	20–100	52	81	310
	France	1 454	42	0.2–222	0.7–740	538	721	23 800
	Germany	960	49	1.3–60	4.3–200	54	84	1 898
	Greece	1	100	–	–	151	151	151
	Hungary	12	8	20–222	67–740	58	622	740
	Ireland	42	43	50–60	167–200	214	320	2 165
	Luxembourg	27	44	20–214	67–713	52	167	713
	Poland	94	24	5–100	16–333	68	154	1 183
	Romania	152	30	3–100	10–333	74	151	2 430
	Slovakia	56	39	40–50	133–167	70	165	441
	Slovenia	35	26	100–200	333–667	68	421	667
	Spain	5	0	100–200	333–667	0	533	667
	United Kingdom	366	88	10–10	33–33	703	707	18 310
	European Union	246	48	5–100	17–333	120	160	3 482
	Overall	**3 757**	**48**	**0.2–222**	**0.7–740**	**341**	**443**	**23 800**
Western Pacific	China, Hong Kong SAR	4	0	2.5–2.5	5–5	0	5	5
	Japan	103	97	0.2–1	0.5–2	104	104	583
	Singapore	41	34	10–10	30–35	39	62	609
	Overall	**148**	**77**	**0.2–10**	**0.5–35**	**83**	**90**	**609**
Global		**4 854**	**52**	**0.2–222**	**0.5–740**	**310**	**392**	**23 800**

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; No.: number; UB: upper bound; SAR: Special Administrative Region
[a] Data submitted from January 2011 to August 2016.
[b] Percentage of samples above the LOD/LOQ.
[c] LB mean, samples below LOQ entered as zero values.
[d] UB mean, samples below LOQ entered as LOQ value.

2009). Bryła et al. (2016) found that the ratio of bound to free fumonisins for maize products varied from 0.06 to 25, with a mean ratio of 3.

More studies are required to better understand the occurrence of bound fumonisins in different cereals, the impact of processing on these bound mycotoxins and their bioavailability after consumption.

4. Food consumption and dietary exposure assessment

4.1 Concentrations in food used in the dietary exposure estimates

Dietary exposure to this group of mycotoxins was last evaluated at the seventy-fourth JECFA meeting. Only concentration data submitted to the GEMS/Food contaminants database since 1 January 2011 were included in this current exposure assessment. Data extracted originated from 19 countries, representing nine of the 17 GEMS/Food cluster diets, and represented products sampled between 2000 and 2016. The data are described in detail in section 3.1. The exposure was calculated for FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$).

The concentration data were checked and recoded, if necessary, using the food classification of the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss) (section 4.2). This resulted in the optimal mapping between the products analysed and those consumed. To use the data for the exposure assessment with the GEMS/Food cluster diets (section 4.2), the products analysed were subsequently mapped to the most appropriate level 3 GEMS/Food cluster diet codes. This level describes mainly raw commodities but also contains codes for processed foods such as breakfast cereals, bread, pasta, etc.

Based on the outcomes of the toxicological assessments by the Committee, it was determined that only long-term dietary exposure assessments were required to evaluate fumonisins. Therefore, mean concentrations were calculated per food (group). In the long term, fluctuations in concentrations are expected to level out to a mean concentration. The concentration data were reported as a numerical value above the LOQ or between the LOD and LOQ, or as undetectable.[1] Two scenarios were considered regarding the mean fumonisin concentrations used in the exposure assessment: samples with an undetectable result were assumed to contain either no fumonisins (LB scenario) or fumonisins at the LOQ (UB scenario). Concentrations reported as numerical values were used as such to derive the LB and UB mean concentrations. All concentrations were converted to the same unit (µg/kg).

To avoid a gross overestimation of the UB exposure, food groups with only undetectable levels, and which were expected to contain no fumonisins, were excluded from the exposure assessment. These included "eggs and egg products", "fish and other seafood", "milk and dairy products", "nuts and oilseeds", "starchy roots and tubers" and "vegetable and vegetable products (including fungi)". Particular foods within a specific food group that were not expected to contain fumonisins and for which no detectable levels were reported were also

[1] It is not always clear whether undetectable values relate to concentrations below the LOD or below the LOQ. In the exposure assessment, undetectable values were therefore defined as values below the LOD or LOQ.

excluded from the exposure assessment. For example, all the foods within the food group "fruit and fruit products" were excluded except (dried) fig, for which detectable concentrations were reported. The samples (n = 36) of other dried fruit types, including raisins, cranberries, sultanas and apricots, were all reported as containing undetectable levels of fumonisins. Similarly, all alcoholic beverages, except beer and beer-like beverages, within the food group "alcoholic beverages" were excluded. The other alcoholic beverage samples (n = 50) were mostly wine or wine-like drinks (n = 47) and stout, gin and vodka (n = 1/each), and all had undetectable levels of fumonisins. Analysed products that were not sufficiently described were also excluded.

The Committee determined that analyses with LODs or LOQs lower than 250 and 750 µg/kg for FB_1, and 550 and 2000 µg/kg for total fumonisins, respectively, represented reliable results. Therefore, samples with LODs or LOQs above these levels were not considered in the exposure assessment (section 3.1).

The GEMS/Food contaminants database contained fumonisin concentrations that were labelled as obtained via random and targeted sampling. Analytical results from random sampling were compared with those from targeted sampling to determine if there was a bias in the results from targeted sampling. Because no differences in contamination were observed, data from targeted sampling were included in the exposure assessment. The reason why samples were labelled as derived from targeted sampling was not specified in the database.

4.1.1 FB_1

In total, 24 524 out of 25 143 samples analysed for FB_1 were considered in the exposure assessment (52% from the WHO European Region, 24% from the WHO Region of the Americas, 18% from the WHO Western Pacific Region, 4% from the WHO African Region and 2% from the WHO Eastern Mediterranean Region). The data were reported for nine clusters: G05, G06, G07, G08, G09, G10, G11, G13 and G15.

Table 14 lists the LB and UB mean concentrations of FB_1 in the products considered in the exposure assessment with at least 500 analysed samples. To make it easier to compare, products with fewer than 500 analysed samples are also listed if they were reported by the seventy-fourth JECFA (Annex 1, reference *205*). Of the samples considered in the exposure assessment, 36% referred to maize and maize-based products (including maize flour, maize meal and popcorn); 8.2% to wheat and wheat-based products (including wheat flour, wheat germ and wheat bran); 7.1% to infant food; 6.8% to bread and other cooked cereal products; and 6.4% to sorghum and sorghum-based products (including sorghum flour).

Table 14

Summary of data from the GEMS/Food contaminants database on concentrations of FB_1 in products for the GEMS/Food cluster diets

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Barley	No. of individual samples	266	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	99.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.5	–	–	–	–	–	–	0	5.1	0	0	–	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	130	–	–	–	–	–	–	640	69	50	41	–	–	–	–	170	–	–	68
Bread and other cooked cereal products	No. of individual samples	1 373	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	89	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	20	–	–	–	–	–	–	11	13	0	39	0	–	–	–	20	–	–	0.6
	Mean (UB, µg/kg)	73	–	–	–	–	–	–	75	100	5	76	333	–	–	–	190	–	–	39
Beer and beer-like beverages	No. of individual samples	574	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	99.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.02	–	–	–	–	–	–	0	10	0	0.1	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	27	–	–	–	–	–	–	10	30	8.7	0.5	–	–	–	–	–	–	–	32
Buckwheat	No. of individual samples	111	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.8	–	–	–	–	–	–	–	0	–	1.0	–	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	42	–	–	–	–	–	–	–	72	–	41	–	–	–	–	150	–	–	37

Table 14 (continued)

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Cakes, cookies and pies	No. of individual samples	1 371	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	74	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	38	–	–	–	–	0	–	18	60	0	15	76	–	–	–	0	–	–	3.2
	Mean (UB, µg/kg)	89	–	–	–	–	1.7	–	74	120	5	70	100	–	–	–	230	–	–	36
Infant food	No. of individual samples	2 157	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	87.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	14	–	–	–	–	–	–	0	6.9	–	21	14	–	–	–	0	–	–	4.8
	Mean (UB, µg/kg)	85	–	–	–	–	–	–	100	140	–	54	77	–	–	–	270	–	–	45
Figs (dried)	No. of individual samples	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	31	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	31
Maize	No. of individual samples	4 437	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	310	–	–	–	–	180	150	550	70	0	140	52	–	–	–	310	–	–	110
	Mean (UB, µg/kg)	420	–	–	–	–	310	150	700	120	35	170	98	–	–	–	510	–	–	150
Maize flour	No. of individual samples	2 878	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	44	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
	Mean (LB, µg/kg)	200	–	–	–	–	95	–	380	210	0.1	170	120	–	–	–	110	–	–	150
	Mean (UB, µg/kg)	240	–	–	–	–	220	–	410	240	44	190	160	–	–	–	330	–	–	180
Maize meal	No. of individual samples	838	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	321	–	–	–	–	700	140	930	–	0	260	690	–	–	–	130	–	–	12
	Mean (UB, µg/kg)	360	–	–	–	–	740	140	930	–	50	290	690	–	–	–	290	–	–	73
Millet	No. of individual samples	135	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.4	–	–	–	–	–	–	0	0	0	0.7	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	46	–	–	–	–	–	–	100	80	50	39	–	–	–	–	–	–	–	37
Oats	No. of individual samples	468	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	3.0	–	–	–	–	–	–	9.7	0.3	0	1.7	–	–	–	–	0	–	–	0.8
	Mean (UB, µg/kg)	71	–	–	–	–	–	–	69	100	5	50	–	–	–	–	270	–	–	53
Popcorn	No. of individual samples	668	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	65.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	95	–	–	–	–	620	–	60	36	0	50	41	–	–	–	300	–	–	32
	Mean (UB, µg/kg)	170	–	–	–	–	680	–	86	77	50	160	89	–	–	–	460	–	–	61
Rice	No. of individual samples	603	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 14 (continued)

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
	% samples <LOD or LOQ	99.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.2	–	–	–	–	–	–	0	0	0	0.8	38	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	70	–	–	–	–	–	–	69	190	43	46	38	–	–	–	190	–	–	41
Sorghum	No. of individual samples	37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	97.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	11	–	–	–	–	–	–	16	–	0	–	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	490	–	–	–	–	–	–	730	–	50	–	–	–	–	–	–	–	–	30
Sorghum flour	No. of individual samples	1 535	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	88.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	32	–	–	–	–	–	–	–	–	–	–	–	–	32	–	–	–	–	0
	Mean (UB, µg/kg)	54	–	–	–	–	–	–	–	–	–	–	–	–	54	–	–	–	–	32
Soybean (dry)	No. of individual samples	239	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	97.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.7	–	–	–	–	–	–	–	0	–	0.7	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	29	–	–	–	–	–	–	–	97	–	25	–	–	–	–	–	–	–	46
Sweet corn kernels	No. of individual samples	326	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	90.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	11	–	–	–	–	–	–	2.4	64	0	0	–	–	–	–	11	–	–	3.7
	Mean (UB, µg/kg)	84	–	–	–	–	–	–	98	81	50	35	–	–	–	–	180	–	–	43

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Wheat	No. of individual samples	736	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	3.9	–	–	–	–	–	–	9.3	0.4	0	0.2	89	–	–	–	22	–	–	0
	Mean (UB, µg/kg)	200	–	–	–	–	–	–	660	76	50	42	160	–	–	–	490	–	–	48
Wheat flour	No. of individual samples	1 171	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	96.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	6.4	–	–	–	–	–	–	0	0.2	0	0.2	149	–	–	–	0	–	–	0.2
	Mean (UB, µg/kg)	61	–	–	–	–	–	–	89	83	50	49	180	–	–	–	220	–	–	40

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; No.: number; NS: non-specified; UB: upper bound

[a] No concentrations were submitted categorized as groundnuts, shelled.

[b] The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ when calculating mean concentrations. The UB estimates were derived by substituting the LOQ for analytical results below the LOD or LOQ.

[c] Includes fumonisin samples from Singapore and countries recorded in the GEMS/Food contaminants database as part of the WHO European Region.

The products with the highest percentage of contamination were maize and maize-based products, with levels varying between and within clusters (Table 14). The LB and UB mean FB_1 concentrations in, for example, maize meal, the maize-based product with the highest percentage of contamination (71% of detectable results), ranged from 0 to 930 µg/kg and from 50 to 930 µg/kg, respectively, across clusters. FB_1 concentrations were generally lower in non-maize foods, with the highest LB mean concentration of 149 µg/kg in wheat flour reported in cluster G11 (Belgium and the Netherlands). Of these non-maize food samples, 88% had an undetectable FB_1 concentration. Due to this, the LB and UB mean concentrations could differ significantly (Table 14). This was also due to relatively high LOQs reported in the database within several clusters, for example, for barley, sorghum and wheat in cluster G07 (various developed countries) and bread and other cooked cereal products in clusters G11 and G15 (European countries).

4.1.2 Total fumonisins

The concentration of total fumonisins was reported for 3405 samples. After removal of the data for the food groups with only undetectable levels and those that were expected to contain no fumonisins (section 4.1), 3302 samples were used in the exposure assessment of total fumonisins. To increase the number of samples for the exposure assessment, total fumonisin concentration was estimated from FB_1 by assuming that its occurrence represents 68% of the total fumonisins present (section 3.1). This allowed the addition of analytical results for a further 23 557 samples. The Committee also considered using solely the summed total fumonisin concentrations of the samples for which FB_1, FB_2 and FB_3 concentrations were reported separately (section 3.1). However, since these samples only covered three countries and resulted, in some cases, in lower total fumonisin concentrations than for only FB_1, the reported total fumonisin concentrations were used supplemented with FB_1 samples. The possible underestimation as a result of using total fumonisin concentrations based on the sum of FB_1 and FB_2 (without FB_3) was considered negligible; these two mycotoxins accounted, on average, for almost 90% of the total fumonisin concentration in a sample (section 3.1). In addition, only 12% of the samples for which all three fumonisins were reported contained detectable FB_3 concentrations.

In total, data on 26 859 samples were taken into account in this current exposure assessment of total fumonisins (56% from the WHO European Region, 22% from the WHO Region of the Americas, 17% from the WHO Western Pacific Region, 4% from the WHO African Region and 2% from the WHO Eastern Mediterranean Region). The data were reported for the same nine clusters as the FB_1 concentration data (section 4.1.1).

Table 15 lists the LB and UB mean concentrations of total fumonisins in the commodities also listed for FB_1. Of the samples, 36% referred to maize and maize-based products (including maize flour, maize meal and popcorn); 7.7% to wheat and wheat-based products; 6.6% to infant food; 6.0% to bread and other cooked cereal products; and 5.9% to sorghum and sorghum-based products. These percentages are comparable to those for FB_1 (section 4.1.1). As for FB_1, maize and maize-based products had the highest percentage of contamination, with about 68% for maize meal, 45% for maize, 56% for maize flour and 34% for popcorn.

4.2 Food consumption data used in the dietary exposure assessments

The Committee calculated national estimates of exposure to FB_1 and total fumonisins using the CIFOCOss database, which at the time of the assessment in 2016 contained summary statistics of 37 surveys from 26 countries. These surveys covered at least 2 survey days. The database provided summary statistics of food consumption for about 750 items at the highest level of classification. The statistics were available for different age groups per survey.

In addition, the Committee calculated international estimates of exposure to FB_1 and total fumonisins using the GEMS/Food cluster diets for these estimates. These cluster diets provided mean consumption values per capita for 17 clusters of countries. The consumption values are for raw commodities and some processed foods based on FAO food balance sheet data. The GEMS/Food cluster diets are identified by grouping countries that are culturally and economically comparable (Sy et al., 2013).

4.3 Assessments of dietary exposure

4.3.1 National estimates from the scientific literature

Since the evaluation of the dietary exposure to FB_1 and total fumonisins at the seventy-fourth Committee meeting, a number of national evaluations have been published. The Committee considered evaluations performed by Brazil, China (including Hong Kong Special Administrative Region [SAR]), France, Guatemala, Japan, Malawi, the Netherlands, Portugal, the Republic of Korea, Spain, the United Republic of Tanzania, Viet Nam and Zimbabwe. An overview of these evaluations is presented below and summarized in Table 16.

Table 15

Summary of data from the GEMS/Food contaminants database on concentrations of total fumonisins ($FB_1 + FB_2 + FB_3$) in products for the GEMS/Food cluster diets

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Barley	No. of individual samples	295	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	99.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.7	–	–	–	–	–	–	0	7.5	0	0	–	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	230	–	–	–	–	–	–	850	100	67	60	–	–	–	–	260	–	–	86
Bread and other cooked cereal products	No. of individual samples	1 623	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	89.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	29	–	–	–	–	–	–	17	19	0	57	0	–	–	–	28	–	–	0.7
	Mean (UB, µg/kg)	110	–	–	–	–	–	–	110	150	7.4	110	490	–	–	–	310	–	–	58
Beer and beer-like beverages	No. of individual samples	586	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	99.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.02	–	–	–	–	–	–	0	0.01	0	0.2	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	36	–	–	–	–	–	–	15	38	20	0.8	–	–	–	–	–	–	–	47
Buckwheat	No. of individual samples	111	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	1.2	–	–	–	–	–	–	–	0	–	1.5	–	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	62	–	–	–	–	–	–	–	85	–	61	–	–	–	–	220	–	–	55

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Cakes, cookies and pies	No. of individual samples	1 373	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	73.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	56	–	–	–	–	0	–	28	87	0	21	110	–	–	–	0	–	–	4.7
	Mean (UB, µg/kg)	130	–	–	–	–	2.5	–	140	170	7.4	100	150	–	–	–	350	–	–	52
Infant food	No. of individual samples	2 229	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	69.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	19	–	–	–	–	–	–	0	11	–	32	21	–	–	–	0	–	–	6.7
	Mean (UB, µg/kg)	130	–	–	–	–	–	–	160	200	–	79	110	–	–	–	420	–	–	67
Figs (dried)	No. of individual samples	59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	86.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	74	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	59
Maize	No. of individual samples	5 871	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	54.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	390	–	–	–	–	260	220	710	100	0	200	76	–	–	–	300	–	–	130
	Mean (UB, µg/kg)	570	–	–	–	–	460	220	940	170	51	260	140	–	–	–	580	–	–	220
Maize flour	No. of individual samples	2 952	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	43.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	280	–	–	–	–	140	–	560	360	31	250	180	–	–	–	130	–	–	200
	Mean (UB, µg/kg)	350	–	–	–	–	320	–	600	370	84	270	240	–	–	–	460	–	–	270

Table 15 (continued)

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Maize meal	No. of individual samples	933	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	31.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	430	–	–	–	–	1 000	210	1 400	–	0	380	1 000	–	–	–	130	–	–	64
	Mean (UB, µg/kg)	490	–	–	–	–	1 100	210	1 400	–	74	430	1 000	–	–	–	310	–	–	150
Millet	No. of individual samples	135	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	0.5	–	–	–	–	–	–	0	0	0	1.0	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	67	–	–	–	–	–	–	150	120	74	57	–	–	–	–	–	–	–	54
Oats	No. of individual samples	469	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	3.9	–	–	–	–	–	–	12	0.5	0	2.5	–	–	–	–	0	–	–	1.1
	Mean (UB, µg/kg)	110	–	–	–	–	–	–	110	150	7.4	74	–	–	–	–	402	–	–	77
Popcorn	No. of individual samples	691	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	66.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	130	–	–	–	–	910	–	90	53	0	73	61	–	–	–	360	–	–	45
	Mean (UB, µg/kg)	250	–	–	–	–	1 000	–	130	110	74	240	130	–	–	–	610	–	–	110
Rice	No. of individual samples	604	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	99.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
	Mean (LB, µg/kg)	0.3	–	–	–	–	–	–	0	0	0	1.1	56	–	–	–	0	–	–	0
	Mean (UB, µg/kg)	100	–	–	–	–	–	–	100	270	63	68	56	–	–	–	280	–	–	60
Sorghum	No. of individual samples	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	98.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	9.7	–	–	–	–	–	–	17	–	0	–	–	–	–	–	–	–	–	0
	Mean (UB, µg/kg)	590	–	–	–	–	–	–	960	–	55	–	–	–	–	–	–	–	–	44
Sorghum flour	No. of individual samples	1 535	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	88.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	46	–	–	–	–	–	–	–	–	–	–	–	–	47	–	–	–	–	0
	Mean (UB, µg/kg)	79	–	–	–	–	–	–	–	–	–	–	–	–	79	–	–	–	–	47
Soybean (dry)	No. of individual samples	239	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	97.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	1.0	–	–	–	–	–	–	–	17	–	1.1	–	–	–	–	–	–	–	1.1
	Mean (UB, µg/kg)	43	–	–	–	–	–	–	–	140	–	36	–	–	–	–	–	–	–	69
Sweet corn kernels	No. of individual samples	535	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	89.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	14	–	–	–	–	–	–	3.5	95	6.0	0	–	–	–	–	26	–	–	4.8
	Mean (UB, µg/kg)	150	–	–	–	–	–	–	150	120	70	51	–	–	–	–	250	–	–	130

Table 15 (continued)

Food product[a]	Statistic[b]	Global total	Data per GEMS/Food cluster diet																	
			G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17	NS[c]
Wheat	No. of individual samples	896	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	97.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	10	–	–	–	–	–	–	18	0.6	0	0.4	130	–	–	–	50	–	–	0
	Mean (UB, µg/kg)	360	–	–	–	–	–	–	860	110	74	62	230	–	–	–	740	–	–	70
Wheat flour	No. of individual samples	1 192	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	% samples <LOD or LOQ	96.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Mean (LB, µg/kg)	10	–	–	–	–	–	–	0	0.3	0	0.3	220	–	–	–	0	–	–	2.8
	Mean (UB, µg/kg)	92	–	–	–	–	–	–	140	120	60	72	260	–	–	–	430	–	–	61

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; No.: number; NS: non-specified; UB: upper bound

[a] No concentrations were submitted categorized as groundnuts, shelled.

[b] The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ when calculating mean concentrations. The UB estimates were derived by substituting the LOQ for analytical results below the LOD or LOQ.

[c] Includes fumonisin samples from Singapore and countries recorded in the GEMS/Food contaminants database as part of the WHO European Region.

Table 16
Summary of national estimates of dietary exposure to fumonisins from the literature

Fumonisin / Country	Food concentration data used	Consumption data used	Population groups (age range in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	References
FB_1						
China (Huantai, Huaian and Fusui)	Survey mean	FFQ	Adults	1 500–7 700[b,c,d]	Not stated	Sun et al. (2011)
France	LB–UB survey mean	Individual food consumption data (INCA2 – national 7-day food diary)	Children (3–17) Adults (18–79)	15–45 (50–106)[e] 7.5–29 (23–66)	Breakfast cereals, sweet and savoury biscuits and bars, nonalcoholic beverages	Sirot, Fremy & Leblanc (2013)
Netherlands	LB–UB survey mean	Individual food consumption data (national 2-day food consumption survey)	Children (2–6) Persons (7–69)	0–31.2 (5.34–82.7)[b,e] 0–46.9 (10.8–109)	Bread, coffee, tea, beer	Sprong et al. (2016a)
Spain	Survey mean	Consumption statistics	Adolescents (10–17) Adults (18–64)	0.002 (0.017) 0.008 (0.039)	Only brewed coffee included	García-Moraleja et al. (2015b)
Viet Nam	Survey mean	Mean food consumption (two dietary surveys)	Children Adults	1 019 536	Rice	Huong et al. (2016)
Zimbabwe	Survey median	Mean food consumption (FFQ)	Young children (<5) Children (5–8) Adolescents (9–18) Adults (19–60) Older adults (>60)	3 910 5 400 4 400 2 300 2 200	Only maize included	Hove et al. (2016)
FB_2						
France	LB–UB survey mean	Individual food consumption data (INCA2 – national 7-day food diary)	Children (3–17) Adults (18–79)	6.5–24 (30–83)[e] 2.4–10 (16–42)	Sweet and savoury biscuits and bars, nonalcoholic beverages	Sirot, Fremy & Leblanc (2013)
Netherlands	LB–UB survey mean	Individual food consumption data (national 2-day food consumption survey)	Children (2–6) Persons (7–69)	0–30 (0–80)[b,e] 0–46 (0–107)	Bread, coffee, tea, beer	Sprong et al. (2016a)
Spain	Survey mean	Consumption statistics	Adolescents (10–17) Adults (18–64)	0.003 (0.025) 0.012 (0.056)	Only brewed coffee included	García-Moraleja et al. (2015)

Table 16 (continued)

Fumonisin / Country	Food concentration data used	Consumption data used	Population groups (age range in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	References
$FB_1 + FB_2$						
Brazil	Survey mean and P90	Daily consumption levels (household budget survey)	Adults	121 (256)	Corn meal, corn grits and corn flakes (only corn-based products included)	Martins et al. (2012)
Brazil	Survey mean and P90	Daily consumption levels (household budget survey)	Adults	192	Corn meal (only corn-based products included)	Savi et al. (2016)
China (Gansu, Sichuan, Guizhou)	Survey mean	Mean food consumption (Chinese National Survey 2002)	Adults	30–120[c]	Only maize kernels included	Li et al. (2015b)
China (Hebei)	Survey mean	Per capita maize consumption	Adult	80 (1 100)	Only maize kernels included	Li et al. (2015a)
Republic of Korea	Survey mean	P95 daily food consumption	Adults	(2.3)	Only sunsik included	Jung et al. (2015)
Spain (Catalonia)	Survey mean	Mean food consumption (FFQ)	Infants (0–3) Children (4–9) Adolescents (10–19) Adults (20–65) People with coeliac disease Immigrants	156 63.0 36.7 M: 34.5 / F: 63.2 40.9 65.1	Not stated	Cano-Sancho et al. (2012a,b)
United Republic of Tanzania	Survey mean	Mean food consumption (24-hour recall)	Infants (<5 months)	140[b]	Only maize flour included	Magoha et al. (2016)
$FB_1 + FB_2 + FB_3$						
China (Hong Kong SAR)	LB–UB survey mean	Individual food consumption data (two nonconsecutive 24-hour dietary recalls)	Adults (20–84)	1.6–97 (0.8–169)[e]	Cereals and their products	Yau et al. (2016)
Guatemala (Escuintla, Chimaltenango, Chiquimula, Jutiapa, Sacatepéquez, Santa Rosa)	Survey mean	Individual maize consumption (food questionnaire)	Adults (female)	0.03–5.79	Only maize	Torres et al. (2014); Riley et al. (2015b)

Fumonisin / Country	Food concentration data used	Consumption data used	Population groups (age range in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	References
Japan	LB–UB, log-normal distribution survey data	Individual food consumption data (2 consecutive days)	Children (1–6) Older children (7–14) Adolescents (15–19) Adults (>20)	(7.2–10.2) (1.2–4.6) (0.0–0.0) (0.0–0.0)	Popcorn, corn flakes, corn snacks, beer and millet (only food groups included)	Sugita-Konishi et al. (2013)
Malawi	Survey mean	Per capita maize consumption	Adults	3 000–15 000[b]	Only maize included	Matumba et al. (2015)
Netherlands	LB–UB survey mean	Individual food consumption data (national 2-day food consumption survey)	Children (2–6) Persons (7–69)	0–91 (5.3–206)[b,e] 0–138 (10.8–241)	Bread, coffee, tea, beer	Sprong et al. (2016a)
Portugal[f]	Mean (literature)	Mean food consumption (household budget survey data)	Adults (18–64)	109	Corn, corn-based products and corn bread	Abrunhosa et al. (2016)

bw: body weight; F: female; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; FFQ: food frequency questionnaire; LB: lower bound; M: male; P90: 90th percentile; P95: 95th percentile; SAR: Special Administrative Region; UB: upper bound

[a] High consumer estimate refers to the 95th percentile of exposure, except for the two studies from Brazil and the study in Hebei province of China. In these studies, the 90th percentile of exposure and the maximum exposure were reported, respectively.

[b] Reported exposure is median (P50).

[c] Dietary exposure was estimated for three regions. The range represents the minimum and maximum mean or median estimate across the regions.

[d] The original publication reported exposures in ng per day. These estimates were converted to estimates corrected for body weight by dividing them by 60 kg.

[e] The range of dietary exposure estimates refers to the LB and UB estimates of exposure.

[f] The fumonisins assessed were not described. It was therefore assumed that FB_1, FB_2 and FB_3 were included in the exposure estimate.

(a) **Brazil**

In a study conducted in the state of Paraná, Brazil, 100 samples of corn-based products, including corn meal, corn grits, popcorn, corn flour and corn flakes, were analysed for FB_1 and FB_2 (Martins et al., 2012). To assess the exposure to the sum of FB_1 and FB_2, concentrations were added per analysed sample. These summed concentrations were multiplied with national consumption statistics of corn-based foods and divided by 60 kg of body weight. The national consumption statistics were mean daily consumption levels in g/person per day and were obtained from a household budget survey performed in Brazil in 2008/2009.

Two exposure assessments were performed: one using the mean contamination levels of FB_1 and FB_2 and one using the 90th percentile contamination levels. The resulting mean and 90th percentile exposures were 121 and 256 ng/kg bw per day, respectively (Martins et al., 2012). At both exposure levels, corn meal contributed most to the exposure (52% and 59%, respectively), followed by corn grits (35% and 30%, respectively) and corn flakes (both 9%).

Another study performed in Brazil examined the presence of FB_1 and FB_2 in corn-based products and subsequent exposure (Savi et al., 2016). Fifteen samples of corn grain, corn grits, corn meal and corn flour, 25 samples of corn flakes and 10 samples of popcorn were analysed. The mean and 90th percentile daily exposures of the sum of FB_1 and FB_2 were calculated for corn-based food by combining the mean and the 90th percentile contamination levels with the daily consumption derived from the same household budget survey as used by Martins et al. (2012). The estimates were divided by a body weight of 60 kg. The mean exposure ranged from 0.4 ng/kg bw per day via popcorn to 134 ng/kg bw per day via corn meal. The 90th percentile daily exposure equalled 341 ng/kg bw per day via consumption of corn meal (Savi et al., 2016). The overall mean exposure across all corn-based products considered equalled 192 ng/kg bw per day.

(b) **China (including Hong Kong SAR)**

Sun et al. (2011) assessed the exposure to FB_1 via various foods in three areas of China: Huaian (a high-risk area for oesophageal cancer), Fusui (a high-risk area for liver cancer) and Huantai (a low-risk area for both oesophageal and liver cancers). In total, 209 samples of maize, rice, wheat flour, peanuts and plant oil, mainly made from peanut and soybean, were collected from individual households and analysed for FB_1. The concentrations were used to estimate exposure with the use of food consumption data obtained via a food frequency questionnaire (FFQ).

The two areas with high liver cancer rates had higher median daily exposures to FB_1 than the area with lower rates: 138 600 and 460 000 ng per day compared to 92 400 ng per day (Sun et al., 2011). The authors suggest that the

high liver cancer rates were due to the co-occurrence of FB_1 with aflatoxin B_1. The differences in FB_1 exposure were very likely due to differences in climatic conditions that may favour fungal growth (Sun et al., 2011).

Li et al. (2015b) examined the exposure to FB_1 and FB_2 via maize consumption in the Chinese provinces of Gansu (n = 62), Sichuan (n = 15) and Guizhou (n = 69). In total, 146 samples of maize kernels were collected in 2012 and analysed for both fumonisins. The mean analysed concentrations were used to estimate the exposure to the sum of FB_1 and FB_2. For this, an estimated mean consumption of maize of 10 g/person per day was used, based on data from the 2002 Chinese National Survey and a mean adult body weight of 60 kg. The calculated mean exposure ranged from 30 ng/kg bw per day in Gansu up to 120 ng/kg bw per day in Guizhou.

A similar study in 2011–2013 analysed FB_1 and FB_2 in 125 maize kernel samples collected from Hebei province (Li et al., 2015a). The exposure to the sum of FB_1 and FB_2 was estimated by assuming a mean consumption of 10 g per day of maize for a Chinese adult and a mean body weight of 60 kg. Based on a mean contamination level of 469 µg/kg, the exposure equalled 80 ng/kg bw per day. The maximum exposure, based on a maximum analysed level of 6634 µg/kg, was 1100 ng/kg bw per day.

In 2016, the results of a total diet study into the exposure to mycotoxins in Hong Kong SAR were published (Yau et al., 2016). In this study, several mycotoxins, including FB_1, FB_2 and FB_3, were analysed in 60 food samples prepared as consumed. These food samples covered 12 food groups and were collected in each season to give 240 analytical samples. The highest LB and UB mean total fumonisin concentrations were detected in the food group "cereals and their products": 2.6 and 9.8 µg/kg, respectively, which was mainly due to the food "breakfast cereals". Fumonisins were detected in only two other foods, potato chips and corn starch.

Food consumption information was derived from two nonconsecutive 24-hour dietary recalls for each of 5008 adults (20–84 years). The dietary exposure was estimated for the adult population at the individual level by mapping the analysed foods to those reported as consumed. Survey weights were used to align the food consumption cohort to the Hong Kong SAR population. Mean and 95th percentile exposures were estimated. LB and UB mean dietary exposure to total fumonisins in the adult population equalled 1.6 and 97 ng/kg bw per day, respectively. The LB and UB 95th percentile exposures were estimated at 0.8 and 169 ng/kg bw per day, respectively. The LB mean exposure estimate was higher than that estimated for a high consumer. This was due to a higher LB concentration in breakfast cereals, compared to the other samples, combined with a small number of high consumers of this food. Cereals and their products contributed most to the mean exposure to total fumonisins (62–99%).

(c) France

In the Second French Total Diet Study (Sirot, Fremy & Leblanc, 2013), FB_1 and FB_2 were analysed in 1319 food samples, which were prepared as consumed. Exposure to FB_1 and FB_2 was estimated in children (3–17 years) and adults (18–79 years) by combining the national food consumption data from the Second National Individual Survey of 2006–2007 (INCA2) with the occurrence data. The LB and UB exposures were assessed individually, using individual consumption data and body weights. From the resulting exposure distributions, LB and UB mean and 95th percentile exposure levels were obtained.

The LB mean and 95th percentile exposures to FB_1 in adults were 7.5 and 23 ng/kg bw per day, respectively. Corresponding UB estimates were 29 and 66 ng/kg bw per day. Children had a higher exposure to FB_1: LB mean and 95th percentile estimates were 15 and 50 ng/kg bw per day, respectively, and the UB mean and 95th percentile estimates were 45 and 106 ng/kg bw per day, respectively.

The exposure to FB_2 was lower in both age groups. The LB mean and 95th percentile exposures in adults were 2.4 and 16 ng/kg bw per day, respectively. The corresponding UB estimates were 10 and 42 ng/kg bw per day. The exposures in children were, respectively, 6.5 and 30 ng/kg bw per day for the LB mean and 95th percentile and 24 and 83 ng/kg bw per day for the corresponding UB estimates.

Food groups that contributed most to the exposure to FB_1 and FB_2 were bread and dried bread products, sweet and savoury biscuits and bars, and nonalcoholic beverages. In the UB scenario, alcoholic beverages were also important contributors to the exposure in adults.

(d) Guatemala

The Torres et al. (2014) study into the exposure to total fumonisins via maize consumed by women in high-exposure (Jutiapa) and low-exposure communities (Chimaltenango and Escuintla) was repeated using a similar protocol in two other high-exposure communities (Chiquimula and Santa Rosa) and one low-exposure community (Sacatepéquez) (Riley et al., 2015b). In both studies, FB_1, FB_2 and FB_3 were analysed in maize samples sold for human consumption by vendors in all six departments (equivalent to counties in the USA). The samples were collected for analysis in March 2011 to February 2012 (first study, n = 116 maize samples) and February to March 2013 (second study, n = 30). Maize consumption was estimated based on an interview questionnaire. Exposure to total fumonisins was estimated based on individual maize consumption levels, individual body weights, fumonisin contamination in the purchased maize and the effects of processing (nixtamalization). The results were confirmed using UFB_1 in urine collected from individual women at the same time as the maize

samples were collected and the interviews conducted. Box plots showed that the mean and median total fumonisin intakes in the low-exposure communities of Chimaltenango, Escuintla and Sacatepéquez were significantly lower than in the high-exposure communities of Chiquimula, Jutiapa and Santa Rosa (Torres et al., 2014; Riley et al., 2015b). Values for the box plots provided to the Committee by the corresponding author of these studies showed that the mean ± SD estimated total fumonisin exposures in Chimaltenango, Escuintla and Sacatepéquez were 0.81 ± 0.63, 1.04 ± 1.00 and 0.03 ± 0.02 μg/kg bw per day, respectively. The mean ± SD estimated total fumonisin exposures in Chiquimula, Jutiapa and Santa Rosa were 5.79 ± 4.54, 5.02 ± 4.07 and 4.05 ± 2.49 μg/kg bw per day, respectively. In total, 11% (104/935) of the women in the low-exposure communities and 75% (450/598) of the women in the high-exposure communities had estimated total fumonisin exposure that exceeded 2 μg/kg bw per day.

(e) **Japan**

Since 2011, one study into the exposure to total fumonisins has been conducted in Japan (Sugita-Konishi et al., 2013). In total, 1505 samples covering 22 foods were analysed for FB_1, FB_2 and FB_3 over 6 years (2004–2009). The concentrations, in combination with consumption data from the National Health and Nutrition Survey 2007–2010, were used to assess the exposure. The food consumption survey included 17 827 individuals covering four age groups: toddlers and young children (1–6 years), older children (7–14 years), adolescents (15–19 years) and adults (≥20 years). Food consumption data were recorded on 2 consecutive days.

Five out of the eight foods with detectable levels of fumonisins, including popcorn, corn flakes, corn snacks, beer and millet, were included in the exposure assessment. The exposure was estimated using a Monte Carlo simulation. In this simulation, the consumption of the five foods per age group was assumed to follow a log-normal distribution. This was also assumed for the LB and UB fumonisins concentrations per food.

Toddlers, young children and older children were the most exposed age groups: the 95th percentile estimates of exposure were 7.2 (LB) and 10.2 (UB) ng/kg bw per day for toddlers and young children, and 1.2 (LB) and 4.6 (UB) ng/kg bw per day for older children. In adolescents and adults, the LB and UB 95th percentile exposure estimates were zero.

(f) **Malawi**

The exposure to total fumonisins via maize consumption was estimated in four agro-ecological regions of Malawi (Matumba et al., 2015). For this, 90 maize samples were collected and analysed for FB_1, FB_2 and FB_3. Based on a per capita maize consumption of 368 g per day, an adult body weight of 60 kg and the mean

concentration of total fumonisins in maize per region, the exposure per region was calculated. Actual levels of exposure were not reported. Box plots showed that the median exposure ranged from about 3000 to about 15 000 ng/kg bw per day. The authors assumed that non-dehulled maize was consumed. The authors indicated that the exposure would very likely drop by a factor of 3 or more if dehulled maize was consumed (Matumba et al., 2015).

(g) Netherlands

Dietary exposure to total fumonisins in Dutch children (2–6 years) and persons aged 7–69 years was estimated within a mycotoxin-dedicated total diet study (Sprong et al., 2016a). In this study, 88 composite food samples were analysed for FB_1, FB_2 and FB_3. Mean and high percentile exposures were calculated using individual food consumption data from two Dutch National Food Consumption Surveys.

Three of the 88 composite samples contained FB_1 at concentrations above the LOQ (10 µg/kg). FB_2 and FB_3 were not detected at levels above the LOD (3.3 µg/kg). Two out of the three positive samples for FB_1 were breakfast cereals. The third sample was dried fruit (López et al., 2016).

The estimated LB median exposure to total fumonisins was zero for both populations. The corresponding 95th percentile exposure estimates were 5.3 ng/kg bw per day in children aged 2–6 years and 10.8 ng/kg bw per day in the population aged 7–69 years. In this exposure scenario, the exposure to total fumonisins was completely due to FB_1. The UB median exposure to total fumonisins equalled 91 ng/kg bw per day in children aged 2–6 years and 138 ng/kg bw per day in the population aged 7–69 years The corresponding 95th percentile exposures were 206 ng/kg bw per day and 241 ng/kg bw per day, respectively. The UB exposure to total fumonisins was equally distributed among FB_1, FB_2 and FB_3, each accounting for 1/3 of the exposure. In addition, the median and 95th percentile exposures to FB_1 and FB_2 were reported for both populations. These exposures are listed in Table 16. FB_3 exposure was also reported but not included in Table 16 because it was not reported in any of the other national studies.

(h) Portugal

A review of the occurrence of fumonisins in food and feed available on the Portuguese market was published in 2016 (Abrunhosa et al., 2016). Based on the concentration data gathered, the provisional daily intake was estimated using mainly household budget survey data. Exposure was estimated by multiplying the mean concentration per food by its mean consumption and dividing the result by a body weight of 65 kg. The exposures per food were subsequently summed to obtain the total daily exposure. Undetectable samples were assumed to contain

the mycotoxins at half the LOD. The probable daily intake equalled 109 ng/kg bw per day. The main contributors to the exposure were corn (69%), corn-based products (17%) and corn bread (13%). Which fumonisins were included in the exposure assessment was not stated.

(i) Republic of Korea

A study performed in the Republic of Korea examined the presence of FB_1 and FB_2 in 47 samples of sunsik, a mixture of powders of several roasted and ground cereals and legumes, including rice, beans and barley (Jung et al., 2015). To assess the exposure, the analysed mean fumonisin level was multiplied by a 95th percentile daily consumption of 30.61 g and divided by an adult body weight of 64 kg. The exposure to the sum of FB_1 and FB_2 equalled 2.3 ng/kg bw per day.

(j) Spain

A Spanish study was performed in the region of Catalonia to assess the exposure to FB_1 and FB_2 (Cano-Sancho et al., 2012a,b). In total, 928 samples were collected from stores and pooled into 370 food samples that were analysed for both fumonisins. The consumption data were obtained via an FFQ focused on region-specific foods that may have been contaminated with fumonisins. The population groups considered were infants (0–3 years), children (4–9 years), adolescents (10–19 years), adults (20–65 years) and older adults (>65 years). People with coeliac disease and immigrants were also included. Exposure estimates of the sum of FB_1 and FB_2 were obtained through a simple deterministic and a more advanced stochastic approach. Only those obtained with the simple deterministic approach are reported here. In this approach, mean consumptions and mean concentrations per food were used to assess the exposure per food. Exposures per food were subsequently summed and divided by a fixed body weight. Undetectable samples were assumed to contain FB_1 and FB_2 at half the LOD. The mean exposure to the sum of FB_1 and FB_2 was highest in infants, immigrants, male adults and children, in that order, with daily exposures of 156, 65.1, 63.2 and 63.0 ng/kg bw per day, respectively. Female adults and adolescents had the lowest mean daily exposure at 34.5 and 36.7 ng/kg bw per day, respectively. The exposure in people with coeliac disease equalled 40.9 ng/kg bw per day.

Another Spanish study examined exposure to FB_1 and FB_2 via the consumption of brewed coffee (García-Moraleja et al., 2015b). In total, 169 brewed coffee samples were analysed for both fumonisins. The mean concentration was combined with food consumption statistics from the Spanish data in the European Food Safety Authority (EFSA) comprehensive database. Statistics used were the mean, 95th percentile, 97.5th percentile and 99th percentile of consumption for adults and adolescents. No consumption statistics were available for children

from Spain in this database. Undetectable samples were assumed to contain no fumonisins (LB).

Four out of the 169 brewed coffee samples contained FB_1 and FB_2 at detectable levels. The LB mean concentrations were 0.62 and 0.90 µg/kg for FB_1 and FB_2, respectively. Use of these mean levels resulted in an LB mean exposure to FB_1 of 0.008 ng/kg bw per day in adults and 0.002 ng/kg bw per day in adolescents. Corresponding exposure estimates for FB_2 were 0.012 and 0.003 ng/kg bw per day, respectively. The LB 95th percentile of exposure was maximally 0.056 ng/kg bw per day for FB_2 in adults.

(k) United Republic of Tanzania

In 2014, a study into the exposure to FB_1 and FB_2 in infants aged less than 6 months was performed in the United Republic of Tanzania (Magoha et al., 2016). The food consumption of 143 infants was studied via three follow-up visits at 1, 3 and 5 months of age. A 24-hour dietary recall was used to estimate flour consumption in infants who had been introduced to maize foods. For children who started to eat maize flour at the age of 3 months, one maize flour sample was collected from the family at 3 and at 5 months of age. The samples were analysed for FB_1 and FB_2. The exposure was calculated by combining the mean maize flour consumption with the summed fumonisin levels in flour, and then divided by the infant's body weight. The exposures were estimated per infant. In total, 67 infants had started eating maize flour at the age of 3 months. Of these infants, 31% (21 infants) consumed maize flour with detectable levels of fumonisins. The exposure in these infants to the sum of FB_1 and FB_2 ranged from 5 to 880 ng/kg bw per day. Median exposure was 140 ng/kg bw per day.

(l) Viet Nam

A study in northern Viet Nam examined the exposure to FB_1 from maize and rice in two ethnic groups, Ta Phoi and Hop Thanh (Huong et al., 2016). In total, 213 samples of dried maize seeds ($n = 102$) and rice ($n = 111$), including wholesale, retail (from local markets) and household (from individual households) samples, were collected and analysed for FB_1. The dietary exposure was calculated by combining the mean FB_1 levels in maize and rice with the mean amount of food consumed per day (obtained from two dietary surveys) and subsequently dividing by a mean body weight of 49 kg for adults and 11.3 kg for children.

Nine rice samples contained FB_1 at concentrations ranging from 2.3 to 624 ng/g and 24 maize samples contained FB_1 at concentrations ranging from 5.6 to 90 ng/g. Mean concentrations of 69 µg/kg in rice and 2.4 µg/kg in maize were used in the calculation. Overall, the mean FB_1 exposure from both sources was 536 and 1019 ng/kg bw per day in, respectively, adults and children. The main

contributor to the exposure was rice: 99.9% in adults and 100% in children. Rice is consumed much more than maize in northern Viet Nam. Children involved in the study were not fed maize.

(m) Zimbabwe

The exposure to FB_1 and FB_2 via maize was estimated for subsistence farmers in Zimbabwe (Hove et al., 2016). In total, 95 maize meal samples were collected from household stores of randomly selected subsistence farming households and analysed for FB_1 and FB_2. Only the exposure to FB_1 was estimated because FB_2 levels were all below the LOD. Age groups considered were young children (<5 years), children (5–8 years), adolescents (9–18 years), adults (19–60 years) and older adults (≥61 years). Mean food consumption per age group was estimated by means of a quantitative FFQ, including standardized recipe data and typical portion sizes. Body weights were averages based on participant body weights per age categories. A median concentration of FB_1 in maize of 146 µg/kg was used to assess the exposure. The exposure estimates ranged from 2200 ng/kg bw per day in older adults to 5400 ng/kg bw per day in children. The exposure in young children equalled 3910 ng/kg bw per day.

(n) Summary

National estimates of dietary fumonisin exposure described above are summarized in Table 16. In these studies, the mean exposure in European countries to fumonisins was below 250 ng/kg bw per day. High exposures to FB_1 were reported for Zimbabwe (Hove et al., 2016) and China (Sun et al., 2011), with a maximum of 7700 ng/kg bw per day for adults living in the rural province of Huaian. The Committee calculated the exposure per kilogram body weight for China by dividing the reported maximum exposure of 460 000 ng per day by 60 kg. For total fumonisins (FB_1 + FB_2 + FB_3), the highest mean exposures were reported in Malawi, ranging from 3000 to 15 000 ng/kg bw per day (Matumba et al., 2015). The authors observed that if maize was consumed after dehulling, the dietary exposure would drop by a factor of 3 or greater (Matumba et al., 2015). Owing to the large differences in foods included in the published assessments, from only one food (e.g. only maize or coffee) to the whole diet, and differences in methodologies used to assess the exposure (e.g. per capita consumption or individual food consumption data), it is not possible to compare the exposures between the studies.

4.3.2 National estimates derived by the Committee

The Committee estimated national exposures to FB_1 and total fumonisins using data from the CIFOCOss database (section 4.2) combined with the occurrence data described in section 4.1.

The countries included in the CIFOCOss database as well as the countries that submitted the occurrence data were first classified according to the six WHO regions (http://www.who.int/about/regions/en/). WHO region-specific mean concentrations were calculated per food. Also, mean concentrations were calculated based on the occurrence data across all six regions, the global mean. In the exposure assessment, mean consumption statistics corrected for body weight per survey and age group were mapped to the relevant region-specific concentration levels. The mean exposures per food were subsequently summed to obtain the total mean exposure per day. Using region-specific mean concentrations assumes that concentrations of foods available in a specific region are representative for all countries in that region. If no region-specific mean for a food was available, the global mean concentration was used. No weightings were applied to the concentrations.

Exposures were calculated per survey and age group. For the Philippines, only consumption statistics in grams per day were available for three age groups. To obtain exposure estimates corrected for body weight, these consumption statistics were divided by a fixed body weight. For the general population and women of childbearing age, a body weight of 60 kg was used, the same as used to obtain body weight–corrected exposure values based on the GEMS/Food cluster diets (section 4.3.3). For children aged 5 years or less, a body weight of 12 kg was used; this is the standard default value for body weight proposed by EFSA for children aged 1–3 years living in Europe (EFSA, 2012).

For surveys performed in countries in the WHO South-East Asia Region, no region-specific concentration data were available, whereas for those countries in the African and Eastern Mediterranean regions, region-specific data were only available for sorghum. For the WHO Region of the Americas, there were 31 foods with region-specific occurrence data; for the WHO European Region, there were 49 foods with region-specific occurrence data; and for the WHO Western Pacific Region, there were 50 foods with region-specific occurrence data.

The CIFOCOss database includes food consumption data for infants from the EFSA comprehensive database (EFSA, 2011) for two European countries (Bulgaria and Italy). In this age group, the food group "food for infants and small children" was consumed at high levels. Detailed examination of this food group using the data from the EFSA comprehensive database as published on the website[2] showed that the foods consumed were infant formula. The same was true

[2] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

for toddlers from Finland. The concentrations of fumonisins in infant formula reported in the GEMS/Food contaminants database were all undetectable. The exposure to fumonisins via the food group "foods for infants and small children" in these three population groups was therefore assumed to be zero.

The results of the exposure assessment are presented in Table 17. Exposures estimated are mean exposures in ng/kg bw per day and represent chronic dietary exposures. To assess potential high exposure, the mean exposure was multiplied by a factor of 2. This factor approximates the 90th percentile (FAO/WHO, 2009). The highest exposures were in the youngest population groups. "Other children" (3–9 years) from Greece had the highest LB mean exposure to FB_1, at 800 ng/kg bw per day, with a corresponding 90th percentile of exposure of 1600 ng/kg bw per day. The highest LB mean exposure to total fumonisins was in the same population group, at 1200 ng/kg bw per day, with a corresponding 90th percentile of exposure of 2300 ng/kg bw per day. The UB mean exposure to FB_1 was highest in toddlers (1–2 years) from Italy, at 2000 ng/kg bw per day, whereas the UB mean exposure to total fumonisins was highest in toddlers from Germany, at 3200 ng/kg bw per day. Furthermore, FB_1 accounted for on average 70% (range 63–83%) of the LB exposure to total fumonisins. In the UB scenario, this was 67% (range 53–72%).

Major contributors to the LB mean exposure to FB_1 and total fumonisins were "cakes, cookies and pies" (~33%), "cereal-based composite foods" (~31%) and "cereal grains (nonspecified)" (~11%) across all population groups and surveys. Focusing only on infants and toddlers, the age groups with the highest exposure levels, the same foods contributed most, with percentage contributions of about 33%, 13% and 24%, respectively; "foods for infants and small children" contributed around 13%. In the UB scenario, "wheat white bread" was also an important contributor to the exposure across all population groups and surveys (~16%), as was "ready-to-eat meals" (~23%) in infants and toddlers. These two foods are consumed in large amounts, whereas more than 95% of the analysed samples contained FB_1 and total fumonisins at undetectable levels. The Committee noted that the contributions based on the UB estimates may thus overestimate the likely contribution of food groups with no or limited number of detectable concentrations of FB_1 and total fumonisins.

The occurrence data used per region were the same. Differences in exposure within a region were therefore due to differences in consumption data. Because of differences in the way the food consumption data were collected and coded per country, direct country-to-country comparisons in exposure are not feasible. Generally, the highest exposures were in the European region in the UB scenario. Given the high percentage of undetectable results in the dataset (Tables 14 and 15) and the high LOQs reported in the database within several clusters,

Table 17

Estimated mean and high[a] chronic dietary exposure to FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$) for the countries and age groups present in the CIFOCOss database

WHO region / Country	Population[c]	Estimated exposure[b] (ng/kg bw per day)			
		FB_1		Total fumonisins	
		Mean	High	Mean	High
African Region					
Burkina Faso	Adult women	190–910	380–1 800	260–1 300	530–2 500
Uganda	Adult women	470–840	940–1 700	640–1 200	1 300–2 400
Region of the Americas					
Brazil	General population	25–260	49–510	36–380	73–750
USA	Children <6 years	130–530	250–1 100	190–780	370–1 600
	Women of childbearing age	36–140	71–280	52–210	110–420
	General population	46–190	92–380	68–280	140–570
South-East Asia Region					
Bangladesh	Children	79–1 100	160–2 100	110–1 600	220–3 100
	Adult women	17–720	34–1 400	22–1 000	45–2 100
Thailand	General population	17–620	34–1 200	25–900	50–1 800
European Region					
Belgium	Toddlers	270–1 300	540–2 500	380–2 000	760–4 000
	Other children	300–1 100	600–2 100	420–1 600	840–3 300
	Adolescents	100–450	200–910	140–670	290–1 300
	Adults	74–360	150–730	110–530	210–1 100
	Elderly adults	53–270	110–530	77–390	150–770
	Very elderly adults	52–270	100–540	75–400	150–790
Bulgaria	Infants	120–1 400	240–2 900	170–2 100	330–4 300
	Toddlers	270–1 600	540–3 200	390–2 400	770–4 900
	Other children	250–1 300	490–2 500	360–1 900	720–3 700
Cyprus	Adolescents	370–690	740–1 400	530–1 000	1 100–2 100
Czech Republic	Other children	180–520	370–1 000	270–780	530–1 600
	Adolescents	150–400	310–810	220–600	450–1 200
	Adults	75–340	150–670	110–470	220–950
Denmark	Other children	100–600	210–1 200	140–910	280–1 800
	Adolescents	54–350	110–700	75–520	150–1 000
	Adults	34–290	68–580	47–420	94–830
	Elderly adults	27–260	55–520	38–370	76–740
	Very elderly adults	42–280	83–560	57–400	110–800
Finland	Toddlers	340–1 200	680–2 500	450–1 900	900–3 800
	Other children[d]	150–1 100	300–2 200	210–1 700	420–3 400
	Adults	70–290	140–580	100–440	200–890
	Elderly adults	62–250	120–490	86–390	170–770
France	Other children	240–970	470–1 900	330–1 500	660–3 000
	Adolescents	130–580	260–1 200	180–860	370–1 700
	Adults	72–410	140–810	100–600	200–1 200
	Elderly adults	48–360	96–720	69–530	140–1 100

WHO region / Country	Population[c]	Estimated exposure[b] (ng/kg bw per day)			
		FB_1		Total fumonisins	
		Mean	High	Mean	High
	Very elderly adults	43–330	86–660	61–480	120–970
Germany	Toddlers[e]	200–1 700	400–4 100	280–3 200	550–6 400
	Other children[e]	180–700	370–1 400	260–1 100	520–2 200
	Adolescents	86–290	170–570	120–420	250–840
	Adults	77–280	150–550	110–400	220–790
	Elderly adults	67–250	130–500	97–360	200–720
	Very elderly adults	78–240	160–480	110–350	230–700
Greece	Other children	800–1 300	1 600–2 500	1 200–1 900	2 300–3 800
Hungary	Adults	35–360	69–720	51–540	100–1 100
	Elderly adults	39–320	78–640	58–470	120–950
	Very elderly adults	43–390	87–780	64–580	130–1 200
Ireland	Adults	41–440	82–870	59–630	120–1 300
Italy	Infants	64–1 500	130–3 000	87–2 300	170–4 600
	Toddlers	250–2 000	490–3 900	350–3 000	710–6 000
	Other children	210–1 300	410–2 500	300–1 900	600–3 800
	Adolescents	130–750	260–1 500	190–1 100	390–2 200
	Adults	70–520	140–1 000	100–770	210–1 500
	Elderly adults	49–470	98–950	72–700	140–1 400
	Very elderly adults	52–510	100–1 000	76–760	150–1 500
Latvia	Other children	290–760	590–1 500	410–1 100	830–2 300
	Adolescents	210–560	420–1 100	300–830	600–1 700
	Adults	140–400	280–800	200–590	400–1 200
Netherlands	Toddlers	180–690	350–1 400	250–1 100	500–2 200
	Other children	150–510	300–1 000	220–760	430–1 500
	Adults	62–280	120–560	90–400	180–790
Spain	Toddlers	190–1 300	370–2 600	260–1 900	520–3 800
	Other children[f]	120–940	240–1 900	170–1 400	340–2 800
	Adolescents[f]	70–700	150–1 400	110–1 000	220–2 100
	Adults[f]	55–380	110–770	80–560	160–1 100
Sweden	Other children	420–1 000	840–2 000	610–1 500	1 200–3 000
	Adolescents	300–650	610–1 300	440–980	880–2 000
	Adults	210–480	430–970	310–720	620–1 400
United Kingdom	Adults	51–360	100–710	72–520	150–1 000
Western Pacific Region					
Australia	Children (2–6 years)	12–250	24–500	19–370	38–730
	Children (2–16 years)	10–180	20–360	16–270	32–530
China	Children	64–540	130–1 100	95–800	190–1 600
	General population	24–300	49–610	37–450	73–900
Japan	Children	17–510	34–1 000	25–760	50–1 500
	General population	10–300	19–610	14–450	28–900

Table 17 (continued)

WHO region / Country	Population[c]	Estimated exposure[b] (ng/kg bw per day)			
		FB_1		Total fumonisins	
		Mean	High	Mean	High
Philippines	Children (<6)	570–980	1 100–2 000	840–1 500	1 700–2 900
	Children	0–220	0–440	0–330	0–650
	Women of childbearing age	140–370	280–730	210–540	410–1 100
	General population	130–300	270–590	200–440	390–880
Republic of Korea	Children	10–290	20–580	15–420	30–850
	General population	11–170	22–350	16–260	32–510

bw: body weight; CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] The high exposure equals the 90th percentile of exposure. This percentile is an approximation calculated as twice the mean dietary exposure.

[b] The range of dietary exposure estimates refers to LB and UB estimates of mean and high dietary exposure. The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ. The UB estimate was derived by substituting the value of the LOQ for analytical results below the LOD or LOQ.

[c] For European countries, population group descriptors are defined as follows: Infants, <1 year; toddlers, 1–2 years; other children, 3–9 years; adolescents, 10–17 years; adults, 18–64 years; elderly adults, 65–74 years; very elderly adults, ≥75 years (EFSA, 2011). In the other population groups, age is included in the table if noted in the CIFOCOss database.

[d] The CIFOCOss database contains information from two surveys of food consumption by other children (DIPP and STRIP). The results presented here are the lower- and upper-bound dietary exposure estimates across the two surveys.

[e] The CIFOCOss database contains information from three successive surveys of food consumption by toddlers and other children (DONALD 2006, DONALD 2007 and DONALD 2008). The results presented here are the LB and UB dietary exposure estimates across the three surveys.

[f] The CIFOCOss database contains information from different surveys of food consumption by other children (enKID, NUT_INK05), adolescents (AESAN_FIAB, enKID, NIU_INK05) and adults (AESAN, AESAN_FIAB). The results presented here are the LB and UB dietary exposure estimates across the different surveys per age–sex group.

these UB exposures very likely overestimate the exposure, and may be interpreted as worst-case estimates of exposure based on the data available.

4.3.3 International estimates

The international estimates of exposure were assessed by combining consumption data from GEMS/Food cluster diets (section 4.2) with the occurrence data (section 4.1). In the international exposure assessment of FB_1 and total fumonisins at the seventy-fourth meeting of the Committee, processed foods such as breakfast cereals, cereal-based dishes, biscuits, organic cookies, etc., were not included. In this current assessment, these foods were included by mapping the foods analysed to foods coded at level 3 in the GEMS/Food cluster diets. This level also contains codes of processed foods such as breakfast cereals, bread, pasta, etc. Occurrence data on food specifically meant for infants and young children were not included as no consumption data for infants are available in the GEMS/Food cluster diets.

The countries with concentration data on fumonisins were classified according to the individual GEMS/Food cluster diets. Subsequently, LB and UB mean FB_1 and total fumonisin concentration levels per food were calculated per cluster and across clusters, the global mean. This assumes that concentrations of foods available in a specific cluster are representative for all countries in this

cluster. If no cluster-specific mean for a food was available, the global mean was used. No weightings were applied to the concentrations as no relevant information was available. A standard body weight of 60 kg was used to derive an exposure per kilogram of body weight.

Some of the occurrence data were submitted by countries in the WHO European Region, but no information was available to link these data to a specific country in this region. These data were therefore only used to calculate the LB and UB global mean per food. This was also true for data submitted by Singapore, which is not categorized in a cluster.

Exposure estimates of FB_1 and total fumonisins are listed in Table 18. The estimated exposures are mean exposures in ng/kg bw per day and represent chronic dietary exposures. To assess potential high exposure, the mean exposure was multiplied by a factor of 2. This factor approximates the 90th percentile of exposure (FAO/WHO, 2009). The LB mean chronic exposure to FB_1 ranged from 2 (cluster G09; mainly East Asian countries) to 560 ng/kg bw per day (cluster G05; mainly South and Central American countries). The LB mean chronic exposure to total fumonisins ranged from 13 (cluster G09) to 820 ng/kg bw per day (cluster G05). The corresponding 90th percentile estimates of exposure to FB_1 ranged from 5 to 1100 ng/kg bw per day and to total fumonisins from 25 to 1600 ng/kg bw per day. The highest UB mean exposure estimates of FB_1 and total fumonisins were observed in cluster G15 (European countries): 1200 and 2100 ng/kg bw per day, respectively (Table 18). In both scenarios, FB_1 accounted for on average 67% of the exposure to total fumonisins.

The Committee noted that the LB and UB mean and 90th percentile exposure estimates of FB_1 and total fumonisins differed by more than a factor of 10 within clusters G09 and G15, resulting in a UB 90th percentile exposure estimate of 4300 ng/kg bw per day in cluster G15 (Table 18). This was due to the food products rice in cluster G09 and wheat flour in cluster G15. All fumonisin levels in these products were undetectable (Table 14 and Table 15). Assigning the LOQ to these samples resulted in high contamination levels which, combined with the relatively high mean consumption of rice in cluster G09 (262 g per day) and of wheat flour in cluster G15 (206 g per day), resulted in the observed difference in exposure between the LB and UB scenarios.

Maize was the major contributor to the LB exposure to FB_1 (Table 19). In almost all clusters, the contribution of maize (products), which included maize, maize flour and maize oil, exceeded 60%. In two clusters, G09 and G11 (Belgium and the Netherlands), the contribution was less than 5%. This was due to a low concentration of FB_1 in maize flour in cluster G09 (Table 14) and a low mean consumption of this product in cluster G11 (4.2 g per day). Other major contributors to the exposure were wheat (products), which included wheat

Table 18

Mean and high[a] chronic dietary exposure to FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$) according to commodities in the GEMS/Food cluster diets

	Estimated dietary exposure per GEMS/Food cluster diets (ng/kg bw per day)																
Exposure scenario and fumonisin[b]	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
FB_1																	
Mean																	
LB	130	180	340	200	560	190	120	61	2	58	430	300	400	47	74	220	170
UB	480	530	550	560	940	610	500	550	310	300	650	650	660	410	1 200	400	390
High																	
LB	260	350	680	400	1 100	380	250	120	5	120	860	590	800	93	150	450	330
UB	960	1 100	1 100	1 100	1 900	1 200	1 000	1 100	620	610	1 300	1 300	1 300	820	2 301	790	780
Total fumonisins																	
Mean																	
LB	190	260	490	290	820	290	180	89	13	85	630	430	580	68	91	320	240
UB	720	790	790	840	1 400	900	760	780	440	440	940	940	960	600	2 100	570	570
High																	
LB	380	510	970	570	1 600	570	360	180	25	170	1 300	850	1 200	140	180	640	480
UB	1 400	1 600	1 600	1 700	2 800	1 800	1 500	1 600	880	890	1 900	1 900	1 900	1 200	4 300	1 100	1 100

bw: body weight; FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] The high exposure equals the 90th percentile of exposure. This percentile is an approximation calculated as twice the mean dietary exposure.

[b] The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ. The UB estimate was derived by substituting the value of the LOQ for analytical results below the LOD or LOQ.

Table 19

Per cent contribution of top three commodities[a] from GEMS/Food cluster diets to FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$) exposure based on LB[b] estimates of exposure

	Food product[c,d,e,f,g] and per cent contribution					
	FB_1			Total fumonisins		
GEMS/Food cluster diets	1st	2nd	3rd	1st	2nd	3rd
G01	Maize (products): 67%	Wheat (products): 24%	–	Maize (products): 65%	Wheat (products): 27%	–
G02	Maize (products): 75%	Wheat (products): 16%	–	Maize (products): 74%	Wheat (products): 18%	–
G03	Maize (products): 96%	–	–	Maize (products): 96%	–	–
G04	Maize (products): 66%	Food prep, nes: 15%	Wheat (products): 12%	Maize (products): 65%	Food prep, nes: 15%	Wheat (products): 13%
G05	Maize (products): 94%	–	–	Maize (products): 94%	–	–
G06	Maize (products): 77%	Wheat (products): 19%	–	Maize (products): 76%	Wheat (products): 21%	–
G07	Maize (products): 95%	–	–	Maize (products): 95%	–	–
G08	Maize (products): 74%	Food prep, nes: 8%	Barley (products): 5%	Maize (products): 74%	Food prep, nes: 8%	Barley (products): 5%
G09	Food prep, nes: 45%	Sorghum (products): 27%	Barley (products): 6%	Maize (products): 72%	Food prep, nes: 13%	Sorghum (products): 8%
G10	Maize (products): 79%	Food prep, nes: 11%	–	Maize (products): 79%	Food prep, nes: 11%	–
G11	Wheat (products): 95%	–	–	Wheat (products): 95%	–	–
G12	Maize (products): 66%	Food prep, nes: 27%	–	Maize (products): 65%	Food prep, nes: 28%	–
G13	Maize (products): 87%	Sorghum (products): 10%	–	Maize (products): 87%	Sorghum (products): 10%	–
G14	Maize (products): 64%	Wheat (products): 20%	Food prep, nes: 7%	Maize (products): 63%	Wheat (products): 22%	Food prep, nes: 7%
G15	Maize (products): 71%	Food prep, nes: 18%	–	Maize (products): 67%	Food prep, nes: 21%	–
G16	Maize (products): 93%	–	–	Maize (products): 93%	–	–
G17	Maize (products): 63%	Food prep, nes: 29%	Wheat (products): 6%	Maize (products): 62%	Food prep, nes: 29%	Wheat (products): 7%

FB_1: fumonisin B_1; FB_2: fumonisin B_2; FB_3: fumonisin B_3; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; prep: prepared; UB: upper bound

[a] Commodities with a contribution of at least 5%.

[b] The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ.

[c] Maize (products): Maize (level 3 code = 170), Flour of Maize (level 3 code = 171) and Maize oil (level 3 code = 234).

[d] Wheat (products): Wheat (level 3 code = 177) and Flour of Wheat (level 3 code = 160).

[e] Food prep, nes: linked to the FB concentrations analysed in cereal-based composite foods and vegetable-based composite foods (including mushroom-based).

[f] Barley (products): Barley (level 3 code = 167), Pot Barley (level 3 code = 168), Barley Flour and Grits (level 3 code = 169) and Barley Pearled (level 3 code = 186).

[g] Sorghum (products): Sorghum (level 3 code = 173) and Flour of Sorghum (level 3 code = 174).

and wheat flour, and the level 3 food group "Food prepared, nes", comprising mainly the analysed products cereal-based composite foods and vegetable-based composite foods (including mushroom-based). "Food prepared, nes" contributed more than 10% to the exposure to FB_1 in clusters G04 (mainly Caribbean island and Middle Eastern states), G09, G12 (Central American countries), G15 and G17 (Island states) (Table 19). This high contribution was due to a relatively high FB_1 global mean concentration (130 µg/kg)[3] and because of a high mean consumption in clusters G12 and G17.

For total fumonisins, the food commodity maize (products) was also the main contributor to the LB exposure. Maize (products) was the main contributor in G09 because of higher concentrations of total fumonisins in maize (products) compared to FB_1 (Tables 14 and 15). In cluster G11, wheat (products) was the main contributor to the exposure to both FB_1 and total fumonisins (Table 19). In this cluster, the highest FB_1 and total fumonisin concentrations in wheat were reported (Tables 14 and 15).

In the UB scenario, wheat was the major source of exposure to FB_1 and total fumonisins (~40–75%) in nine clusters and rice in three clusters (~20–60%). These differences in contribution compared to the LB scenario, in all the clusters except for cluster G11, were due to a combination of high consumption levels, high percentage of undetectable results and/or high LOQs in these two products. In five clusters, maize remained the main contributor (~35–70%). As for the calculated national estimates of exposure (section 4.3.2), the Committee noted that the contributions based on the UB estimates may overestimate the likely contribution of food groups with no or a limited number of detectable concentrations of FB_1 and total fumonisins. Furthermore, the UB estimations of exposure may also be interpreted as worst-case estimates of the exposure based on the data available.

5. Dose–response analysis and estimation of toxic/carcinogenic risk

5.1 Identification of key data for risk assessment

Since the 2011 evaluation, no new studies have been designed with sufficient rigour to reveal accurate dietary dose–response relationships. The weaknesses

[3] In total, 14 samples were classified as being in the food group "Food prepared, nes" with eight positive samples with an FB_1 concentration ranging from 39.1 to 727 µg/kg. Results were from Belgium, Germany, the United Kingdom and countries classified as in the WHO European Region. The foods were named prepared salads, cereal-based dishes, pizza and pizza-like pies, and grain soup.

usually involved poor characterization of toxins in the diets and/or selection of inappropriate fumonisin levels for evaluating risk of adverse effects in experimental animal models.

At the seventy-fourth meeting, the Committee identified the individual liver scores for the incidence of megalocytic (more correctly, karyocytomegalic) hepatocytes and apoptosis from the Bondy et al. (2010) study as critical and sensitive end-points for the dose–response evaluation of purified FB_1 (Annex 1, reference *206*, "Fumonisins (addendum)", Tables 24 and 25). Bondy et al. (2010) subjectively assigned a numeric grade to lesions other than tumours, from 0 to 5 (where 0 indicated that no lesions were present and 1–5 indicated that lesions were minimal, mild, moderate, marked and severe, respectively). More recently, the results of the same study were reported with the same scoring system with slight differences in the incidence data and one small adjustment to a pathology score (Bondy et al., 2012). The Committee concluded that these slight differences (Bondy et al., 2010 versus Bondy et al., 2012) did not change the overall toxicological assessment performed at the seventy-fourth meeting in 2011 (Annex 1, reference *206*).

6. Comments

6.1 Biochemical aspects

Most of the studies reported since 2011 provide information that confirms the findings and conclusions reported previously (WHO, 2000; Annex 1, references *153* and *206*). This update of the biochemical aspects of fumonisins will focus on new findings or those that extend previous findings or confirm older findings that were uncertain.

A recent feeding study in rats confirmed that only small amounts of hydrolysed FB_1, relative to FB_1, are formed in the gut, where the relative recoveries in faeces of FB_1, partially hydrolysed FB_1 and fully hydrolysed FB_1 were 93.8%, 5.9% and 0.3%, respectively (Hahn et al., 2015).

Very small amounts of FB_1 are excreted in the urine. A recent study in mice showed that following intraperitoneal dosing, the half-life of UFB_1 was less than 24 hours, whereas elevated levels of sphingoid bases and sphingoid base 1-phosphates in blood increased after UFB_1 peaked (<4 hours), and elevated levels were detectable up to at least 120 hours after the last dose of FB_1, with an estimated half-life of between 48 and 72 hours (Riley et al., 2015a). Likewise, a recent study confirmed the rapid absorption and elimination of FB_1 in rats, showing that after a single oral dose, UFB_1 peaked rapidly (12 hours) and then

decreased equally rapidly, with a half-life in the urine of between 24 and 36 hours (Mitchell et al., 2014).

In humans consuming known amounts of fumonisins, FB_1 was detected in the urine soon after exposure began and decreased rapidly after consumption or exposure ceased (Riley et al., 2012). The total urinary excretion of FB_1 in humans was less than 1% of the cumulative dose, a value similar to that reported in animal studies. The estimated half-life in humans was less than 48 hours after they consumed FB_1-containing diets for 3 consecutive days. A study involving 1200 women found that FB_1 was excreted in the urine much more efficiently than FB_2 or FB_3, based on the relative levels of FB_1, FB_2 and FB_3 in the food consumed (Torres et al., 2014).

The free primary amino group in FB_1 is required for inhibition of ceramide synthase. This finding has been confirmed and extended in a recent in vivo study using diets formulated with highly purified FB_1, partially hydrolysed FB_1 (one TCA side-chain removed), fully hydrolysed FB_1 (both TCA side-chains removed) and *N*-(1-deoxy-D-fructos-1-yl) FB_1. The results show that the fumonisin derivatives did not elevate the Sa/So ratio and were significantly less nephrotoxic than FB_1 (Hahn et al., 2015).

Recent studies show that FB_1, as shown previously for fully hydrolysed FB_1, is a substrate for ceramide synthase, forming *N*-acyl-FB_1 derivatives in vivo and in vitro. Considerably more *N*-acyl-hydrolysed FB_1 accumulates in cells compared with *N*-acyl-FB_1. This mimics the relative accumulation of fully hydrolysed FB_1 and FB_1 (Harrer et al., 2013; Harrer, Humpf & Voss, 2015). Very little of the total FB_1 in the male rat kidney (the most sensitive target organ) was metabolized to *N*-acyl-FB_1, whereas in the liver, approximately half of the total fumonisin consisted of the *N*-acyl-FB_1 derivatives.

The *N*-acyl-FB_1 derivatives are more cytotoxic in vitro than FB_1. The in vivo toxicity of the *N*-acyl-FB_1 derivatives and *N*-acyl-hydrolysed FB_1 derivatives is not known; however, hydrolysed FB_1 is much less toxic than FB_1, as shown in both previously reviewed (Annex 1, reference *206*) and more recent feeding studies (Grenier et al., 2012; Voss & Riley, 2013; Harrer, Humpf & Voss, 2015; Masching et al., 2016).

FB_1 is a potent and specific inhibitor of ceramide synthases. There has been growing evidence for the important role of ceramides, sphingoid bases and sphingoid base 1-phosphates as cellular mediators in the development of human diseases, but there is no evidence of FB_1-induced ceramide synthase inhibition in any human disease, nor is there evidence that FB_1-induced ceramide synthase inhibition is in itself an adverse effect. As in the previous JECFA evaluations, there were many animal studies providing evidence that fumonisin inhibits ceramide biosynthesis and stimulates sphingoid base phosphorylation. Recent studies in mice (Riley et al., 2015a) show that the elevated levels of sphinganine

and sphinganine 1-phosphate in liver and kidney are paralleled by increased levels of sphinganine 1-phosphate in mouse blood spots in a dose-dependent fashion following oral exposure to FB_1. The increased sphinganine 1-phosphate in blood spots, liver and kidney is positively correlated with the UFB_1 level. The Committee noted that disruption of lipid metabolism consequent to inhibition of ceramide synthases appears to play an important and early role in fumonisin toxicity and carcinogenicity in animal models.

The development of mice lacking ceramide synthase 2 has led to a better understanding of how decreased ceramide biosynthesis and increased sphinganine are involved in the development of liver lesions, liver cancer, mitochondrial dysfunction, increased oxidative stress and biochemical/structural changes in membranes (Pewzner-Jung et al., 2010a,b; Silva et al., 2012; Zigdon et al., 2013; Novgorodov et al., 2014). The Committee concluded that these findings provide additional mechanistic support for involvement of disrupted sphingolipid metabolism in the FB_1-induced increased oxidative damage to lipids, proteins and DNA, liver damage and liver cancer.

6.2 Toxicological studies

In the previous evaluations, the Committee concluded that FB_1 was not acutely toxic. There have been no new acute toxicity studies reported since 2011.

Since 2011, three short-term rat studies have been conducted with purified FB_1. None of these studies was a dose–response feeding study (Denli et al., 2015; Hahn et al., 2015; Riedel et al., 2015).

In addition, the Committee reviewed the 2012 study by Bondy et al. (2012), which is the final report of the short-term study with purified FB_1 in mice that was provided to the Committee in 2011 as a preliminary report of unpublished data (Bondy et al., 2010). In the study, the effects of dietary FB_1 exposure on the mouse liver were characterized after 26 weeks of exposure to 0, 5, 50 or 150 mg FB_1/kg diet. Comparison of the incidence of and pathology scores for megalocytic (karyocytomegalic) hepatocytes and apoptosis in the preliminary and final reports showed slight differences in the incidence data due to the addition of four mice: one additional mouse in the control group (0 mg/kg bw per day), one additional mouse in the low-dose group (0.4 mg/kg bw per day) and two additional mice in the high-dose group (12 mg/kg bw per day). There was also one mouse in the mid-dose group (4 mg/kg bw per day) for which the pathology score was adjusted from zero to one. The Committee concluded that these slight differences (Bondy et al., 2010 versus Bondy et al., 2012) would not change the overall toxicological assessment performed by the previous Committee.

Other short-term toxicity studies have been conducted using fumonisin obtained from a variety of sources and are described briefly below, but they are not further considered in the assessment owing to poor characterization of the test material or other study limitations.

One study in mice involved only one high dose level of FB_1 of unspecified purity (Alizadeh et al., 2015), and the other mouse study used repeated intraperitoneal injection of a partially purified fumonisin preparation (Sozmen et al., 2014). A single dose level feeding study in male rats used *F. verticillioides* culture material (Venancio et al., 2014), and another feeding study in male rats used culture material diets at high dose levels (50, 100 or 200 mg FB_1/kg diet) (Khalil et al., 2015). In a third rat study, male rats were fed diets containing FB_1 prepared from maize naturally contaminated with fumonisins at 0.22, 1.8, 3.6 or 4.2 mg FB_1/kg diet (Voss et al., 2013). Two studies were conducted in pigs using fungal culture material or partially purified fumonisins. One of the studies was a multi-dose feeding study (0, 3, 6 or 9 mg FB_1/kg diet) (Souto et al., 2015); the diets were prepared using *F. verticillioides* culture material. The other study in pigs involved only a single dose level of partially purified fumonisins (Lalles et al., 2010).

The studies conducted on genotoxicity since 2011 support the conclusions of the 2001 and 2011 evaluations with regard to the lack of evidence for fumonisin-induced DNA damage being a consequence of direct interaction with DNA or metabolism to a DNA-reactive metabolite. The more recent studies also suggest that the source of ROS is disruption of mitochondrial integrity (Domijan & Abramov, 2011; Zigdon et al., 2013; Novgorodov et al., 2014). The Committee concluded that the weight of the evidence is that fumonisins are not DNA reactive, nor are they metabolized to DNA-reactive metabolites, but increased oxidative stress may play a role in the DNA damage observed in some in vivo studies.

Since 2011, there has been one oral gavage dose–response developmental toxicity study in mice (n = 2–4 per dose group) using pure FB_1 (0, 5, 10, 15, 25 and 50 mg/kg bw per day), which showed the induction of NTDs at all doses greater than 5 mg/kg bw per day (Riley et al., 2015a).

Another study in mice found that intragastric intubation of pure FB_1 at 12.5 mg/kg bw per day induced NTDs in the litters from four of seven treated dams (Liao et al., 2014).

In a study in rats (n = 2 or 3 per dose group) fed methyl donor–sufficient or methyl donor–deficient diets for 30 days with and without oral gavage of 0.004 mg FB_1/kg bw per day and then mated and fed the same diets plus 0.004 mg FB_1/kg bw per day for an additional 30 days, FB_1-only treatment and FB_1-plus methyl donor–deficient diet treatment were associated with a multitude of changes, including histone modifications in the fetal livers (Pellanda et al.,

2012). In addition, several in vitro studies have shown that fumonisins can cause epigenetic effects and subsequent effects on gene expression that might alter the risk of disease (Chuturgoon, Phulukdaree & Moodley, 2014b; Sancak & Ozden, 2015; Gardner et al., 2016).

In 2011, the Committee considered the effects on the immune system to be relevant to the risk assessment; however, all of the studies were performed using single doses. Since 2011, there have been additional in vivo studies documenting the immunotoxic effects of fumonisins, and in particular the ability to alter the response to infectious agents. Only one study was done using pure FB_1, and that study used subcutaneous injection in mice (Tafesse et al., 2015). There were also six in vivo studies in pigs. One study used intragastric gavage of *F. verticillioides* culture material extracts (Malik, Toth & Nagy, 2012), and four other studies used *F. verticillioides* culture material extracts to formulate diets containing FB_1, which were fed to pigs at a single dose level (Pósa et al., 2009, 2011, 2013, 2016). In the last pig study evaluated, naturally contaminated maize was used to prepare diets containing 12 mg FB_1 plus FB_2/kg diet in order to investigate their effects on pigs co-exposed to *Salmonella enterica*, with mostly negative results (Burel et al., 2013).

In previous evaluations, the most notable neurological effect of fumonisin was the induction of ELEM, a disease believed to be the result of vascular dysregulation. Since the last evaluation, there have been only a few studies investigating the potential neurotoxicity of fumonisin. In mice, a single intraperitoneal injection of 8 mg pure FB_1/kg bw sensitized the mice to pentylenetetrazol-induced seizures (Poersch et al., 2015). In another study, rats were fed diets containing FB_1 and FB_2 prepared using *F. verticillioides* culture material at a concentration of 0, 1 or 3 mg/kg; effects on the myenteric neurons were observed (Sousa et al., 2014). A study conducted in pigs reported effects on protein content in brain regions (Gbore, 2013).

In 2011, there were a large number of in vitro and in vivo studies investigating the combined effects of fumonisins and other mycotoxins. In the present evaluation, the new co-exposure studies for fumonisins and aflatoxins are covered separately (see "Co-exposure of fumonisins with aflatoxins", pages 879–960). The studies describing the combined effects of in vivo and in vitro co-exposure of fumonisins with mycotoxins other than aflatoxins were also evaluated; as in 2011 (Annex 1, reference *206*), the current evaluation included numerous in vivo and in vitro studies showing a wide range of responses suggesting antagonistic, additive and more-than-additive (synergistic) responses. Many of the studies involved only a single dose level of individual mycotoxins, and therefore accurate quantitative assessment of interactions was not possible.

In 2011, the Committee noted that the nephrotoxicity of culture material was much greater than the nephrotoxicity of pure FB_1. No studies have been conducted since 2011 that specifically addressed this concern.

Since 2011, two in vivo feeding studies, one using naturally contaminated maize (Voss et al., 2013) and the other using *F. verticillioides* culture material (Souto et al., 2015), produced toxicological results in rats and pigs, respectively, consistent with the view that pure FB_1, naturally contaminated maize containing FB_1 and *F. verticillioides* culture material containing FB_1 are not toxicologically equivalent.

The Committee also noted that the reason for the differences in the toxicity of pure FB_1, culture material containing fumonisins and naturally contaminated maize is likely related to differences in the chemical composition of the various test agents. For example, before the availability of pure FB_1, many animal feeding experiments were conducted by incorporating culture material of *F. verticillioides* grown mainly on autoclaved maize kernels. Many studies were reported with cultures inoculated with *F. moniliforme* (now *F. verticillioides*) MRC 826. Although most studies report analytical data for FB_1 only, it is now known that the processing required to prepare culture material alters not just the growth of *F. verticillioides*, but also the ratios of various secondary metabolites, including mycelial proteins and fungal metabolites. Although these metabolites would be present in naturally contaminated grains, they would not be present in the same relative amounts. Thus, in the Committee's opinion, although the use of *F. verticillioides* maize culture material has been viewed as representative of naturally contaminated maize, they are not the same, and therefore it should not be surprising that the toxicological profiles of pure fumonisins, culture material containing fumonisins and naturally contaminated maize containing fumonisins are different.

6.3 Observations in domestic animals/veterinary toxicology

Since 2011, there have been few available published reports on field disease outbreaks in farm and domestic animals involving fumonisins. Nonetheless, farm animals are frequently used in studies of fumonisin toxicity because of the fact that mycotoxin-contaminated feed is often suspected of being involved in field performance problems and the susceptibility of poultry and other farm animals to infectious agents. The Committee noted that development of reporting systems designed to identify suspected mycotoxin involvement in farm/domestic animal disease outbreaks could be informative for identifying areas where the risk for mycotoxins as a contributing factor in human diseases may also be increased.

6.4 Observations in humans

Since the last JECFA evaluation, biomarkers of fumonisin exposure have been used increasingly to estimate human exposure worldwide. UFB_1 is the most commonly used biomarker of exposure and has been validated in multiple human studies. UFB_1 has also been used as a biomarker to evaluate the effectiveness of dietary interventions designed to help decrease fumonisin exposure in humans. UFB_1 is reflective of recent fumonisin exposure, but in areas where maize is a dietary staple and exposure is likely to occur at every meal and year-round, UFB_1 levels may be indicative of an individual's chronic exposure.

FB_1 was found in the urine of exclusively breastfed infants, suggesting that human breast milk could be an important source of exposure in young children (Njumbe Ediage et al., 2013). Although previous animal-based data demonstrated an insignificant feed-to-milk transfer, one study in humans has reported the detection of high levels of FB_1 in breast milk from Tanzanian women (Magoha et al., 2014a). The Committee considered the method used in the Magoha et al. (2014a) study to quantify the FB_1 in breast milk to be inadequate for this matrix.

In an effort to develop mechanism-based biomarkers of fumonisin, a method to quantify phosphorylated sphingoid bases in human blood spots was developed based on the fact that RBCs are the main storage reservoir for sphinganine 1-phosphate and sphingosine 1-phosphate (Riley et al., 2015a). A human study in Guatemala (Riley et al., 2015b) showed that there was a positive and statistically significant correlation between UFB_1 (biomarker of exposure) and the blood spot levels of sphinganine 1-phosphate and the Sa 1-P/So 1-P ratio (biomarkers of effect) in humans consuming diets containing high levels of fumonisins. For both the Sa 1-P/So 1-P ratio and the sphinganine 1-phosphate concentration, the first statistically significant increases occurred in the UFB_1 concentration range of >0.5 to <1.0 ng FB_1/mL. A UFB_1 concentration of 0.5 ng/mL was estimated to be equivalent to an exposure of 1.67 µg FB_1/kg bw per day. The Committee concluded that these data support the hypothesis that daily exposure to high levels of FB_1 is likely to result in inhibition of ceramide synthase in humans, similar to what has been described in many animal studies.

A limited number of epidemiological studies have been published since the last JECFA evaluation on the associations between fumonisin exposure and health outcomes in humans. In an ecological study conducted in the Islamic Republic of Iran, FB_1 contamination in rice, but not in maize, was associated with an increased risk of oesophageal cancer (Alizadeh et al., 2012). However, the Committee decided that no causal relationship could be derived because of the lack of control for other risk factors of oesophageal cancer. One nested case–control study investigated the contribution of fumonisin exposure to the risk of

hepatocellular cancer in two cohorts in China; a significant association was not found (Persson et al., 2012).

Two prospective epidemiological studies were conducted in the United Republic of Tanzania investigating the association of mycotoxin exposure and childhood growth. In one study, a significant negative association was found between UFB_1 and the LAZ and length velocity among young children followed up until 6–14 months of age (Shirima et al., 2015). In the other study, exposure to fumonisin from maize-based foods, either alone or together with aflatoxin, was not significantly associated with stunting or underweight among infants followed up until 5 months of age (Magoha et al., 2016). However, the result of this study was compromised by low statistical power and the limitation of exposure characterization.

6.5 Levels and patterns of contamination in food

Data since the last assessment of fumonisins during the seventy-fourth meeting of the Committee in 2011 (Annex 1, reference *206*) were included with information collated from two sources. First, the GEMS/Food contaminants database was screened for fumonisin data (FB_1, FB_2, FB_3 and total fumonisins) submitted from January 2011 until August 2016; second, a literature search was conducted on fumonisin occurrence data published during the same time period. The dataset from the GEMS/Food contaminants database contained 56 702 records and included samples collected from 2000 to 2016. The majority of records were for FB_1 and FB_2 (25 143 and 19 990, respectively), with a smaller dataset for FB_3 and total fumonisins (8164 and 3405, respectively). Data for total fumonisins were calculated based on the sum of FB_1, FB_2 and FB_3 (7580 samples). The distribution of total fumonisins (FB_1, FB_2 and FB_3) across the food categories was similar to that of FB_1. The overall ratio between the three fumonisins was 68:20:12.

The majority of samples were cereals and cereal products (approximately 80%), and the highest occurrence and highest concentrations were detected in samples of cereals and cereal-based products and the other food categories that may contain cereals. Of the cereals and cereal-based products, maize and maize-based products had the highest concentrations of FB_1. For maize samples, the occurrence above the LOQ was just over 50%, with an LB mean of 310 µg/kg, a UB mean of 392 µg/kg and a maximum of 23 800 µg/kg. The data originated predominantly from Europe, followed by Canada and Japan. Higher mean concentrations of FB_1 were reported for maize and maize products in the literature from Africa, South America and other countries in the WHO Western Pacific Region (Rocha et al., 2009; Adetunji et al., 2014; Fu et al., 2015; Phuong et al., 2015).

Sorghum had intermediate levels of FB_1 occurrence (12% above LOQ) and concentration (LB mean 31 µg/kg), whereas all other cereal grains had a much lower occurrence (<5%) and LB means below 3 µg/kg. However, wheat, barley and oats all had maximum concentrations between 500 and 1000 µg/kg, indicating that higher concentrations can occasionally occur in these cereals.

The non-cereal-based category samples contained predominantly undetectable or low concentrations of FB_1; this corresponded to results within the published literature. FB_1 was undetectable (LOQ: 30–67 µg/kg) in milk and dairy products and rarely detected in meat and meat products (occurrence 2.6% [LOQ: 5–33 µg/kg] and LB mean 3.1 µg/kg), indicating that transfer into animal products is negligible. This is in agreement with the previous assessment of the Committee at its seventy-fourth meeting in 2011 (Annex 1, reference *206*). Consequently, an evaluation of the occurrence of fumonisins in feed was not conducted at the current meeting.

Overall, the estimated global means from the GEMS/Food contaminants database reported here are lower than those in the Committee's previous assessment in 2011 (Annex 1, reference *206*). For example, the global FB_1 LB mean reported in this evaluation for maize is 310 µg/kg, compared with 1237 µg/kg in 2011. It is not possible to directly compare the datasets, as there is evidence that differences observed are largely due to a major shift in the geographical distribution of reported samples.

Fumonisins exist in various forms within food, such as hydrolysed and matrix-bound forms. The partially and fully hydrolysed forms of fumonisins are usually present as a low proportion of the parent fumonisins. Fumonisins can also be non-covalently bound with proteins and complex carbohydrates. Previous studies have shown that the level of bound fumonisins is usually higher than the level of the free forms, with one study reporting that the ratio of bound to free fumonisins for maize products varied from 0.06 to 25, with a mean ratio of 3 (Bryła et al., 2016). There are limited data on the occurrence of bound fumonisins in different cereals, the impact of processing on these bound mycotoxins and their bioavailability after consumption.

6.6 Food consumption and dietary exposure estimates

The Committee evaluated the chronic dietary exposure to FB_1 and total fumonisins ($FB_1 + FB_2 + FB_3$). For this, it reviewed a number of national evaluations of dietary exposure to fumonisins that have been published since its last evaluation of fumonisins at its seventy-fourth meeting in 2011. The Committee also estimated the dietary exposure to fumonisins based on occurrence data submitted to the GEMS/Food contaminants database combined with food consumption data

from either the GEMS/Food cluster diets (international estimates) or the FAO/WHO CIFOCOss database (national estimates).

The Committee considered national evaluations performed by Brazil, China (including Hong Kong SAR), France, Guatemala, Japan, Malawi, the Netherlands, Portugal, Republic of Korea, Spain, United Republic of Tanzania, Viet Nam and Zimbabwe. In these studies, the mean exposure to FB_1 and total fumonisins in European countries was generally below 250 ng/kg bw per day. High exposures to FB_1 were reported for Zimbabwe (Hove et al., 2016) and China (Sun et al., 2011), with a maximum of 7700 ng/kg bw per day for adults living in the rural province of Huaian. For total fumonisins, the highest mean exposures were reported in Malawi, ranging from 3000 to 15 000 ng/kg bw per day (Matumba et al., 2015). No mean concentrations of total fumonisins were reported in the Malawi study. Based on the per capita consumption of 382 g of maize and an adult body weight of 60 kg used in the study to calculate the exposure, the Committee calculated a mean concentration of 2400 µg/kg to obtain the highest reported exposure level of 15 000 ng/kg bw per day. This concentration is a factor of 6 higher than the highest UB mean concentration reported in the occurrence data for maize from the GEMS/Food contaminants database. The authors observed that if maize was considered to be consumed after dehulling, the dietary exposure would drop by a factor of 3 or even greater (Matumba et al., 2015). Owing to the large differences in foods included in the published assessments, ranging from only one food (e.g. only maize or coffee) to the whole diet, and differences in methodologies used to assess the exposure (e.g. per capita consumption or individual food consumption data), it is not possible to compare the exposures between the studies.

The Committee subsequently estimated national exposures to FB_1 and total fumonisins from all food sources using national food consumption data available from the CIFOCOss database combined with FB_1 and total fumonisins data submitted to the GEMS/Food contaminants database from January 2011 until August 2016. WHO region–specific LB and UB mean concentrations were calculated per food (group). To map the concentration data to the foods recorded in the CIFOCOss database, the countries present in the CIFOCOss database were grouped according to WHO region. Exposures were corrected for individual body weights. To assess potential high exposure, the mean exposures were multiplied by a factor of 2. This factor approximates the 90th percentile of exposure (FAO/WHO, 2009).

The highest national exposures to FB_1 and total fumonisins were observed in the youngest age groups. In the LB scenario, the highest mean exposure to FB_1 and total fumonisins was observed in the group "other children" (3–9 years) from Greece, at 800 and 1200 ng/kg bw per day, respectively. The UB mean exposure estimate for FB_1 was highest in toddlers (1–2 years) from Italy, at 2000 ng/kg

bw per day, whereas the UB mean exposure estimate for total fumonisins was highest in toddlers from Germany, at 3200 ng/kg bw per day (Table 20). For the high national exposure estimates for children and adults, see Table 20. Major contributors to the LB mean exposures to both FB_1 and total fumonisins were the food groups "cakes, cookies and pies", "cereal-based composite foods" and "cereal grains (non-specified)". Focusing only on infants and toddlers, the food group "foods for infants and small children" was also a relevant contributor to the LB mean exposure.

The Committee also calculated international exposure estimates for FB_1 and total fumonisins from all dietary sources using the same occurrence data mapped to food consumption data of the 17 GEMS/Food cluster diets. For this, LB and UB mean fumonisin concentrations per food (group) were calculated per cluster and across all clusters. The international LB mean exposure estimates for FB_1 and total fumonisins ranged from 2 ng/kg bw per day (cluster G09; mainly East Asian countries) to 560 ng/kg bw per day (cluster G05: mainly South and Central America) and from 13 ng/kg bw per day (cluster G09) to 820 ng/kg bw per day (cluster G05), respectively (Table 20). The highest UB mean exposure estimates for FB_1 and total fumonisins were observed in cluster G15 (European countries): 1200 and 2100 ng/kg bw per day, respectively. The high exposures, estimated by multiplying the mean exposures by a factor of 2, are listed in Table 20. In the LB scenario, maize contributed more than 60% to the international exposure to FB_1 and total fumonisins in all clusters except two, owing to a low concentration in maize (cluster G09) or a low consumption of maize (cluster G11; Belgium and the Netherlands). In this last cluster, wheat contributed 95% of the fumonisin exposure. In the UB scenario, wheat was also an important source of exposure in additional clusters.

The national and international exposures estimated by the Committee according to the UB scenario should be interpreted with care. Of the data considered in the exposure assessment for FB_1, 74% (n = 18 157) of the samples were reported to contain FB_1 below the LOD or LOQ. Because of this, the UB exposure estimates may be considered as worst-case estimates based on the data available to the Committee for the exposure assessment. Also, the high LOQs reported in the database contributed to this overestimation. For France and the Netherlands, recent national exposure estimates were available from the public literature covering a wide range of dietary sources (Sirot, Fremy & Leblanc, 2013; Sprong et al., 2016a), which used (partly) the same underlying consumption data from the CIFOCOss database. These national estimates were considerably lower than those estimated by the Committee in the LB scenario. The reported LB mean FB_1 exposure in children from France was 15.5 ng/kg bw per day, compared with 30–240 ng/kg bw per day estimated by the Committee. For the Netherlands, the corresponding estimates in children were 0 and 150–180 ng/kg

Table 20

Summary of the mean and high (90th percentile) national and international estimates of chronic dietary exposure to FB_1 and total fumonisins (FB_1 + FB_2 + FB_3) calculated by the Committee

Type of estimation and population[a]	Exposure (ng/kg bw per day)							
	FB_1				Total fumonisins			
	Mean		High		Mean		High	
	LB[b]	UB[b]	LB	UB	LB	UB	LB	UB
National estimates								
Children	0–800	180–2 000	0–1 600	360–3 900	0–1 200	270–3 200	0–2 300	530–6 400
Adults	17–470	140–910	34–950	280–1 800	22–640	210–1 300	45–1 300	420–2 500
International estimates								
Adults	2–560	300–1 200	5–1 100	610–2 300	13–820	440–2 100	25–1 600	880–4 300

bw: body weight

[a] For the purpose of the summary table, "children" were taken to be any population group described as infants, toddlers, children or adolescents. "Adults" were taken to be any population group described as adults (18–64 years), elderly adults (65–74 years) or very elderly adults (>75 years).

[b] The LB estimates were derived by substituting zero for analytical results below the LOD or LOQ when calculating mean concentration values. The UB estimate was derived by substituting the value of the LOQ for analytical results below the LOD or LOQ.

bw per day, respectively. For total fumonisins, the estimated LB mean exposure in Dutch children was 0 ng/kg bw per day in the national study, compared with 220–250 ng/kg bw per day as calculated by the Committee. In the French study, no exposure estimates for total fumonisins were reported (Sirot, Fremy & Leblanc, 2013). These differences in exposure were due to higher levels of fumonisins in the GEMS/Food contaminants database compared with those used in the national studies. Furthermore, foods as recorded in the food consumption databases were analysed for fumonisins in these studies. This allowed for a more precise mapping of foods consumed to those analysed than was possible with the data available to the Committee. These foods were also, if relevant, prepared as consumed before analysis. The effect of processing on the levels of fumonisins in food was reviewed during the seventy-fourth meeting of the Committee (Annex 1, reference *206*). The Committee concluded that thermal heating may result in a reduction of fumonisin levels in heated food. However, the Committee also observed that the results from the different studies were variable and that further studies were required to determine the fate of fumonisins and their reactions in heated food. In contrast, the data in the two national studies were collected during a limited period of time: June 2007 to January 2009 in the French study (Sirot et al., 2009) and the autumn/winter period of 2013 in the Dutch study (Sprong et al., 2016b). Mycotoxin levels have a high seasonal and annual variation, as they are highly dependent on climatic conditions. It is therefore uncertain how well the national estimates represent the exposure to fumonisins over a longer period of time. In

the assessment of the Committee, a more extended period of data sampling was covered, including the years 2000–2014 for the WHO European Region.

At the seventy-fourth meeting of the Committee in 2011, the exposure to FB_1 and total fumonisins was also estimated using the GEMS/Food cluster diets combined with concentration data from the GEMS/Food contaminants database (Annex 1, reference *206*). The LB mean estimated exposures to FB_1 ranged from 100 to 6100 ng/kg bw per day. Corresponding estimates for total fumonisins were 200–8200 ng/kg bw per day. The current exposure estimations tended to be lower: 2–560 ng/kg bw per day for FB_1 and 13–820 ng/kg bw per day for total fumonisins (Table 20), despite the fact that processed foods were also included in the current assessment. In both assessments, maize contributed the most to the exposure. Comparing the mean FB_1 levels in this food showed that the levels used in the present assessment were lower than those used in 2011: 270 µg/kg (average of maize, maize flour and maize meal) compared with 1237 µg/kg. For total fumonisins, the levels were 360 and 1651 µg/kg, respectively. In the present assessment, the maize samples used from the GEMS/Food contaminants database were from Brazil, Canada, China (including Hong Kong SAR), Japan, Republic of Korea, Singapore, the USA and 19 countries belonging to the WHO European Region. No maize data were available from countries belonging to the African, Eastern Mediterranean or South-East Asia WHO regions. In 2011, very high levels of FB_1 and total fumonisins were reported for the regional clusters A and G: LB mean FB_1 levels were 4322 and 2971 µg/kg, and LB mean total fumonisin levels were 5921 and 4071 µg/kg, respectively. Countries belonging to these regional clusters belonged to the African (cluster A) and the South-East Asia (cluster G) WHO regions. It can therefore not be ruled out that owing to the absence of information on FB_1 and total fumonisin levels in maize from countries belonging to these two regions, some national exposures may have been underestimated, as well as the current international estimates for the clusters that represent these regions, such as G01, G03, G04 and G13. In 2014, as a result of the fumonisin exposure assessment of the Committee at its seventy-fourth meeting, maximum levels (MLs) of fumonisins (FB_1 + FB_2) in maize and maize flour/meal were set by the Codex Alimentarius Commission at 4000 and 2000 µg/kg, respectively. It is not possible with the current dataset to determine whether these MLs have already resulted in a decrease in fumonisin levels and thus contributed to the lower exposure estimates. At the 2011 meeting, no exposure to FB_1 and total fumonisins was estimated based on national food consumption data as available in the CIFOCOss database.

The Committee concluded, based on the calculated national and international exposure estimates (Table 20), that the LB mean and high (90th percentile) chronic FB_1 exposures in adults were maximally 0.56 and 1.1 µg/kg bw per day, respectively. For total fumonisins, the corresponding exposure estimates

were 0.82 and 1.6 μg/kg bw per day (Table 20). Given the uncertainty regarding the large percentage (around 70%) of samples with a fumonisin level below the LOD or LOQ (so-called "non-detect" samples), the UB mean and high exposures were estimated to be as high as 1.2 and 2.3 μg/kg bw per day for FB_1 and 2.1 and 4.3 μg/kg bw per day for total fumonisins, respectively. In children, the LB mean and high chronic FB_1 exposures were maximally 0.8 and 1.6 μg/kg bw per day, and for total fumonisins, maximally 1.2 and 2.3 μg/kg bw per day, respectively. In this population group, the UB mean and high exposures were estimated to be as high as 1.6 and 3.9 μg/kg bw per day for FB_1 and 3.2 and 6.4 μg/kg bw per day for total fumonisins, respectively. Because of the absence of information on fumonisin levels in maize of countries belonging to the African, Eastern Mediterranean and South-East Asia WHO regions in the current assessment, the national and international exposures related to these regions may have been underestimated. Maize is the predominant source of LB exposure to FB_1 and total fumonisins in most cluster diets. In the UB scenario, wheat was also an important contributor to the exposure to fumonisins in some clusters.

6.7 Dose–response analysis

The Committee reviewed the previous dose–response analysis in light of the updated Bondy et al. (2012) study and confirmed the previous analysis.

7. Evaluation

The Committee reaffirmed the conclusions of the seventy-fourth meeting that fumonisins are associated with a wide range of toxic effects, and the liver and kidney are the most sensitive target organs. The Committee reviewed the studies that have become available since the 2011 evaluation and concluded that the 2010 study by Bondy et al. (2010), subsequently updated in 2012, remained the most relevant for the evaluation. The Committee evaluated the updated Bondy et al. (2012) data and concluded that they would not change the overall toxicological assessment performed previously by the Committee. Thus, the established group PMTDI of 2 μg/kg bw for FB_1, FB_2 and FB_3, alone or in combination, was retained by the current Committee.

The Committee noted the paucity of new data on the occurrence of fumonisins in food submitted to the GEMS/Food contaminants database since 2011 by all WHO regions except the European Region, as opposed to the data used in the previous evaluation (2011). Owing to these differences in the datasets between 2011 and the current evaluation, a direct comparison was not possible.

The Committee noted that there are limited data on the occurrence of bound fumonisins in different cereals, the impact of processing on these bound mycotoxins and their bioavailability after consumption.

LB mean and high (90th percentile) chronic FB_1 exposures in adults were maximally 0.56 and 1.1 µg/kg bw per day, respectively. For total fumonisins, the corresponding exposure estimates were 0.82 and 1.6 µg/kg bw per day. The UB mean and high exposures were estimated to be as high as 1.2 and 2.3 µg/kg bw per day for FB_1, respectively, and as high as 2.1 and 4.3 µg/kg bw per day for total fumonisins, respectively. In children, the LB mean and high chronic FB_1 exposures were maximally 0.8 and 1.6 µg/kg bw per day, respectively, and for total fumonisins, maximally 1.2 and 2.3 µg/kg bw per day, respectively. In this population group, the UB mean and high exposures were estimated to be as high as 1.6 and 3.9 µg/kg bw per day for FB_1, respectively, and as high as 3.2 and 6.4 µg/kg bw per day for total fumonisins, respectively. Maize is the predominant source of LB exposure to FB_1 and total fumonisins in most cluster diets. In the UB scenario, wheat was also an important contributor to the exposure to fumonisins in some clusters.

Comparison of the estimates of exposure to FB_1 and total fumonisins with the group PMTDI indicates that the group PMTDI was not exceeded at the LB mean exposure level in both children and adults. Assuming that all non-detect samples contained fumonisins at the LOQ, the UB mean exposure to total fumonisins in children exceeded the PMTDI in several countries. This was also true for the high (90th percentile) exposure, independent of the fumonisin concentration assigned to the non-detect samples. For adults, only the UB high exposure exceeded the PMTDI. The Committee noted that, owing to the high percentage of non-detect samples in the concentration database (around 70%) and the wide range of LOQs reported in the GEMS/Food contaminants database for fumonisins, the UB estimates may be interpreted as a worst-case estimate of exposure based on the data available.

The Committee noted that the international exposure estimates for FB_1 and total fumonisins were lower than those estimated by the Committee at its seventy-fourth meeting in 2011. In the current assessment, a larger part of the occurrence data was from countries belonging to the WHO European Region compared with 2011, resulting in lower overall fumonisin levels in maize. In the current assessment, no information on fumonisin levels in maize was available from countries belonging to the African, Eastern Mediterranean or South-East Asia WHO regions, where higher fumonisin concentrations are typically detected. Given these limitations of the occurrence data used in the exposure assessment and high exposures reported in the literature in some countries, it is likely that the exposures to fumonisins in areas where maize is a staple food and high contamination with fumonisins can occur are higher than those estimated

by the Committee at this meeting, as can be seen in the previous evaluation, which was based on a larger and more representative dataset.

Co-exposure of fumonisins with aflatoxins is covered separately.

7.1 Recommendations

The Committee noted the need for data on FB_1 in breast milk using analytical methods with appropriate specificity and sensitivity in order to further evaluate this potential exposure route.

The Committee recommended that exposure to fumonisin be reduced, particularly in areas where maize is the major dietary staple food and where high contamination can occur.

The Committee advises the development of surveillance programmes for regions for which little current information on occurrence of fumonisins in the GEMS/Food contaminants database exists, carefully considering the impact of these programmes on food security. The Committee recommended that these countries be encouraged to submit fumonisin concentration data to the GEMS/Food contaminants database.

The Committee recommended that countries be encouraged to analyse fumonisins in food samples using analytical methods with appropriate sensitivity to reduce the uncertainty in the exposure assessment, especially for maize and wheat.

The Committee recommends that additional studies be conducted to better understand the occurrence of bound fumonisins in different cereals, the impact of processing on these bound mycotoxins and their bioavailability after consumption.

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Glycidyl esters

First draft prepared by
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1. Explanation 577
2. Biological data 578
2.1 Biochemical aspects of glycidyl esters 578
2.2 Biochemical aspects of glycidol 582
2.2.1 Absorption, distribution and excretion 582
2.3 Toxicological studies of glycidyl esters 584
2.3.1 Genotoxicity (glycidyl esters and comparison to glycidol) 584
2.4 Toxicological studies of glycidol 585
2.4.1 Acute toxicity 585
2.4.2 Short-term studies of toxicity 585
(a) Mice 585
(b) Rats 587
2.4.3 Long-term studies of toxicity and carcinogenicity 588
(a) Mice 588
(b) Rats 590
(c) Hamsters 592
2.4.4 Genotoxicity 592
(a) Glycidol, in vitro 592
(b) Glycidol, in vivo 595
2.4.5 Reproductive and developmental toxicity 595
(a) Reproductive toxicity 595
(b) Developmental toxicity 597
2.4.6 Special studies 598
(a) Covalent binding to nucleic acids and/or proteins 598
(b) Immunotoxicity 599
(c) Neurotoxicity 599

2.5 Observations in domestic animals/veterinary toxicology 600
2.6 Observations in humans 600
2.6.1 Biomarkers of exposure 601
3. Analytical methods 601
3.1 Chemistry 601
3.1.1 Formation of glycidyl esters 602
3.2 Description of analytical methods 603
3.2.1 Introduction 603
3.2.2 Indirect methods 604
3.2.3 Direct methods 606
3.2.4 Reference methods 607
3.2.5 Quality assurance considerations 608
4. Sampling protocols 609
5. Effects of processing 609
5.1 Introduction 609
5.2 Degumming/washing 610
5.3 Neutralization 611
5.4 Bleaching 611
5.5 Deodorization 611
5.6 Effect of frying 611
6. Prevention and control 612
6.1 Preharvest control 612
6.1.1 Harvest and storage conditions 612
6.1.2 Reduction of precursors – Optimization of refining processes 613
6.1.3 New deodorization conditions 613
6.1.4 Utilization of adsorption materials 613
7. Levels and patterns of contamination in food commodities 614
8. Food consumption and dietary exposure estimates 616
8.1 Introduction 616
8.2 Methods for evaluating dietary exposures to glycidyl esters 616
8.2.1 Data sources 616
(a) Concentration data 616
(b) Consumption data 617
(c) Literature review 617
8.2.2 Overview of methods for the glycidyl ester/glycidol dietary exposure assessment 618
8.2.3 Data in the GEMS/Food contaminants database 619
8.2.4 National assessments of dietary exposure using concentration data from the GEMS/Food contaminants database 622
(a) Concentration data from Japan 622
(b) Concentration data from the USA 623
8.2.5 International assessments of dietary exposure using concentration data from the GEMS/Food contaminants database 623
8.2.6 Assessment for infants fed infant formula or follow-on formula 626
(a) Concentration data 626
(b) Methods for estimating dietary exposure to glycidol in infant formula 628
8.3 Assessments of dietary exposure 630
8.3.1 National estimates 630

(a) Japan 630
(b) USA 631
(c) Germany 632
(d) Europe 632
8.3.2 International exposure estimates 633
8.3.3 Estimated dietary exposures for infants 634
8.4 Limitations and uncertainties in the dietary exposure assessment 635
9. Dose–response analysis and estimation of toxic/carcinogenic risk 637
9.1 Identification of key data for risk assessment 637
9.1.1 Pivotal data from biochemical and toxicological studies 637
9.2 General modelling considerations 637
9.2.1 Selection of data 637
9.2.2 Measure of exposure 637
9.2.3 Measure of response 638
9.2.4 Selection of mathematical model 638
10. Comments 641
10.1 Biochemical aspects 641
10.1.1 Glycidyl esters 641
10.1.2 Glycidol 642
10.2 Toxicological studies 643
10.2.1 Glycidyl esters 643
10.2.2 Glycidol 643
10.3 Observations in humans 645
10.4 Analytical methods 645
10.5 Sampling protocols 647
10.6 Effects of processing 647
10.7 Prevention and control 648
10.8 Levels and patterns of contamination in food commodities 649
10.9 Food consumption and dietary exposure assessment 650
10.10 Dose–response analysis 651
11. Evaluation 652
11.1 Recommendations 653
12. References 654
Appendix 1 663

1. Explanation

Glycidyl esters are processing-induced contaminants primarily found in refined fats and oils and foods containing fats and oils. Initial research related to glycidyl esters was largely performed as part of the investigation into 3-monochloro-1,2-propanediol (3-MCPD) esters. During MCPD ester analysis, variable MCPD concentrations were obtained, leading to a proposal that additional compounds were present in edible oils and converted to 3-MCPD during sample analysis (Weisshaar & Perz, 2010). The presence of additional processing-induced

contaminants, glycidyl esters, in refined edible oils was later confirmed. Initially, it was assumed that 3-MCPD esters and glycidyl esters were formed by similar processes, but it is now known that their mechanisms of formation are different, with glycidyl ester formation directly associated with elevated temperatures (>240 °C) and time at these elevated temperatures (Destaillats et al., 2012a). Glycidyl esters are generally formed from diacylglycerols, with no requirement for the presence of chlorinated compounds. Formation of glycidyl esters occurs following intramolecular rearrangement, elimination of a fatty acid and epoxide formation (Fig. 1).

Glycidyl esters have not been evaluated previously by the Committee. The present evaluation was conducted in response to a request from the Codex Committee on Contaminants in Foods for a full evaluation of glycidyl esters.

A comprehensive search of peer-reviewed literature was conducted in PubMed (toxicological) and Scopus (occurrence) for glycidyl esters and glycidol, taking the recent opinion by the European Food Safety Authority (EFSA) (2016) into consideration, as well as secondary literature (reports and reviews). Only recent occurrence data (2012–2016) were evaluated, as there has been considerable improvement in the analysis of glycidyl esters and because changes in oil processing have led to a decrease in the levels of glycidyl esters in the finished oils. Data generated prior to this date were considered less reliable and few in number.

The International Agency for Research on Cancer (IARC) classified glycidol as probably being carcinogenic to humans (Group 2A; IARC, 2000), and glycidyl oleate and glycidyl stearate as not classifiable as to their carcinogenicity in humans (Group 3; IARC, 1987; Bakhiya et al., 2011; Habermeyer, Guth & Eisenbrand, 2011; MacMahon, 2016).

2. Biological data

2.1 Biochemical aspects of glycidyl esters

Seven different glycidyl esters (glycidyl laurate, myristate, palmitate, stearate, oleate, linoleate and linolenate) were shown to be rapidly (within 15 minutes) and fully degraded by lipase AP12 (from *Aspergillus niger*) in a static in vitro system with gastric electrolyte solution at pH 4.8, with a strong influence of pH on lipase activity. In the absence of lipase, glycidyl esters were relatively stable. In a dynamic model (TNO gastrointestinal model, TIM) simulating the different gastrointestinal compartments, taking into account different physiological conditions such as pH values, enzymes and peristaltic movement, the efficient

Fig. 1
Glycidyl monoester with examples of fatty acid chains: i) lauric acid, ii) stearic acid and iii) linoleic acid

Examples of possible R groups (fatty acid chains)

degradation of the seven different glycidyl esters was confirmed using milk as a food matrix. The major hydrolysis product for deuterated glycidyl oleate and glycidyl linoleate was glycidol (92%). The chain length of the fatty acids (12–18 carbons) did not significantly impact the kinetics. With regard to possible metabolites, only small amounts of glycerol were detected in strongly acidic conditions, whereas neither 2- nor 3-MCPD was detected in any scenario. The authors concluded that glycidyl esters are rapidly digested by gut lipases to form glycidol (Frank et al., 2013).

Gut content samples from stomach, duodenum or caecum of non-treated F344 gpt delta rats (triplicates from nine males) were incubated with glycidol, glycidyl oleate or glycidyl linoleate (20 µmol/mL) for 30 minutes at 37 °C. Mainly unchanged parent compounds were recovered in all the gut content samples. When the samples were incubated with glycidyl esters, small amounts of glycidol were recovered. 3-MCPD concentrations were mainly below the lower limit of quantification (LOQ) of 0.45 nmol/mL (Onami et al., 2015).

Male Wistar rats (n = 8; 8–9 weeks old) received radiolabelled [2-^{14}C]-glycidyl palmitate (1.2 MBq) and glycidyl-[9,10-^{3}H]palmitate (4.9 MBq),

administered together by gavage to result in a "virtual double tag" of a single dose of 0.67 mmol/kg body weight (209.4 mg/kg body weight [bw], corresponding to 50 mg/kg bw of the glycidol moiety). Blood kinetics over 24 hours, excretion after 7 days and tissue distribution after 24 hours and 7 days (n = 4) were investigated. The activities of ^{14}C and ^{3}H in plasma were at a maximum 2 hours after dosing (approximately 1.5% of the dose), while activities in erythrocytes remained relatively stable over 24 hours (maximum 2% of the dose). Seven days after dosing, 41.3% of the ^{14}C had been excreted in urine, 32.9% in expired air and 21.6% in faeces; 9.1% remained in tissues and organs. The corresponding excretion rates for the ^{3}H radiolabel were 50.8% in faeces, 20.5% in expired air and 7.7% in urine; 23.3% remained in tissues and organs. For either radiolabel, highest concentrations were seen in liver, skeletal muscle, bone and erythrocytes (^{3}H radiolabel was also seen in plasma) at 24 hours and 7 days after administration. It was not determined if the tissue retention associated with the radiolabels was related to covalent binding or metabolic processes (Appel et al., 2013).

In a comparative kinetics study, male Wistar rats (n = 16; 8–9 weeks old) received a single equimolar dose of 0.67 mmol/kg bw of glycidol (50 mg/kg bw; 96% pure) or glycidyl palmitate (209.4 mg/kg bw; 98% pure) in corn oil by gavage. Two control animals received the vehicle (corn oil). Concentrations of the haemoglobin adduct *N*-(1,2-dihydroxypropyl)valine (diHOPrVal) in blood reached the same steady-state level with both substances. However, this level was reached approximately 4–8 hours later with glycidyl palmitate than with glycidol. Concentrations of 2,3-dihydroxypropyl mercapturic acid (DHPMA) excreted in urine were also similar for both substances at the three sampling time points (0–8, 8–24 and 24–48 hours), reaching a recovery level of approximately 14% of the dose as DHPMA 48 hours after administration. Based on the measurements of these two biomarkers, the authors concluded that glycidyl palmitate was rapidly and efficiently hydrolysed to glycidol (Appel et al., 2013).

Fasted male F344 rats (n = 3; 172–218 g; 9 weeks old) received a single dose of vehicle or of glycidol, glycidyl oleate or glycidyl linoleate (all reported as 98% pure) in 0.03% Tween 80 at equimolar doses of 510 µmol/kg bw (37.7 mg/kg bw for glycidol) by gavage. In all treatment groups, 3-MCPD was detected in the serum at the only tested time point of 30 minutes after administration. In the glycidol treatment group, the mean serum concentration of 3-MCPD was 88 nmol/mL and of glycidol was 22 nmol/mL. In the glycidyl ester treatment groups, the corresponding serum levels were lower: 3-MCPD at 1.4–11 nmol/mL and glycidol at less than 2.7 nmol/mL (below the lower LOQ). Neither glycidyl ester was detectable in the serum (lower LOQ of 0.06 nmol/mL) (Onami et al., 2015).

This study shows that glycidol and the tested glycidyl esters are metabolized to 3-MCPD in male rats. However, with only one sampling time

point and without any information on the characteristics of the area under the concentration–time curve (AUC), no quantitative comparisons could be made.

Non-fasted male Crl:CD(SD) rats (n = 189; 197–249 g; 7 weeks old) and non-fasted male cynomolgus monkeys (n = 18; 3.49–5.69 kg; 6 years old) were administered a single gavage or intravenous dose of glycidol (75 mg/kg bw dissolved in olive oil; 100% purity) or a single equimolar gavage dose of glycidyl linoleate (341 mg/kg bw dissolved in water; 96.3–96.7% purity). Glycidol and glycidyl linoleate concentrations in plasma were monitored for 24 hours in rats and 96 hours in monkeys. Three animals were used for each time point.

Glycidyl linoleate was nondetectable in either species at any sampling time point (below the lower LOQ of 5 ng/mL); in contrast, glycidol was detected in rats and monkeys following administration of either glycidol or glycidyl linoleate. In rats, maximum glycidol concentrations in plasma were reached 15–30 minutes after oral dosing and were nondetectable (below the LOQ of 0.2 µg/mL) after 24 hours for both substances (Table 1). Similar blood kinetics for glycidol were observed for the administered glycidyl linoleate compared with administered glycidol in the rat.

Following oral administration of glycidol or glycidyl linoleate in monkeys, glycidol also peaked quickly (after about 50–90 minutes) in plasma and declined within 24 hours to below the limit of detection (LOD). However, the AUC for glycidol in plasma was only 56% following oral administration of glycidyl linoleate versus glycidol. Times to reach maximum concentrations (T_{max}) in blood were 4 times longer in cynomolgus monkeys than in rats. Maximum concentrations (C_{max}) after oral dosing with glycidol and glycidyl linoleate were also significantly reduced for cynomolgus monkeys compared with rats (factor 4 for glycidol and factor 17 for glycidyl linoleate). Although only a small number of monkeys was available at each sampling time point (n = 3) and only free glycidol (not bound, e.g. to haemoglobin) was measured in blood, the authors suggested that the pharmacokinetic differences between rats and monkeys might be attributable to differences in lingual or gastric lipase activity, stomach pH or epoxide metabolism.

In the same study, C_{max} and AUC were also reported for lower orally administered doses (single dose of glycidol at 0.492, 1.64 or 4.92 mg/kg bw and equimolar glycidyl linoleate at 2.24, 7.46 or 22.4 mg/kg bw). In rats, glycidol was detected in plasma following a single oral dose of 1.64 or 4.92 mg/kg bw glycidol or equimolar glycidyl linoleate (7.46 or 22.4 mg/kg bw), but not at the lowest tested dose level (LOQ of 0.2 µg/mL). In monkeys, glycidol was only detected in plasma following oral administration of 4.92 mg/kg bw glycidol and not at lower doses or at either dose level of glycidyl linoleate (Wakabayashi et al., 2012).

Table 1
Toxicokinetic parameters of glycidol in plasma after a single oral (gavage) or intravenous dose of glycidol or equimolar glycidyl linoleate to male rats or male monkeys

Species	Compound	Dose (mg/kg bw)	Route	C_0 (µg/mL)	C_{max} (µg/mL)	T_{max} (h)	$t_{½\ initial}$ (h)	AUC (h·µg/mL) (%)[a]
Rat	Glycidol	75	Intravenous	100	nc	nc	0.367	47.1
	Glycidol	75	Oral	nc	33.6	0.25	1.28	32.4 (69%)
	Glycidyl linoleate	341	Oral	nc	26.0	0.5	1.51	41.6
Monkey	Glycidol	75	Intravenous	76.6	nc	nc	0.409	47.7
	Glycidol	75	Oral	nc	8.60	0.83	1.48	16.4 (34%)
	Glycidyl linoleate	341	Oral	nc	1.46	1.8	19.9	9.11

AUC: area under the concentration–time curve; bw: body weight; C_0: concentration at beginning; C_{max}: maximum concentration; nc: not calculated; $t_{½\ initial}$: initial plasma elimination half-life; T_{max}: time to reach C_{max}
[a] Systemic bioavailability of glycidol in plasma as a percentage, calculated as AUC of glycidol following administration by gavage compared with intravenous administration.
Source: Wakabayashi et al. (2012)

2.2 Biochemical aspects of glycidol

2.2.1 Absorption, distribution and excretion

Approximately 87–92% of orally administered [1,3-^{14}C]glycidol (155.4 × 10^6 Bq/mmol) was absorbed from the gastrointestinal tract of male Fischer 344 rats (n = 8–11; 190–229 g; 10–11 weeks old) administered a single dose of 37.5 or 75 mg/kg bw by gavage or intravenously. Similar disposition kinetics were observed for oral and intravenous dosing, with 40–48% of the radioactivity excreted in urine, 5–12% excreted in faeces and 26–32% exhaled; 7–8% was retained in tissues 72 hours after administration (9–12% 24 hours after administration). Highest concentrations of radioactivity were in blood cells, thyroid, liver, kidney and spleen. It was not determined if the tissue retention associated with the radiolabel was related to covalent binding or metabolic processes. Glycidol was extensively metabolized, as indicated by the metabolite profile in pooled urine. This metabolite profile was similar for both doses and routes and revealed 15 different metabolites: one major metabolite (14–21% of the dose), four lesser metabolites (2–8% each) and 10 minor metabolites (≤1% each). However, the structures were not further identified. Only the occurrence of β-chlorolactic acid in urine was specifically investigated because it is the oxidative metabolite of α-chlorohydrin (3-MCPD) assumed to be formed from glycidol and gastric HCl. Only 0.02% of the pooled urinary radioactivity co-eluted with β-chlorolactic acid following gavage or intravenous administration (Nomeir et al., 1995).

Absolute systemic bioavailability of glycidol was estimated as 69% in male Crl:CD(SD) rats and 34% in male cynomolgus monkeys (Table 1; see

Wakabayashi et al. (2012) for further information on kinetics of glycidol in section 2.1).

Glycidol was shown to be conjugated with glutathione and excreted in urine as *S*-(2,3-dihydroxypropyl)cysteine and DHPMA following intraperitoneal administration of glycidol to male ICI/Swiss mice (200 mg/kg bw per day; n = 5) and male Wistar rats (100 mg/kg bw per day; n = 3) for 10 days (Jones, 1975).

Urinary excretion of DHPMA in male Wistar rats was also observed following a single oral (gavage) dose of 50 mg/kg bw glycidol (Appel et al., 2013; section 2.1).

Hepatic glutathione was significantly decreased in male Wistar rats (up to 90% depletion compared with controls) at between 0.5 and 12 hours after a single administration of 500 µL/kg bw of glycidol by gavage (dose not stated; assuming undiluted glycidol of a density of 1.11 g/cm^3 converts the volume to a dose of 560 µg/kg bw). Hepatic glutathione depletion was significant for 150–750 µL/kg bw (i.e. 180–840 µg/kg bw) 2 hours after administration. Levels returned to control values after 24 hours (Montaldo, Dore & Congiu, 1984).

In vitro investigations with [U-^{14}C]glycidol incubated with rat liver supernatant for 3 hours resulted in the formation of *S*-(2,3-dihydroxy[U-^{14}C]-propyl)glutathione (50–60% of the radioactivity) and [U-^{14}C]glycerol (30–35% of the radioactivity), indicating that glycidol is detoxified in rats by conjugation with glutathione and by hydrolysis to glycerol (Jones, 1975).

These findings were confirmed by Patel, Wood & Leibman (1980), who demonstrated hydrolysis of glycidol to glycerol in rat liver and lung microsome preparations, and conjugation of glycidol with glutathione as measured by glutathione consumption after glycidol addition to rat liver and lung cytosol fractions. The formation of glycerol from glycidol was catalysed by epoxide hydrolases (Patel, Wood & Leibman, 1980).

Glycidol hydrolyses spontaneously to glycerol under acidic conditions, and conjugates within a few hours with glutathione in a buffered solution at pH 7–8 (but not at pH 6) (Jones, 1975).

Due to its epoxide structure, glycidol has alkylating properties and reacts directly with cellular macromolecules like proteins or DNA (NTP, 1990). It is notable that glycidol is surprisingly stable in blood, urine, in vitro test solutions and food.

^{36}Cl-labelled β-chlorolactic acid was identified in the urine of male Wistar rats (n not stated) that received ^{36}Cl-labelled saline via intraperitoneal administration for 2 days prior to an oral dose of glycidol at 100 mg/kg bw. No other radiolabelled urinary metabolites were found (Jones & O'Brien, 1980).

The metabolic pathway proposed for glycidyl esters based on in vivo studies in rodents and monkeys and in vitro studies is shown in Fig. 2.

Fig. 2
Proposed metabolic pathway of glycidyl esters

ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; EH: epoxide hydrolase; GSH: glutathione; GST: glutathione *S*-transferase; HCl: hydrochloric acid; 3-MCPD: 3-chloropropane-1,2-diol
Dotted lines represent proposed pathways.
Source: Adapted from Jones (1975), Lynch et al. (1998), NTP (1990); cited in Appel et al. (2013)

2.3 Toxicological studies of glycidyl esters

No oral repeated-dose studies in rodents were identified for glycidyl esters.

2.3.1 Genotoxicity (glycidyl esters and comparison to glycidol)

In order to compare the genotoxic activity of glycidol (100% purity, in water) and glycidyl linoleate (96.7% purity, in dimethyl sulfoxide [DMSO]), a variety of genotoxicity end-points were tested following good laboratory practice (GLP) and Organisation for Economic Co-operation and Development (OECD) test guidelines. Results for glycidyl linoleate are tabulated in Table 2, and for glycidol in Table 6 and Table 7 (see below). Although glycidol was positive in vitro in all tested bacterial strains (*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA with and without metabolic activation), glycidyl linoleate only tested positive in TA100 and TA1535 with and without metabolic activation, and in WP2uvrA with metabolic activation. In the in vitro

Table 2
Results of glycidyl linoleate genotoxicity assays

End-point	Test system	Concentration[a]	Result: Without exogenous metabolic system	Result: With exogenous metabolic system	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA100	156 µg/plate	Positive	Positive 78 µg/plate	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA1535	10 µg/plate	Positive	Positive	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA98	156 µg/plate	Negative	Negative	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA1537	156 µg/plate	Negative	Negative	Ikeda et al. (2012)
Reverse mutation	*E. coli* WP2uvrA	313 µg/plate	Negative	Positive	Ikeda et al. (2012)
Chromosomal aberrations	Chinese hamster lung cells	3 400 µg/mL	Negative	Negative	Ikeda et al. (2012)
In vivo					
Micronucleus formation	ICR mice, bone marrow	1 000 mg/kg bw × 2 (gavage)	Negative[b]	–	Ikeda et al. (2012)

bw: body weight
[a] Lowest effective dose or highest ineffective dose.
[b] Signs of bone marrow toxicity were observed.

chromosomal aberration test with Chinese hamster lung cells, glycidol induced structural aberrations (mainly chromatid breaks and chromatid exchange) but no numerical aberrations, whereas glycidyl linoleate was negative for both end-points. When tested in vivo, neither substance induced micronuclei in the bone marrow of mice (Ikeda et al., 2012).

2.4 Toxicological studies of glycidol

2.4.1 Acute toxicity

The oral median lethal dose (LD_{50}) of glycidol was 450 mg/kg bw in mice and 420–850 mg/kg bw in rats. The intraperitoneal LD_{50} of glycidol was 200–350 mg/kg bw in rats (Table 3).

2.4.2 Short-term studies of toxicity

(a) Mice

Glycidol (94% purity; the main impurities were 2.8% diglycidyl ether, 1.2% 3-methoxy-1,2-propanediol, 1.1% 2,6-dimethanol-1,4-dioxane, 0.4% 3-MCPD and 0.1% methanol) in distilled water was administered by gavage to B6C3F1 mice for 16 days, 13 weeks or 2 years (NTP, 1990). The shorter studies focused on dose-range finding for the chronic carcinogenicity study.

Table 3
Acute toxicity of glycidol

Species	Sex	Group size	Route	LD_{50} (mg/kg bw)	Reference
Mouse (Long–Evans)	Male	5–6	Oral	450	Hine et al. (1956)
Rat (Webster)	Male	5–6	Oral	850	Hine et al. (1956)
Rat (SD)	Male	5	Oral	760	Thompson & Gibson (1984)
Rat (SD)	Female	10	Oral	420	Thompson & Hiles (1981)
Rat (SD)	Female	5	Oral	640	Thompson & Gibson (1984)
Rat (SD)	Male	5	i.p.	350	Thompson & Gibson (1984)
Rat (SD)	Female	10	i.p.	200	Thompson & Hiles (1981)
Rat (SD)	Female	5	i.p.	210	Thompson & Gibson (1984)

bw: body weight; i.p.: intraperitoneal; LD_{50}: median lethal dose; SD: Sprague Dawley

Glycidol (94% purity; for the main impurities see above) in distilled water was administered to B6C3F1 mice for 16 days (groups of five males and five females, 8 weeks old) by gavage at doses of 0, 37.5, 75, 150, 300 or 600 mg/kg bw per day for 5 days a week (corresponding to average daily doses of 0, 26.8, 53.6, 107.1, 214.3 and 428.6 mg/kg bw per day). Body weight was assessed 1 week before dosing and on days 8 and 16. All vehicle controls and surviving mice at 214.3 mg/kg bw per day (two males and three females) and 107.1 mg/kg bw per day (four males and three females) were necropsied. Various tissues of the necropsied mice were histologically examined. Sperm count/motility was not assessed; clinical parameters were probably also not assessed.

All the mice at 428.6 mg/kg bw per day and 40% at 214.3 mg/kg bw per day died by day 4 of the study (NTP, 1990). Focal demyelination in the medulla and thalamus of the brain occurred in all female mice at 214.3 mg/kg bw per day. Inactivity and ruffled hair coats were observed in about half the male and female mice at 214.3 and 428.6 mg/kg bw per day (NTP, 1990).

Glycidol (94% purity; for the main impurities see above) in distilled water was administered to B6C3F1 mice for 13 weeks (groups of 10 males and 10 females; 8 weeks old) by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw per day for 5 days a week (corresponding to an average daily dose of 0, 13.6, 29.5, 53.6, 107.1 or 214.3 mg/kg bw per day). Body weight was assessed weekly and all the animals were necropsied. Various tissues of animals at 0, 107.1 and 214.3 mg/kg bw per day, brain of animals at 53.6 mg/kg bw per day and testes of animals in all dose groups were histologically examined. At study end, sperm count and motility were analysed in five males in the 0, 13.6, 53.6 and 107.1 mg/kg bw per day groups. Clinical parameters were not reported.

All mice at 214.3 mg/kg bw per day died by the second week and 4/10 males and 3/10 females at 107.1 mg/kg bw per day died before the end of the

study. Renal tubular degeneration was observed in male mice at 214.3 mg/kg bw per day. Demyelination in the medulla and thalamus of the brain was significantly increased at 107.1 mg/kg bw per day in males and at 214.3 mg/kg bw per day in females. Sperm count was significantly decreased at 53.6 and 107.1 mg/kg bw per day (43% and 50% reduction compared with controls, respectively; no data were given for the 214.3 mg/kg bw per day group) along with reduced sperm motility (NTP, 1990).

(b) Rats

Glycidol (94% purity; for the main impurities, see section 2.4.2(a)) in distilled water was administered by gavage to F344/N rats for 16 days, 13 weeks or 2 years (NTP, 1990). The shorter studies focused on dose-range finding for the chronic carcinogenicity study.

Glycidol (94% purity; for the main impurities, see section 2.4.2(a)) in distilled water was administered by gavage to groups of five male and five female F344/N rats (7 weeks old) for 16 days at doses of 0, 37.5, 75, 150, 300 or 600 mg/kg bw per day for 5 days a week (corresponding to an average daily dose of 0, 26.8, 53.6, 107.1, 214.3 and 428.6 mg/kg bw per day). Body weight was assessed 1 week before dosing and on days 8 and 16, and all the rats were necropsied. Histological examinations of various tissues were performed on all rats at 0 and 214.3 mg/kg bw per day. No sperm count or motility was assessed and clinical parameters were not reported.

All the rats at 428.6 mg/kg bw per day died between days 3 and 13. Four of five males at 214.3 mg/kg bw per day developed oedema and degeneration of the epididymal stroma, whereas atrophy of the testis and granulomatous inflammation of the epididymis were seen in the fifth male rat (NTP, 1990).

Glycidol (94% purity; for the main impurities see section 2.4.2(a)) in distilled water was administered by gavage to groups of 10 male and 10 female F344/N rats (7 weeks old) for 13 weeks at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw per day for 5 days a week (corresponding to an average daily dose of 0, 17.9, 35.7, 71.4, 142.9 and 285.7 mg/kg bw per day). Body weight was assessed weekly. All the animals were necropsied. Histological examination of various tissues was performed in all rats at 0, 142.9 and 285.7 mg/kg bw per day as well as of the brain of animals at 71.4 mg/kg bw per day and the testes of animals at all doses. At study end, sperm count and motility were analysed in five males in the 0, 17.9, 71.4 and 142.9 mg/kg bw per day groups.

All the rats at 285.7 mg/kg bw per day died by week 2, and 3/10 males and 1/10 females at 142.9 mg/kg bw per day died before the end of the study. Sperm count and motility were significantly reduced in all dose groups. A 36% reduction in sperm count was already seen at the lowest tested dose of 17.9 mg/kg

bw per day. Testicular atrophy of moderate to marked severity occurred in 10/10 males at 142.9 mg/kg bw per day and 9/10 males at 285.7 mg/kg bw per day.

Renal tubular cell degeneration and/or necrosis increased significantly in males and females at 285.7 mg/kg bw per day (6/10 males and 10/10 females). Significantly increased necrosis of the granular cell layer of the cerebellum was seen in males at 285.7 mg/kg bw per day and in females at 142.9 and 285.7 mg/kg bw per day. Minimally severe demyelination in the medulla was seen at the two higher dose levels (not stated as statistically significant). Significant lymphoid necrosis of the thymus was reported in females at 285.7 mg/kg bw per day (NTP, 1990).

2.4.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In a 2-year carcinogenicity study, glycidol (94% purity; for main impurities see section 2.4.2(a)) in distilled water was administered by gavage to B6C3F1 mice (50/sex per group; 8 weeks old) at doses of 0, 25 or 50 mg/kg bw per day for 5 days a week (corresponding to an average daily dose of 0, 17.9 and 35.7 mg/kg bw per day) for 103 weeks. Body weight, clinical chemistry, necropsy and histology of various tissues in all animals were assessed (NTP, 1990).

Mortality was 46–50% in glycidol-dosed male mice (34% in controls) and 46–66% in glycidol-dosed female mice (42% in controls). Compared with controls, mortality was significantly increased after week 101 in female mice at 35.7 mg/kg bw per day. Mean body weight was decreased in females at 17.9 mg/kg bw per day after week 28 and at 35.7 mg/kg bw per day after week 56, whereas body weight in males was similar or increased compared with controls. No compound-related clinical signs were observed. The following significantly increased incidences of nonneoplastic lesions were seen: epithelial hyperplasia of the forestomach in highest-dose male and female mice; cysts of the preputial gland in highest-dose male mice; and kidney cysts in male mice at both doses.

Statistically significant increases in tumour incidences were seen in both sexes. Table 4 shows the site and incidence of neoplasms that the National Toxicology Program (NTP) considered substance-related based on statistical analyses (mainly incidental tumour test, Cochran–Armitage trend test, Fisher exact test and life table test) and historical control data from earlier NTP studies. NTP concluded that there was "clear evidence for carcinogenic activity" in male mice based on increased incidences of neoplasms of the Harderian gland, forestomach, skin, liver and lung and in female mice based on increased incidences of neoplasms of the Harderian gland, mammary gland, uterus, subcutaneous tissue and skin.

Table 4
Neoplasms in B6C3F1 mice associated with the 2-year gavage administration of glycidol

	No. and incidence of neoplasms per dose[a,b]					
	Males			Females		
Finding	0 mg/kg bw per day	17.9 mg/kg bw per day	35.7 mg/kg bw per day	0 mg/kg bw per day	17.9 mg/kg bw per day	35.7 mg/kg bw per day
Harderian gland[c] adenoma or adenocarcinoma	8/46 (17)[##]	12/41 (29)	22/44 (50)**	4/46 (9)[##]	11/43 (26)*	17/43 (40)**
Mammary gland adenoma, fibroadenoma or adenocarcinoma	NA	NA	NA	2/50 (4)[##]	6/50 (12)	15/50 (30)*
Forestomach squamous cell papilloma or carcinoma	1/50 (2)[##]	2/50 (4)	10/50 (20)**	3/50 (6)	1/50 (2)	4/50 (8)
Uterus carcinoma or adenocarcinoma	NA	NA	NA	0/50 (0)	3/50 (6)	3/50 (6)
Subcutaneous tissue sarcoma or fibrosarcoma	11/50 (22)[#d]	3/50 (6)*[d]	4/50 (8)*[d]	0/50 (0)[##]	3/50 (6)	9/50 (18)**
Skin squamous cell papilloma or carcinoma	0/50 (0)[#]	0/50 (0)	4/50 (8)	0/50 (0)	0/50 (0)	2/50 (4)
Liver adenoma or carcinoma	24/50 (48)[#]	31/50 (62)	35/50 (70)*	9/50 (18)	7/50 (14)	14/50 (28)
Lung alveolar/bronchiolar adenoma or carcinoma	13/50 (26)	11/50 (22)	21/50 (42)	6/50 (12)	10/50 (20)	8/50 (16)

bw: body weight; NA: not applicable/not available; No.: number; #: $P < 0.05$, ##: $P < 0.01$ (Cochran–Armitage trend test); *: $P < 0.05$, **: $P < 0.01$ (Fisher exact test)
[a] Tumour incidence expressed as the number of tumour-bearing animals/number of animals examined and, in parentheses, the incidence expressed as a percentage. Statistical analyses by Cochran–Armitage trend test, Fisher exact test, incidental tumour test and life table test. (For ease of readability, the significance levels of only the currently more commonly used Cochran–Armitage and Fisher exact tests are displayed.)
[b] 0, 25 and 50 mg/kg bw per day for 5 days a week were corrected for noncontinuous dosing.
[c] The denominators for the incidence of Harderian gland tumours are the actual number of Harderian glands that could be microscopically examined.
[d] Lower tumour incidence in the higher-dose group, i.e. a statistically significant negative finding.
Source: NTP (1990)

A no-observed-adverse-effect level (NOAEL) was not identified in this study.

The Committee noted the study limitations: only two dose levels of glycidol (and a control group) were tested.

NTP further evaluated the carcinogenicity of glycidol in a transgenic mouse model haploinsufficient for the $p16^{Ink4a}$ and $p19^{Arf}$ tumour suppressor genes. Groups of 15 male and 15 female $p16^{Ink4a}/p19^{Arf}$ mice received glycidol at doses of 0, 25, 50, 100 or 200 mg/kg bw in water by gavage 5 times per week for 40 weeks (corresponding to average daily doses of 0, 17.9, 35.7, 71.4 and 142.9 mg/kg bw per day when corrected for noncontinuous dosing). There was "clear evidence for carcinogenic activity" of glycidol in males based on significantly increased incidences of histiocytic sarcomas and alveolar/bronchiolar adenoma and "some evidence" in females based on alveolar/bronchiolar adenoma (NTP, 2007).

In contrast, glycidol was not carcinogenic in transgenic $p53^{+/-}$ mice after administration of 0, 25 or 50 mg/kg bw per day by gavage for 6 months (Tennant et al., 1999, cited in NTP, 2007).

In a poorly reported subcutaneous carcinogenicity study in mice, glycidyl esters (laureate, oleate and stearate, in tricaprylin; purity not stated) were injected into female BALB/c or Swiss-Webster mice as 1–2 injections per week (doses of 0.005–10 mg/animal) for a total of 26–104 injections and observed for up to 18 months. Subcutaneous sarcomas at the injection site were increased in the glycidyl ester groups compared with controls although high mortality and pulmonary and other tumours were also observed in controls (Swern et al., 1970).

Due to limitations in study design and reporting, the study was determined to be difficult to interpret. The Committee considered it not suitable as a pivotal study for the present evaluation.

(b) Rats

In a 2-year carcinogenicity study, glycidol (94% purity; for main impurities see section 2.4.2(a)) in distilled water was administered by gavage to F344/N rats (50/sex per group; 7 weeks old at study start) at doses of 0, 37.5 or 75 mg/kg bw per day for 5 days a week (corresponding to average daily doses of 0, 26.8 and 53.6 mg/kg bw per day). Body weight, clinical chemistry, necropsy and histology of various tissues were assessed for all animals.

Survival of males and females was significantly lower than controls (significant after weeks 75/84 for low-dose males/females and after weeks 60/64 for high-dose males/females). At study end, 16 males and 28 females in the control group were alive versus four females at 26.8 mg/kg bw per day and none in the higher-dose treatment group. Most early deaths in males were due to mesotheliomas in the tunica vaginalis that frequently metastasized to the peritoneum. Early deaths in females were mainly associated with mammary gland neoplasms.

Mean body weight was generally reduced in treated animals, and ranged from 80% to 95% of controls in treated males and from 90% to 97% of controls in treated females. No compound-related clinical signs were observed. Significantly increased incidences of hyperkeratosis and epithelial dysplasia of the forestomach and fibrosis of the spleen were seen in both sexes of both treatment groups.

Significantly increased tumour incidences were seen in various tissues at the lowest dose. Table 5 shows the site and incidence of neoplasms that NTP associated with glycidol treatment, which also takes into account the high mortality from statistical analyses and control data from previous NTP studies. NTP concluded that there was "clear evidence of carcinogenic activity" in male rats based on increased incidences of mesotheliomas of the tunica vaginalis, fibroadenomas of the mammary gland, gliomas of the brain and neoplasms of the forestomach, intestine, skin, Zymbal gland and thyroid gland. Incidences of interstitial cell tumours in testis (Leydig cell tumours) were high (94–100%) in

Table 5
Neoplasms in F344/N rats associated with the 2-year gavage administration of glycidol

	No. and incidence of neoplasms per concentration of glycidol[a,b]					
	Males			Females		
Finding	0 mg/kg bw per day	26.8 mg/kg bw per day	53.6 mg/kg bw per day	0 mg/kg bw per day	26.8 mg/kg bw per day	53.6 mg/kg bw per day
Tunica vaginalis / peritoneum – mesothelioma	3/49 (6)[##]	34/50 (68)**	39/47 (83)**	NA	NA	NA
Mammary gland						
Fibroadenoma	3/45 (7)[##]	8/39 (21)	7/17 (41)**	14/49 (29)[##]	32/46 (70)**	29/44 (66)**
Adenocarcinoma	NA	NA	NA	1/50 (2)[##]	11/48 (23)**	16/48 (33)**
Brain glioma	0/46 (0)[##]	5/50 (10)*	6/30 (20)**	0/49 (0)	4/46 (9)	4/46 (9)
Oral mucosa papilloma or carcinoma	3/48 (6)	2/50 (4)	5/44 (11)	1/46 (2)[##]	3/37 (8)	7/26 (27)**
Forestomach papilloma or carcinoma	1/46 (2)[##]	2/50 (4)	6/32 (19)*	0/47 (0)[##]	4/38 (11)*	11/30 (37)**
Intestine– adenomatous polyp or adenocarcinoma	0/47 (0)	1/50 (2)	4/37 (11)	NA	NA	NA
Skin sebaceous gland adenoma, basal cell tumour or sebaceous gland adenocarcinoma	0/45 (0)[##]	5/41 (12)*	4/18 (22)**	NA	NA	NA
Zymbal gland carcinoma	1/49 (2)[#]	3/50 (6)	6/48 (13)	NA	NA	NA
Clitoral gland adenoma, adenocarcinoma or carcinoma	NA	NA	NA	5/49 (10)[#]	9/47 (19)	12/45 (27)*
Thyroid gland follicular cell adenoma or carcinoma	1/46 (2)[##]	4/42 (10)	6/19 (32)**	0/49 (0)[#]	1/38 (3)	3/35 (9)
Haematopoietic system – leukaemia	25/48 (52)	33/50 (66)	21/44 (48)	13/49 (27)[#]	14/44 (32)	20/41 (49)*

bw: body weight; NA: not applicable or no tabulated statistically evaluated data available in the NTP report; No.: number; [#]: $P < 0.05$, [##]: $P < 0.01$ (Cochran–Armitage trend test); *: $P < 0.05$, **: $P < 0.01$ (Fisher exact test); [§]: *P*-values are missing in the report (for exact *P*-values, see NTP, 1990)

[a] Tumour incidence is expressed as the number of tumour-bearing animals alive in each group at the time the first tumour was observed in any of the three groups (i.e. taking into account the high mortality rates).

[b] 0, 37.5 or 75 mg/kg bw per day for 5 days a week were corrected for noncontinuous dosing.

Source: NTP (1990)

all male groups including controls, with 46/49, 50/50 and 49/49 for glycidol doses of 0, 26.8 and 53.6 mg/kg bw per day, respectively, and occurred for the first time at week 44.

In female rats, NTP concluded that there was "clear evidence of carcinogenic activity" based on increased incidences of fibroadenomas and adenosarcomas of the mammary gland, gliomas of the brain, neoplasms of the oral mucosa, forestomach, clitoral gland and thyroid gland, and leukaemia.

No NOAEL was identified (NTP, 1990).

The Committee noted that the study has some limitations: only two dose levels of glycidol (and a control group) were tested, and the high mortality rate. Poor survival was considered in the statistical analyses, by applying the Cochran–Armitage trend test and Fisher exact test not to the 50 animals in the study but to the number alive at the time the first tumour was observed in any of the three

groups. Using this approach, early deaths (and hence a smaller probability of developing a tumour) are given a smaller weight. However, intrinsic difficulties of not knowing the date of onset of a tumour (with the exception of those considered as rapidly lethal, e.g. mesothelioma in the tunica vaginalis and mammary gland neoplasms) add uncertainties to the interpretation of the data.

(c) Hamsters

In a limited study, 20 male and 20 female Syrian golden hamsters, 10 weeks of age, received 12 mg glycidol (96% purity) dissolved in corn oil and ethyl acetate by gavage (approximately 100 mg/kg bw according to the authors) twice a week for 60 weeks. The total cumulative dose per animal was 1.45 g glycidol. The control group consisted of 12 male and 12 female hamsters. Survival, lesions and neoplasms of the major organs were assessed (no details stated). Survival rates were similar and no statistically significant increases in tumour incidences of any target organ were seen. The authors (Lijinsky & Kovatch, 1992) and IARC (2000) nevertheless described haemangiosarcomas in the spleen in 2/19 males and in 4/20 females versus 0/12 in male and 0/12 in female controls.

2.4.4 Genotoxicity

(a) Glycidol, in vitro

Glycidol was clearly genotoxic in vitro, based on comprehensive studies (Table 6; see also DFG, 1999; IARC, 2000; EFSA, 2016). Glycidol tested positive in most in vitro tests in bacteria and mammalian cells with and without metabolic activation. Glycidol was particularly potent in *S. typhimurium* TA100 and TA1535 and *E. coli* WP2uvrA, which detect point mutations (base-pair substitution), and less potent in TA97, TA98 and TA1537, which detect frameshift mutations.

Glycidol was also positive in a battery of in vitro genotoxicity tests with mammalian cells, in most cases also without S9 mix, as well as in the heritable translocation test with *Drosophila melanogaster*. Glycidol induced structural chromosomal aberrations in Chinese hamster lung cells (Ikeda et al., 2012), primary DNA damage in the comet assay with Chinese hamster ovary (CHO) cells (El Ramy et al., 2007) and kidney cell lines NRK-52E and HEK-293 (Ozcagli et al., 2016), mutations in mouse lymphoma L5178Y/TK$^{+/-}$ cells (NTP, 1990), and sister chromatid exchange in CHO cells (NTP, 1990).

Glycidol was cytotoxic and mutagenic in the hypoxanthine phosphoribosyltransferase (HPRT) assay with wildtype and repair-deficient variants of CHO cells. Glycidol induced in these cells strand breaks that were repaired within the observation time (1–4 hours), whereas in repair-deficient cell lines, repair time was significantly delayed. Notably, glycidol was stable under the

Table 6
Results of in vitro glycidol genotoxicity assays

End-point	Test system	Concentration[a]	Result: Without exogenous metabolic system	Result: With exogenous metabolic system	Reference[b]
Differential toxicity	*E. coli* WP2/WP100 *rec* assay	54 µg/well	Positive	NT	McCarroll, Piper & Keech, (1981)
Differential toxicity	*E. coli* WP2/WP100 *rec* assay	10 000 µg/plate	Positive	NT	Mamber, Bryson & Katz (1984)
Reverse mutation	*E. coli* WP2uvrA	156 µg/plate	Positive 78 µg/plate	Positive	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA100	100 µg/plate	Positive	NT	Wade, Moyer & Hine (1979)
Reverse mutation	*S. typhimurium* TA100	61.7 µg/plate	Positive	Positive	Thompson et al. (1981)
Reverse mutation	*S. typhimurium* TA100	33 µg/plate	Positive	Positive	Canter et al. (1986)
Reverse mutation	*S. typhimurium* TA100	25 µg/plate	Positive	NT	Claxton et al. (1991)
Reverse mutation	*S. typhimurium* TA100	20 µg/plate	Positive 4.9 µg/plate	Positive	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA1535	20.6 µg/plate	Positive	Positive	Thompson et al. (1981)
Reverse mutation	*S. typhimurium* TA1535	500 µg/plate	Positive	Positive	Mamber, Bryson & Katz (1984)
Reverse mutation	*S. typhimurium* TA1535	3 µg/plate	Positive	Positive	Canter et al. (1986)
Reverse mutation	*S. typhimurium* TA1535	1.2 µg/plate	Positive	Positive	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA1537	1 670 µg/plate	Positive[c]	Positive	NTP (1990)
Reverse mutation	*S. typhimurium* TA1537	2 500 µg/plate	Positive	Positive 1 250 µg/plate	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA98 (spot test)	10 000 µg/plate	Negative	NT	Wade, Moyer & Hine (1979)
Reverse mutation	*S. typhimurium* TA98	3 333 µg/plate	Positive	Positive	NTP (1990)
Reverse mutation	*S. typhimurium* TA98	2 500 µg/plate	Positive 1 250 µg/plate	Positive	Ikeda et al. (2012)
Reverse mutation	*S. typhimurium* TA97	333 µg/plate	Positive	Positive	NTP (1990)
Reverse mutation	*E. coli* (Sd-4)	740 µg/plate	Positive	NT	Hussain & Osterman-Golkar (1984)
Forward mutation	*Klebsiella pneumoniae*	14.8 µg/mL	Positive	NT	Voogd, van der Stel & Jacobs (1981)
Forward mutation	*Schizosaccharomyces pombe*	74 µg/mL	Positive	Positive	Migliore, Rossi & Loprieno (1982)
Reverse mutation	*Neurospora crassa*	37 000 µg/mL (15 min)[d]	Positive	NT	Kolmark & Giles (1955)
Sex-linked recessive lethal mutations	*D. melanogaster*	1 230 mg/kg feed	Positive	NT	Foureman et al. (1994)
Heritable translocation test	*D. melanogaster*	1 230 mg/kg feed	Positive	NT	Foureman et al. (1994)
Gene mutation	Chinese hamster lung V79 cells, 6-thioguanine resistance in vitro	0.15 µg/mL	Positive	NT	Smith, Cohen & Lawson (1990)
Gene mutation	Mouse lymphoma L5178Y cells, *tk* locus in vitro	8 µg/mL	Positive	Positive	Thompson et al. (1981)

Table 6 (continued)

End-point	Test system	Concentration[a]	Result: Without exogenous metabolic system	Result: With exogenous metabolic system	Reference[b]
Gene mutation	Mouse lymphoma L5178Y cells, *tk* locus in vitro	1.43 µg/mL	Positive	NT	NTP (1990)
Sister chromatid exchange	CHO cells in vitro	1.11 µg/mL	Positive	Positive	NTP (1990)
Sister chromatid exchange	Chinese hamster V79 cells in vitro	92.6 µg/mL	Positive	NT	Von der Hude, Carstensen & Obe (1991)
Chromosomal aberrations	CHO cells in vitro	12.5 µg/mL	Positive	Positive	NTP (1990)
Chromosomal aberrations	Chinese hamster lung cells in vitro	250 µg/mL	Positive[e] 100 µg/mL	Positive[e]	Ikeda et al. (2012)
Gene mutation, (HPRT assay)	CHO cells in vitro, wildtype and BER-deficient cells	20 mmol/L per hour	Positive	NT	Aasa et al. (2016)
DNA strand breaks	CHO cells in vitro, wildtype and BER-deficient cells	10 mmol/L per hour	Positive (later repaired)	NT	Aasa et al. (2016)
DNA breaks (comet assay)	CHO cells in vitro	20 µg/mL	Positive	NT	El Ramy et al. (2007)
DNA breaks (comet assay)	Rat kidney proximal tubular epithelial cell line NRK-52E	20 µg/mL	Positive	NT	Ozcagli et al. (2016)
DNA breaks (comet assay)	Embryonic kidney cell line HEK-293	20 µg/mL	Positive	NT	Ozcagli et al. (2016)
Sister chromatid exchange	Human lymphocytes in vitro	3.7 µg/mL	Positive	NT	Norppa et al. (1981)
Chromosomal aberrations	Human lymphocytes in vitro	29.6 µg/mL	Positive	NT	Norppa et al. (1981)

BER: base-excision repair; CHO: Chinese hamster ovary; HPRT: hypoxanthine phosphoribosyltransferase; NT: not tested; NTP: National Toxicology Program
[a] Lowest effective dose or highest ineffective dose.
[b] References before 2000 are as tabulated by IARC (2000).
[c] Described as "weakly positive" by the NTP (1990).
[d] One dose tested; time-dependent response.
[e] Positive for structural aberrations only; numerical aberrations were negative.

incubation conditions (2–4 hours, 37 °C, different media including water) (Aasa et al., 2016).

Glycidol is considered a direct-acting mutagen. Due to its epoxide structure, glycidol has alkylating properties and reacts directly with cellular nucleophiles, for example, by forming DNA adducts, which has been shown in vitro with purified DNA (NTP, 1990; Segal, Solomon & Mukai, 1990; IARC, 2000; EFSA, 2016).

(b) Glycidol, in vivo

Glycidol induced in vivo micronuclei (NTP, 1990), chromosomal aberrations and sister chromatid exchanges in mouse bone marrow (Sinsheimer et al., 1993) after intraperitoneal administration (Table 7). Administration by gavage induced micronuclei in mouse bone marrow only at the intermediate dose; however, the absence of bone marrow toxicity at all tested doses (up to half of the LD_{50}) indicates that glycidol probably did not reach bone marrow or only in traces (Ikeda et al., 2012).

Chromosomal aberration tests in rat bone marrow gave negative or positive results with glycidol administration by gavage and intraperitoneal administration, respectively (Thompson & Hiles, 1981; Thompson & Gibson, 1984). Orally administered glycidol induced DNA breaks in the in vivo comet assay in the kidney and urinary bladder of Sprague Dawley rats (Wada et al., 2014).

2.4.5 Reproductive and developmental toxicity

(a) Reproductive toxicity

Glycidol (purity not stated, in water) was administered by gavage to groups of five male Wistar rats at 100 or 200 mg/kg bw per day for 5 days or at 100 mg/kg bw per day for 14 days. Groups of five male Wistar rats also received other related compounds, for example, 3-MCPD (purity not stated), by gavage at a single dose of 100 mg/kg bw or at 20 mg/kg bw per day for 5 days. Male fertility was assessed as number of females found inseminated, number of litters and litter size. Histopathological examinations of testis, epididymis and ductus deferens were conducted.

Administration of glycidol at 200 mg/kg bw per day for 5 days resulted in reversible infertility for 2–4 weeks. There were no discernible histological changes except for spermatocoeles, a large one in the cauda epididymis and a small one in a ductulus efferens. Spermatogenic activity in testes was normal and the epididymides were filled with spermatozoa. With the other tested dose regimens, no significant effects on male fertility or histopathology were seen.

3-MCPD was found to be more potent than glycidol. Administration of 3-MCPD at doses of 20 mg/kg bw per day for 5 days resulted in sterility that was reversible after 2 weeks, and a single dose of 100 mg/kg bw produced long-term sterility (observation time of 52 weeks). Histopathological changes were seen in the ductuli efferentes and caput epididymis (Cooper, Jones & Jackson, 1974).

The window of susceptibility regarding reproductive effects of glycidol in female mice was investigated by administering glycidol at a single dose of 0 or 250 mg/kg bw to groups of 23–31 female mice 1, 6, 9 or 25 hours post-mating (mating period of 30 minutes; route and strain not stated but intraperitoneal

Table 7
Results of in vivo glycidol genotoxicity assays

Test system	Test object	Dose (mg/kg bw)	Study details	Result	Reference
Mouse					
Micronucleus formation	B6C3F1 mouse, bone marrow	150 i.p. × 2 (highest tested dose)	In phosphate-buffered saline, *n* = 5 males	Positive	NTP (1990)
Micronucleus formation	ICR mouse, bone marrow	0, 50, 100, 200 gavage × 2	In water, OECD TG 474, *n* = 6 males	Negative (only 100 mg/kg bw was positive)[a]	Ikeda et al. (2012)
Chromosomal aberrations and sister chromatid exchanges	CD-1 mouse, bone marrow	100 i.p.	In DMSO, *n* = 4 males, *R*- and *S*-isomer tested separately	Positive (both isomers)	Sinsheimer et al. (1993)
Rat					
Chromosomal aberrations	SD rat, bone marrow	650–730 oral	Gavage (in water) *n* = 3 males	Negative[b]	Thompson & Gibson (1984)
Chromosomal aberrations	SD rat, bone marrow	600 oral (460–540 oral negative)	Gavage (in water) *n* = 3 females	Positive[b]	Thompson & Gibson (1984)
Chromosomal aberrations	SD rat, bone marrow	320–340 i.p. (290 i.p. negative)	*n* = 3 males	Positive[b]	Thompson & Gibson (1984)
Chromosomal aberrations	SD rat, bone marrow	150 i.p. (180–200 i.p. negative)	*n* = 3 females	Only lowest dose positive (not dose-related)[b]	Thompson & Gibson (1984)
Chromosomal aberrations	SD rat, bone marrow	226 oral × 5 145 i.p. × 5	Gavage (in water) *n* = 6 females	Negative	Thompson & Hiles (1981)
DNA breaks (comet assay)	SD rat, urinary bladder	400 oral × 2 (200 oral × 2 negative)	Gavage (in physiological saline), *n* = 5 males	Positive	Wada et al. (2014)
DNA breaks (comet assay)	SD rat, liver	200 or 400 oral × 2	Gavage (in physiological saline), *n* = 5 males	Positive	Wada et al. (2014)

DMSO: dimethyl sulfoxide; i.p. intraperitoneal; OECD TG: Organisation for Economic Co-operation and Development test guideline; NTP: National Toxicology Program; SD: Sprague Dawley
[a] None of the tested doses were toxic to bone marrow as there was no reduction in the proportion of immature erythrocytes among total erythrocytes in bone marrow.
[b] Mitotic index was not statistically significantly different from control.

dosing was described in previous work by the same authors with (C3H × C57BL)-F1 mice). On gestation day 17, females were killed and examined for resorptions, early gestation deaths, late gestation deaths and fetal defects. Compared with controls, resorptions were significantly increased in all treatment groups. In the 1- and 6-hour post-mating treatment groups, the number of living embryos was significantly decreased and anomalies in live fetuses, mainly hydrops (oedema) and eye defects, were significantly increased (Rutledge et al., 1992).

In a study investigating the effect of a number of chemicals on the reproductive capacity of female mice, glycidol was administered at a single intraperitoneal dose of 0 (water) or 300 mg/kg bw to groups of 34 female (SEC × C57BL6)F1 mice. When mated with untreated male (C3H/R1 × C57BL10)-

F1 mice the following day and again during most of the females' reproductive lifespan (17 breeding intervals, at least 347 days post-treatment), there was no significant difference in the number of offspring or litter size between the treated and control groups. No other end-points were assessed (Bishop et al., 1997).

In a study investigating the antifertility activity of several compounds, glycidol was administered by intraperitoneal injection to groups of five male Wistar rats at a dose equimolar to 5 mg/kg bw 3-MCPD (corresponding to approximately 3.5 mg/kg bw glycidol) daily for 14 days. Sperm motility was significantly reduced compared with controls, but no differences were observed in number of sperm and fertility assessed as pregnancy rate of mated dams, litter size and embryo weight. In comparison, administration of 3-MCPD resulted in complete infertility in this study (Brown-Woodman, White & Ridley, 1979).

Glycidol was injected into one uterine horn of pregnant Sprague Dawley rats (group size not specified) at doses of 10, 100 or 1000 μg per fetus on gestation day 13. The embryos in the other uterine horn received the vehicle, 0.9% sodium chloride. Dams were killed on gestation day 20 and examined for resorption. Compared with controls, resorption rates were increased at all doses. Significant teratogenicity was seen at the highest dose with malformations in 44% of the surviving fetuses, mainly in the forelimbs, hindlimbs and low set ears (Slott & Hales, 1985).

The Committee noted that none of these studies on reproduction/fertility meets modern standards due to their unconventional experimental design.

(b) Developmental toxicity

Glycidol (purity not stated) was administered by gavage to pregnant CD1 mice at doses of 0, 100, 150 or 200 mg/kg bw per day during gestation days 6–15 (30–37 females per dose, pooled data from five replicas). On gestation day 18, the dams were killed and assessed for number of implantation sites and condition of conception. The live fetuses were assessed for weight, sex and external anomalies.

At 200 mg/kg bw per day, five of 30 dams died or were killed moribund. At 200 mg/kg bw per day, an increased number of stunted fetuses (almost half of which had cleft palate) were seen, but all belonged to the same litter. This was not considered a compound-related finding as no other teratogenic effects were seen at any dose and there were no significant effects on the number of pregnant dams, number of implants, number of dams with resorptions, number of fetal deaths, number of live fetuses per dam or the average fetal weight.

The NOAEL was 150 mg/kg bw per day (Marks, Gerling & Staples, 1982).

2.4.6 Special studies

(a) Covalent binding to nucleic acids and/or proteins

The kinetics of diHOPrVal–haemoglobin adduct formation and elimination were investigated in vivo in rats and in vitro in rat and human blood samples.

Male Sprague Dawley rats (n = 3; 7 weeks old; 271–308 kg bw) received a single gavage dose of glycidol dissolved in water at 0, 4.92, 12, 30 or 75 mg/kg bw. (The highest dose corresponds to the high dose in the NTP (1990) study, and the lowest dose to the highest dose in the Wakabayashi et al. (2012) kinetics study.) Whole blood from the abdominal aorta was drawn 24 hours after dosing. A dose-dependent increase in diHOPrVal levels was observed; all dose groups showed a statistically significant ($P < 0.05$) difference compared with controls.

In a second experiment, male rats (n = 3) were administered glycidol (in water) at a single gavage dose of 12 mg/kg bw. Blood was sampled at 1, 10, 20 or 40 days. A linear decrease in diHOPrVal levels, compatible with the normal turnover of rat erythrocytes (lifespan: 61 days), was seen. The authors calculated a first-order elimination rate constant (k_{el}) of 0.000 11 and indicated that the diHOPrVal adduct was chemically stable.

The authors also estimated the second-order rate constant (k_{val}) for the reaction of glycidol with *N*-terminal valine in rat and human haemoglobin in in vitro experiments with whole blood. Human blood samples were obtained from nonsmoking volunteers and rat blood samples from fasted (12 hours) male rats (described above). Whole blood samples were incubated with glycidol (0, 50, 100, 200 or 400 µmol/L) for 1 hour at 37 °C. A dose-dependent formation of diHOPrVal was observed in blood samples of rats and humans. The second-order rate constant k_{val} was calculated as 6.7 ± 1.1 and 5.6 ± 1.3 pmol/g globin per µmol/L per hour in rat and human blood, respectively, indicating no species differences between rats and humans.

The authors suggested that diHOPrVal is a useful biomarker for the quantification of glycidol exposure (Honda et al., 2014).

diHOPrVal was shown to be formed in the blood of male Wistar rats following a single oral dose of glycidol (50 mg/kg bw) or equimolar glycidyl palmitate (209.4 mg/kg bw) by gavage in corn oil (Appel et al., 2013; description in section 2.1).

Glycidol was suspected to be the most likely precursor of diHOPrVal adducts in haemoglobin in rats (3–4 males or females) fed fried feed for 1–2 months (feed was soaked in water, formed into pancakes and fried in a hot pan without fat). There was a 50% higher mean diHOPrVal adduct level in unreduced haemoglobin compared with animals fed standard feed (1–1.8 versus 0.6–1.3 pmol/g globin, statistically significant effect). No specific information on glycidol concentrations in feed was provided (Hindso Landin et al., 2000).

(b) Immunotoxicity

In a study investigating the immunotoxic effects of glycidol, female B6C3F1 mice (n = 8; 8–10 weeks old) received glycidol by gavage (in sterile water; purity not stated) at doses of 0, 25, 125 or 250 mg/kg bw per day for 14 days or cyclophosphamide via intraperitoneal administration as the positive control. Body weight, clinical signs, haematology, weight and gross pathology of the major organs and immunotoxicity (many functional assays and three host resistance models) were assessed.

All treated animals survived. There were significant increases in absolute and relative kidney weights at 125 and 250 mg/kg bw per day and in relative liver weight at the highest dose. Haemoglobin and haematocrit parameters were significantly reduced in the high-dose animals; leukocyte cell counts were unaffected. The following immune modulatory end-points were reported as being significantly different in the mid- and/or high-dose groups compared with controls: decrease in splenocyte immunoglobulin M (IgM) antibody-forming cell response to sheep red blood cells; decreased spleen natural killer cell activity; decreased B cell proliferative responses to anti-IgM F(ab′)2 fragment and/or interleukin-4; decreased number and percentage of B cells and absolute number of CD4+ T cells in the spleen, and decreased host resistance to the B16F10 melanoma tumour model. Glycidol had no effect on host susceptibility to either *Listeria monocytogenes* or *Streptococcus pneumoniae*, suggesting that cell-mediated immunity functions were not affected.

The NOAEL was 25 mg/kg bw per day (Guo et al., 2000).

(c) Neurotoxicity

Glycidol (97.6% purity, dissolved in water) was administered to groups of 16 male Sprague Dawley rats (5 weeks old) by gavage at doses of 0, 30 or 200 mg/kg bw per day for 28 days. Body weight, feed consumption, gait, brain weight and histopathology, thyroid hormones and some genes (presumably those involved in neurotoxicity) were assessed. Statistically significant effects, including decreases in body weight gain, feed consumption and relative and absolute brain weight, as well as abnormal gait, axonopathy in the central and peripheral nervous system, and increased serum levels of triiodothyronine (T_3) and thyroid-stimulating hormone (TSH), were seen only at 200 mg/kg bw per day (Akane et al., 2014a). Genes related to axonogenesis and synaptic transmission were downregulated in the hippocampal dentate gyrus, cingulate cortex and cerebellar vermis at 200 mg/kg bw per day (Akane et al., 2014b).

In an oral developmental neurotoxicity study, glycidol (97.6% purity) was administered in drinking-water to groups of 12 pregnant Sprague Dawley rats from gestation day 6 until postnatal day 21. Concentrations of glycidol in

the drinking-water were 0, 100, 300 or 1000 mg/L. (The authors stated that these doses were equivalent to 0, 18.5, 48.8 and 108.8 mg/kg bw per day, respectively, without explaining whether these were measured or calculated using conversion factors.) Dams and half of the offspring (31–33 males and 11 females) were killed on postnatal day 21 and the remaining offspring on postnatal 77. The number of implantation sites, number of live offspring and ratio of male to female offspring were assessed. Gait abnormalities in dams and offspring were the only neurological end-point assessed. Other examined parameters of this non-OECD study were body and brain weight of all dams and offspring at both necropsy days; histopathology and molecular analyses of brain, trigeminal nerve, sciatic nerve and spinal cord in all dams and 11 male offspring at both necropsy days (1 male per dam, without information on selection criteria); and thyroid hormones (postnatal day 21).

All high-dose dams showed severe gait abnormalities and axon injury in the peripheral and central nervous system. No significant differences in the investigated reproductive end-points were seen in dams. In the offspring, body weight gain was significantly reduced in mid- and high-dose males and in high-dose females. Gait was normal in offspring at all tested doses. Histopathological changes in the hippocampal dental gyrus in offspring was reported at 108.8 mg/kg bw per day.

According to the authors, the NOAEL for maternal toxicity was 48.8 mg/kg bw per day and the NOAEL for offspring toxicity was 18.5 mg/kg bw per day (Akane et al., 2013).

Male offspring at 0, 48.8 and 108.8 mg/kg bw per day underwent gene expression analysis in four brain regions on postnatal day 21. Changes were seen mainly at 108.8 mg/kg bw per day. The authors concluded that gene expression profiles suggest that developmental exposure to glycidol affected the plasticity of neuronal networks in the broad brain areas, and that dentate gyrus neurogenesis may be the sensitive target of this type of toxicity (Akane et al., 2014c).

2.5 Observations in domestic animals/veterinary toxicology

No information was available.

2.6 Observations in humans

No clinical or epidemiological studies were available.

2.6.1 Biomarkers of exposure

The haemoglobin adduct diHOPrVal was detected in blood samples of 14 diacylglycerol oil users and 42 non-users (controls were asked not to consume diacylglycerol oil for 4 months prior to blood sampling). All volunteers were employees of the Kao Corporation, which markets diacylglycerol oil. Smoking habits were recorded in this study. No significant difference in mean diHOPrVal concentration was seen between the two groups (6.9 versus 7.3 pmol/g globin), which is consistent with what was previously reported for fewer users (Honda et al., 2011). However, it is unclear from the study what amounts of glycidol and glycidyl esters were ingested with the diacylglycerol oil (Honda et al., 2012). diHOPrVal appears not to be specific for glycidol, as epichlorohydrin, 3-MCPD and smoking cigarettes, for example, also lead to the diHOPrVal–haemoglobin adduct (Hindso Landin et al., 2000).

DHPMA was measured in spot urine in 54 non-smokers and 44 smokers from northern Bavaria, Germany, with a median of 206 (range 114–369) µg/g creatinine and 217 (range 165–342) µg/g creatinine, respectively. Based on rat metabolism, the authors hypothesized that the source of DHPMA was 3-MCPD or glycidol or their esters or an endogenous source because of the good correlation with creatinine and the high background level compared with the five other alkyl-mercapturic acids also analysed in the spot urine (Eckert, Drexler & Goen, 2010; Eckert et al., 2011).

3. Analytical methods

3.1 Chemistry

Glycidol esters, glycidyl esters or glycidyl fatty acid esters (commonly referred to as glycidyl esters) are related to monoacylglycerols and monochloropropanediol esters in that they all have a glycerol backbone esterified to a fatty acid chain. Monoacylglycerols have hydroxyl groups in the sn-1/sn-2 or sn-1/sn-3 positions; the MCPD esters have a chlorine substitution at one of the hydroxyl groups; and the glycidyl esters have an epoxide ring bridging the sn-1/sn-2 positions (α-carbon) (Fig. 1). Initial research related to glycidyl esters was largely performed as part of the investigations into 3-MCPD and related compounds (Matthäus et al., 2011a; Freudenstein, Weking & Matthäus, 2013). Studies that indirectly analysed MCPD esters determined varying MCPD concentrations. Consequently, Weisshaar & Perz (2010) proposed that other compounds present in edible oils (e.g. glycidyl esters) were being converted to 3-MCPD during sample analysis. The presence of glycidyl esters in edible processed oils was confirmed by Kuhlmann (2011), who

also developed a method to accurately determine both MCPD esters and glycidyl esters without generating any artefacts. Smith (1950) had previously found that glycidol converted to MCPD isomers on treatment with hydrochloric acid. This discovery led to research that focused on isolating the proposed esters of glycidol from triacylglycerols in edible oil samples using size exclusion or gel permeation chromatography. Analysis of the un-derivatized sample fractions using gas chromatography–mass spectrometry (GC-MS) confirmed the presence of glycidyl esters in oil (Weisshaar & Perz, 2010). Similar to the MCPD esters, many glycidyl ester species are possible owing to the different fatty acid chains bound to the glycidol structure. In addition, glycidyl ester composition corresponds to the types of fatty acids present in the source oil.

3.1.1 Formation of glycidyl esters

Glycidyl esters and MCPD esters were originally thought to be formed via similar reaction pathways. However, experimentation with temperature and time, to elucidate the formation pathway of each group of compounds, confirmed that the pathways did in fact differ. Glycidyl ester formation is directly related to elevated temperatures and time, whereas the relationship between MCPD ester formation and temperature is not as clear (Hrncirik & van Duijn, 2011). Temperatures between 250 and 270 °C favour glycidyl ester formation (Pudel et al., 2011). Glycidyl esters are primarily produced not from triacylglycerols but from diacylglycerols, although monoacylglycerols are also, to a limited extent, implicated in their formation (Destaillats et al., 2012a; Stadler, 2015). Because monoacylglycerols are removed during oil deodorization, their contribution to overall glycidyl ester production is thought to be low (Goh & Timms, 1985; Craft et al., 2012). The presence of more than 3–4% diacylglycerols in lipids results in an exponential increase in the relative glycidyl ester formation during deodorization of edible oils (Craft et al., 2012). Glycidyl esters are known to be present in processed palm oils at high levels relative to other processed oil types (e.g. rapeseed oil). This corresponds to the higher diacylglycerol content (5% in palm oil versus 2% in rapeseed oil) (Freudenstein, Weking & Matthäus, 2013; Šmidrkal et al., 2016).

At the high temperatures required for deodorization, intramolecular rearrangement of diacylglycerols occurs as the result of proton loss from a hydroxyl group to the neighbouring carboxyl group, leading to the formation of an acyloxonium intermediate (Destaillats et al., 2012a). This intermediate can rearrange through charge transfer, resulting in fatty acid cleavage and formation of an epoxide ring (Destaillats et al., 2012a). Glycidyl ester formation via vicinal dehydration of monoacylglycerols followed by epoxide formation has been proposed for those monoacylglycerols substituted at the sn-1 or sn-3

position (Hamlet et al., 2011; Šmidrkal et al., 2011; Destaillats et al., 2012a). In experiments examining formation mechanisms of glycidyl esters, it was also observed that at moderate temperatures (150–200 °C), oil deodorization resulted in the formation of oxopropyl esters, but at higher temperatures (>230/240 °C), formation of glycidyl esters increased at an exponential rate (Pudel et al., 2011; Craft & Destaillats, 2014).

Exposure of food to high temperatures also occurs during deep frying in oil. Unlike deodorization, which is principally a thermal process, deep frying results in hydrolytic and oxidative reactions in addition to thermal reactions (Aniolowska & Kita, 2015, 2016). When the impact of frying with palm oil was investigated, inverse relationships between glycidyl ester formation and both frying temperature and time were identified (Aniolowska & Kita, 2016). Rather than being a route of glycidyl ester formation, frying appears to be a mechanism for glycidyl ester reduction.

Glycidyl esters were found to form from MCPD esters during alkaline treatment for fatty acid cleavage in the presence of inorganic chloride (e.g. sodium chloride) (Kuhlmann, 2011). Similarly, the glycidyl ester epoxide ring may open during exposure to acid and MCPD can be formed in the presence of sodium chloride (Kaze et al., 2011). Analysts should be aware that bidirectional conversion of glycidyl esters to MCPD can occur during sample preparation for indirect analytical methods.

3.2 Description of analytical methods

3.2.1 Introduction

Both indirect and direct methods of analysis are used to determine glycidyl ester concentrations in edible oils and foods. The indirect methods, which are used most frequently, require several chemical reactions to be completed to measure derivatized products of glycidyl esters, and require only a limited number of analytical standards. Analytes are measured using GC-MS following formation of derivatives. In contrast, direct measurement allows for the determination of glycidyl esters without the need for ester cleavage, conversion of glycidol into suitable analytes, or derivatization. Owing to the reactivity and thermal instability of the epoxide group, direct analysis is generally performed with liquid chromatography–mass spectrometry (LC-MS). Measuring glycidyl esters using the direct approach, however, requires analytical standards for each of the esters. As in the case with MCPD esters, prior to extraction, samples must be uniform and may require homogenization before being prepared for analysis (Küsters et al., 2011).

Both approaches used for glycidyl ester analysis involve the routine use of internal standards. These are generally present as deuterated analogues, although ^{13}C analogues, used as surrogates, also allow for correction of possible losses or conversion that may occur during sample preparation. In addition, the use of internal standards may help account for matrix impacts encountered during the analysis.

3.2.2 Indirect methods

Similar to the indirect measurement of MCPD esters, the esters are cleaved from the glycidol moiety during indirect analysis of glycidyl esters, although the epoxide ring in glycidyl esters requires additional considerations. In order to form stable intermediates, the glycidol moiety is reacted with a nucleophilic agent (e.g. sodium chloride or bromide, etc.) before or after hydrolysis of the ester group (Kuhlmann, 2011; Küsters et al., 2011; Crews et al., 2013). Different approaches address this issue. One approach involves differential analysis; this is similar to the approach used for MCPD ester analysis where, following ester cleavage, a second aliquot of each sample is prepared and an additional step performed on this second aliquot to convert the glycidol components to 3-MCPD. The glycidol content is then determined as the difference in amount of 3-MCPD measured in each of the two aliquots (Shimizu et al., 2010; AOCS, 2013c). Other researchers have elected to open the epoxide ring under acidic conditions, followed by the conversion of glycidyl esters to monobromopropanediol esters immediately after extraction (Ermacora & Hrncirik, 2013). Still others have converted the glycidyl esters to 3-methoxypropane-1,2-diol prior to ester cleavage (Küsters et al., 2011).

For those indirect methods that rely on differential analysis, which involves comparing the results obtained for MCPD alone and those based on the sum of MCPD plus the MCPD induced through the conversion of glycidyl esters, a conversion factor must be applied. The conversion factor (0.67) allows for the difference between the molecular weight of MCPD (110.54 g/mol) and that of glycidol (74.08 g/mol) (Weisshaar & Perz, 2010). The results obtained using this approach are based on the assumption that complete conversion of glycidol to MCPD has occurred and that the only source of the additional MCPD is from glycidyl esters (Kuhlmann, 2011; Masukawa et al., 2011).

The general principles applied to the indirect analysis of glycidyl esters include adding isotopically labelled MCPD, MCPD esters or glycidyl esters to samples, followed by extraction with solvent. As with MCPD analysis, different solvents are used for sample extraction, for example, methyl-*tert*-butyl ether, diethyl ether and tetrahydrofuran (Kuhlmann, 2011; Küsters et al., 2011; Ermacora & Hrncirik, 2013; Wöhrlin et al., 2015).

The hydrolysis reaction that cleaves the fatty acid esters from the glycidyl esters has been successfully performed in acidic conditions using sulfuric acid/methanol (Ermacora & Hrncirik, 2013; Wöhrlin et al., 2015); in alkaline conditions using sodium hydroxide (Weisshaar, 2008; Kuhlmann, 2011; Küsters et al., 2011; Karl et al., 2016; Li et al., 2016); and in a low pH environment (pH 5) using enzymatic cleavage (Miyazaki & Koyama, 2016). As with the MCPD sample preparation, reaction conditions for acidic or alkaline ester cleavage are critical. Kuhlmann (2011) reported that cooling samples for the duration of the cleavage reaction to −22 °C to −25 °C under alkaline conditions required longer reaction times. Following completion of the cleavage of fatty acid methyl esters from the glycidyl esters, the reaction was stopped by balancing the pH in the reaction vessel. In some cases, sodium bromide was present to allow for the formation of monobromopropanediol rather than MCPD. For those samples that had been brominated at the start of sample preparation, sodium sulfate was used to dry the extract (i.e. remove traces of water) (Kuhlmann, 2011; Ermacora & Hrncirik, 2013). In samples where esters were cleaved enzymatically, the conversion of glycidol to monobromopropanediol was similarly accomplished with sodium bromide (Koyama et al., 2016; Miyazaki & Koyama, 2016).

The glycidol-related compounds obtained using indirect analytical methods are generally derivatized for successful analysis. Derivatization is most frequently conducted using phenylboronic acid with GC-MS for analysis (Shimizu et al., 2010; Kuhlmann, 2011; Küsters et al., 2011; Ermacora & Hrncirik, 2013; Wöhrlin et al., 2015; Miyazaki & Koyama, 2016). Non-polar capillary columns are routinely used for the analysis of glycidol derivatives and oven temperature gradients vary from method to method for analyte separation (Hrncirik, Zelinkova & Ermacora, 2011). Both split/splitless and programmed temperature vaporization (PTV) injector systems are used for these analyses (Kuhlmann, 2011; Küsters et al., 2011; Karl et al., 2016). The ions used to confirm and measure monobromopropanediol concentrations are *m/z* 147 as the quantification ion with *m/z* 240 as a qualifier (Ermacora & Hrncirik, 2013; Miyazaki & Koyama, 2016). Analysis of glycidyl esters as 3-methoxypropane-1,2-diol used *m/z* 147 as the quantification ion with *m/z* 192 as the qualifying ion (Küsters et al., 2011).

In addition to the more widely adopted methods, ^{1}H nuclear magnetic resonance spectroscopy has been proposed as an alternative method for glycidyl ester analysis (Song et al., 2015). This method involves dissolution of the glycidyl esters in deuterated chloroform and benzene as an internal standard. It requires very little preparation prior to analysis (Song et al., 2015).

The LODs reported using indirect methods are consistently low (25–60 µg/kg), with some authors reporting different LODs in oil and fat (15 µg/kg) relative to fat-rich foods (65 µg/kg) (Kuhlmann, 2011; Küsters et al., 2011; Ermacora & Hrncirik, 2013; Wöhrlin et al., 2015). Comparison of LODs is

confounded by the reporting of results that could be based on fat content versus product weight (i.e. total weight).

3.2.3 Direct methods

The literature also includes studies on the analysis of intact glycidyl esters, where glycidyl esters are isolated from samples and analysed without cleavage of esters. Reporting of results using direct methods tends to be based on five or seven glycidyl esters, by addressing the dominant fatty acids (palmitic acid, linolenic acid, linoleic acid, oleic acid, stearic acid, lauric acid and myristic acid) in oils (MacMahon, 2016). Originally, methods developed for the direct analysis of glycidyl esters were performed with liquid chromatography coupled to time-of-flight (LC-TOF) mass spectrometry systems using diluted samples without additional sample preparation (Haines et al., 2011). Although this work allowed for direct measurement of glycidyl esters, it resulted in high LODs and extensive instrument maintenance. Analysis of seven glycidyl esters in oil samples without any cleanup prior to LC-MS analysis with a single quadrupole instrument gave results that compared well with a method that used cleanup (Blumhorst, Venkitasubramanian & Collison, 2011).

To lower the LODs, glycidyl esters need to be separated from oil constituents. A two-stage cleanup protocol using solid-phase extraction (SPE) with C-18 and silica adsorbents has led to the successful analysis of glycidyl esters (Masukawa et al., 2010; Hori et al., 2012; MacMahon et al., 2013; Becalski et al., 2015a). Although most methods involve only two initial cleanup steps – using C-18 SPE, followed by silica (Masukawa et al., 2010; Becalski et al., 2012; MacMahon et al., 2013) – Hori et al. (2012) reported the successful use of the same steps in reverse. Becalski et al. (2012) introduced a pre-concentration step on a larger silica column for better removal of interferences and to achieve lower LODs. The solvent used to elute glycidyl esters from cleanup cartridges differs between research groups; solvents include methanol, chloroform, acetonitrile, ethyl acetate, ethyl acetate/hexane and diethyl ether/hexane (Masukawa et al., 2010; Becalski et al., 2012; Hori et al., 2012; MacMahon et al., 2013). Gel permeation chromatography has been used to separate the lipid from the glycidyl esters, although those oils with very high levels of monoacylglycerols and diacylglycerols required further cleanup using silica (Dubois et al., 2011).

In addition to the LC-TOF mass spectrometric analyses, glycidyl esters have been analysed using liquid chromatography or ultra-fast pressure liquid chromatography (UFLC) coupled to triple quadrupole, single quadrupole or QTrap mass spectrometers (Masukawa et al., 2010; Blumhorst, Venkitasubramanian & Collison, 2011; Shiro et al., 2011; Becalski et al., 2012; Hori et al., 2012; Blumhorst et al., 2013; MacMahon et al., 2013). Instruments

have been operated using both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) operating in the positive ion mode for glycidyl ester analysis, with separation consistently achieved using gradient elution on C-18 columns (Blumhorst, Venkitasubramanian & Collison, 2011; Masukawa et al., 2011; Becalski et al., 2012; Hori et al., 2012; MacMahon, Begley & Diachenko, 2013; MacMahon et al., 2013).

A collaborative study has also been performed to determine glycidyl esters (palmitate, stearate, oleate, linoleate and linolenate) in oil using a direct method. The protocol involved SPE cleanup with both C-18 and silica cartridges and analysis performed using liquid chromatography coupled with single quadrupole mass spectrometers. Results obtained using the method were considered acceptable by the study directors (recoveries 100.2–109.0%; repeatability 6.85–19.88%) (Blumhorst et al., 2013).

Unlike indirect analyses where a limited number of standards are required for quantitative analysis, quantification of glycidyl esters using direct methods requires a large number of analytical standards especially when stable isotope dilution analysis is used, with a corresponding increase in ions to be monitored. Owing to the diversity in fatty acid chain length, monitoring of a large number of ions is required. The ions selected for analysis range from *m/z* 256.2 to 399.2, and if multiple reaction monitoring (MRM) is applied, transitions must be considered and may lead to measurement using nonspecific ions (e.g. 313.2 → 57) (Masukawa et al., 2010; Blumhorst, Venkitasubramanian & Collison, 2011; Dubois et al., 2011; Becalski et al., 2012; MacMahon et al., 2013; Thürer & Granvogl, 2014).

Direct analysis of glycidyl esters has been proposed using GC-MS. This method involved greater sample handling prior to analysis with extraction performed with acetonitrile, followed by cleanup with heptane and normal phase liquid chromatography and GC-MS analysis with a polar column (Steenbergen et al., 2013).

LODs vary between individual glycidyl esters and between direct methods used, but generally range from 10 to 16 µg/kg (Blumhorst, Venkitasubramanian & Collison, 2011; Haines et al., 2011; Shiro et al., 2011; Becalski et al., 2012; Hori et al., 2012; MacMahon et al., 2013; Steenbergen et al., 2013).

3.2.4 Reference methods

The American Oil Chemists' Society (AOCS) has developed three indirect official methods for the analysis of glycidyl esters exclusively in oils and fats. One of these methods quantifies glycidol by the difference in the 3-MCPD concentration measured in paired samples in which glycidol is converted to MCPD in one of the samples and not the other; therefore, the result is based on MCPD ester/

MCPD concentrations alone (AOCS, 2013c). This method is the basis for the international standard method for fats and oils (ISO 18363-1) (AOCS, 2013c; British Standards Institution, 2015). This indirect approach, by difference in concentration, assumes that no other MCPD-forming substances are present (AOCS, 2013c). Other methods allow for the concurrent determination of MCPD esters and glycidyl esters, as their respective free forms, by conversion of the glycidol to monobromopropanediol (AOCS Cd 29a-13 and Cd 29b-13) (AOCS, 2013a,b,c). Method Cd 29a-13 uses acidic hydrolysis whereas method Cd 29b-13 uses alkaline hydrolysis at a temperature of approximately –25 °C.

In addition, a method for the direct detection of glycidyl esters in edible oil has been developed jointly by the AOCS and the Japan Oil Chemists' Society (JOCS). Similar to other methods reported in the literature, the method requires two stages of SPE cleanup followed by LC-analysis (AOCS Cd 28-10) (AOCS & JOCS, 2012).

Although there are collaborative studies of methods for the determination of glycidyl esters in fats and oils, none have been published on the determination of these contaminants in foods. Recent conference presentations (AOCS, 2016; Euro Fed Lipid, 2016) have detailed a collaborative study for the detection of 3-MCPD esters in mayonnaise and margarines. In view of the absence of collaborative studies on other food matrices, interpreting analytical data arising from complex food analysis should be done with caution. Furthermore, there is a high degree of uncertainty in comparing the reported levels in the same foods from different regions because of the lack of comparisons between laboratories and the absence of information on sample sharing or data arising from proficiency testing schemes.

3.2.5 Quality assurance considerations

Analyte loss during sample preparation is of concern to all analysts, and developing approaches to correct for losses is critical for accurate measurement. The most frequently adopted method to account for losses is the addition of surrogate standards, generally from stable isotope analogues of the compound(s) of interest, prior to initiating sample preparation. Deuterated (e.g. d_5-) analogues of MCPD are routinely used for this purpose when glycidyl ester determination is performed indirectly, although direct glycidyl ester determination involves the use of glycidyl ester surrogates with both deuterated and ^{13}C analogues (Dubois et al., 2011; Hori et al., 2012; MacMahon et al., 2013; Becalski et al., 2015a,b). Additional approaches to ensure that analyses are of acceptable quality involve the inclusion of (1) reagent blanks to determine contributions to analyte concentrations from the laboratory or reagents used; (2) samples fortified with the analyte and treated as samples of unknown concentration to determine recovery

through processing; (3) participation in proficiency testing programmes; and (4) measurement of analytes in reference materials (Küsters et al., 2011; Hori et al., 2012; AOCS, 2013b,c). Annual proficiency tests and quality control test materials have recently become available for glycidyl ester analysis in vegetable oil from the Food Analysis Performance Assessment Scheme (FAPAS).

4. Sampling protocols

Although the Codex Alimentarius Commission has not established sampling protocols specifically for glycidyl esters, general guidelines on sampling have been developed (FAO/WHO, 2004). The European Union has been addressing methods of sampling for a number of food processing–induced chemical contaminants, though initially only including free 3-MCPD (European Commission, 2001, 2007, 2011, 2014). Although the European directives and regulations utilize principles established by Codex for sample collection, etc., they have been updated and amended several times and currently include glycidyl esters (European Commission, 2007, 2011, 2014).

Sample collection must be performed by qualified individuals using containers that are clean and nonreactive and that protect samples from contamination or damage during transport and storage (European Commission, 2001, 2007). Sampling of commercial food products must ensure that the samples collected are representative of the lot. Therefore, collection of multiple samples (incremental samples) from within the lot is recommended and may be used to form an aggregate sample from which laboratory samples are taken for analysis (European Commission, 2007). Prior to the subsampling for laboratory analysis, homogenization of the aggregate sample should be performed consistent with GLP. Sample collection must be focused on food commodities that are relevant to glycidyl esters. Specific foods have been identified in the recent European regulations and include vegetable oil/fats, specialized nutritional products (e.g. infant formulas), bakery products including bread and rolls, canned meat and fish, potato or cereal-based products and those foods containing or prepared with vegetable oils (European Commission, 2014).

5. Effects of processing

5.1 Introduction

Glycidyl esters are present in processed oils and fats and food prepared using these products. These compounds are, however, not generally present in crude or

unrefined oils at elevated levels. As a result, glycidyl esters are considered to be food processing–induced compounds where the processing of oils/fats leads to their production. The steps required to refine or purify an oil or fat, for example, deodorization and degumming, may contribute to the formation or mitigation, respectively, of these food contaminants.

Preparation of refined oils from the crude form involves several steps that include degumming, neutralization, bleaching and deodorization (Pudel et al., 2011). Degumming of oil removes phospholipids and is generally performed at relatively low temperatures (80–120 °C) (Pudel et al., 2011; Šmidrkal et al., 2011). Neutralization involves interaction of the oil with sodium carbonate or bicarbonate to lower the acidity (increase the pH) prior to deodorization (Freudenstein, Weking & Matthäus, 2013). Bleaching involves exposing oils to bleaching clays to remove phospholipids from the oil (Hrncirik & van Duijn, 2011). The final stage of oil refinement is known as deodorization; in this stage, in addition to acid treatment, oils are heated at elevated temperatures (>150 °C) (Matthäus et al., 2011b; Pudel et al., 2011).

The preparation process has been investigated to determine its effect on the formation of glycidyl esters and MCPD esters, although the majority of research has focused on MCPD esters owing to their earlier discovery.

Several researchers have established that deodorization, during which oils are exposed to elevated temperatures, is the critical step in the production of both glycidyl esters and 3-MCPD esters (Pudel et al., 2011; Destaillats et al., 2012a,b; Freudenstein, Weking & Matthäus, 2013; Matthäus & Pudel, 2013).

Processing of palm oil has been shown to result in higher glycidyl ester and MCPD ester levels than after processing of other oils despite similar processing. This indicates that oil constituents impact the formation of glycidyl esters and MCPD esters (Destaillats et al., 2012a,b). Glycidyl ester values were significantly higher after deodorization of non-degummed palm oil samples, suggesting that the removal of the precursors during degumming is beneficial.

5.2 Degumming/washing

Adding either water or acid leads to the precipitation of phospholipids from the treated oil (Pudel et al., 2011). Phospholipids, which have been identified as possible precursors of MCPD esters, have been removed from oil using a combination of water and ethanol (Craft et al., 2012). Matthäus et al. (2011a) also observed a reduction in glycidyl ester concentrations following washing with water and ethanol, although the observed decrease in 3-MCPD ester concentrations was greater.

5.3 Neutralization

Oils are exposed to elevated temperatures during deodorization. At elevated temperatures, fatty acids are cleaved from the acylglycerols present in the oils (Destaillats et al., 2012a). Although fatty acids are weak acids, when they are exposed to the temperatures used for deodorization (i.e., 150–250 °C), they behave like strong acids and react with other compounds present, including sodium chloride, which contributes to lowering the pH (Šmidrkal et al., 2011). Because glycidyl ester formation is favoured at lower pH, the effects of neutralization prior to deodorization have been examined. Both washing with water and/or adding alkaline compounds (e.g. sodium carbonate, sodium bicarbonate, sodium hydroxide, potassium hydroxide) have proven to be effective in significantly lowering glycidyl ester formation (Pudel et al., 2011; Freudenstein, Weking & Matthäus, 2013).

5.4 Bleaching

Pigments and phospholipids can also be effectively removed through the exposure of oils to bleaching earth and moderate heat (60–90 °C) during the bleaching process (Pudel et al., 2011).

5.5 Deodorization

Glycidyl ester formation is directly related to temperature and time at elevated temperatures, whereas this relationship between temperature and MCPD ester formation was not as clear (Hrncirik & van Duijn, 2011). Formation of glycidyl esters is favoured at temperatures between 250 and 270 °C (Pudel et al., 2011). In addition, oils exposed to elevated temperatures (e.g. 230 °C) for longer periods (e.g. 5 hours) have been shown to have increased glycidyl ester concentrations relative to those oils subjected to high temperatures for a shorter duration (1–3 hours) (Hrncirik & van Duijn, 2011). Although oxopropyl esters can be formed as a result of deodorization at lower temperatures, the formation of glycidyl esters is favoured at temperatures above 240 °C (Destaillats et al., 2012a).

5.6 Effect of frying

During deep frying in oil, food is subjected to high temperatures that can affect glycidyl ester concentrations in the foods. Unlike deodorization, which is principally a thermal process, frying involves both oxidative and thermal reactions (Aniolowska & Kita, 2015, 2016). Investigating the impact of frying

with palm oil, Aniolowska & Kita (2016) identified inverse relationships between glycidyl ester formation and both frying temperature and time.

6. Prevention and control

Formation of glycidyl esters is a function of the composition of crude oils and the processing conditions used to refine them. The type of oil processed impacts the capacity for glycidyl ester formation, with palm oil producing greater amounts of glycidyl esters than other oil types, similar to what has been observed for 3-MCPD esters (Weisshaar & Perz, 2010). Oils high in diacylglycerols, which are known to be glycidyl ester precursors, are expected to produce higher levels of glycidyl esters, although other compounds, such as glycerol and phospholipids (Matthäus et al., 2011a) and monoglycerides and triglycerides (Shimizu, Vosmann & Matthäus, 2012), may also contribute to their formation. The presence of chlorinated compounds contributes to the formation of 3-MCPD esters although they are not anticipated to impact glycidyl ester formation (Nagy et al., 2011).

6.1 Preharvest control

Strategies to prevent and control glycidyl esters in final oil products include:

- selection of raw material with low precursor content;
- removal of precursors using chemical treatment at mid-range temperatures;
- deodorization at neutral pH below 240 °C;
- adoption of dual deodorization protocols; and
- utilization of adsorbents to remove glycidyl esters post-treatment.

6.1.1 Harvest and storage conditions

Growth and storage conditions of the crops used to produce oil affect the presence of precursors in crops. In some cases, such conditions may be beyond the control of producers (e.g. climate, fertilizer, harvest, etc.) (Matthäus, 2012).

Appropriate postharvest measures that reduce the bruising of fruit, which results in formation of diacylglycerols and free fatty acids, may improve the quality of the fruit being used to prepare the oil and may result in lower glycidyl ester concentrations in the final oil (Poku, 2002; Gibon, Greyt & Kellens, 2007). The enzymatic activity in fruit increases with time, leading to over-ripened or damaged

fruit with higher diacylglycerol content (Gibon, Greyt & Kellens, 2007; Craft et al., 2012). This suggests that rapid processing of undamaged fruit would lead to improved oil quality with a lower potential for the formation of glycidyl esters.

6.1.2 Reduction of precursors – Optimization of refining processes

Processing that removes glycidyl ester precursors prior to oil deodorization contributes to the mitigation of these contaminants in processed oils. Much of the research to determine the best approaches for prevention and control has focused on investigating palm oil because of the high glycidyl ester levels in this oil. Degumming to reduce glycidyl ester precursors (e.g. diacylglycerols, phospholipids) has been shown to lower glycidyl ester concentrations in refined oils (Pudel et al., 2011). Similarly, degumming with phosphoric acid combined with activated clays lowers 3-MCPD ester levels (Ramli et al., 2011). Successful reduction of 3-MCPD diester levels has also been observed after washing crude palm oil with a 1:1 mixture of ethanol and water prior to bench-top deodorization (Craft et al., 2012).

Glycidyl ester levels were lower in oils subjected to degumming, neutralization and bleaching prior to deodorization (Pudel et al., 2011). Applying potassium hydroxide during neutralization resulted in a greater reduction in glycidyl ester levels than when sodium hydroxide was used (Pudel et al., 2011).

Temperatures applied during deodorization can impact the glycidyl ester content of refined oils as can the length of time the elevated temperatures are retained (Hrncirik & van Duijn, 2011). Exposure of oil to formic acid during deodorization has also been shown to reduce glycidyl ester content in the final product (Matthäus et al., 2011a).

6.1.3 New deodorization conditions

Although lowering deodorization temperatures can reduce the glycidyl ester content of oil, it may reduce the quality of the final product. Dual deodorization – deodorization at a high temperature (250–270 °C) for a short time followed by a second heat process at a lower temperature (200 °C) for longer – has been proposed as a way to improve the quality of the oil (Matthäus et al., 2011b). Combining shorter and longer periods of deodorization leads to reduced glycidyl ester concentrations in oil relative to oils exposed to the conventional single stage deodorization (Matthäus et al., 2011b).

6.1.4 Utilization of adsorption materials

A different approach to reducing glycidyl ester concentrations in oils, while ensuring that the final product is of high quality, involves removing glycidyl esters via adsorption after deodorization (Strijowski, Heinz & Franke, 2011).

Of the adsorbents tested for this application, only calcined zeolite and synthetic magnesium silicate were able to reduce glycidyl ester concentrations effectively. Using activated bleaching earth (ABE) resulted in chemical transformation of glycidyl esters rather than their adsorption (Shimizu et al., 2012).

The Committee noted the commitment of the European fats and oils industry trade organization (FEDIOL; the EU Vegetable Oil and Proteinmeal Industry) to continue to reduce the levels of 3-MCPD esters in refined vegetable oils and encouraged the organization to continue reducing this contaminant using reasonable approaches to mitigation (FEDIOL, 2016).

7. Levels and patterns of contamination in food commodities

Globally, oils and fats are regional in their production and may be consumed in relatively higher proportions in the production area than in importing countries. The variety of oils consumed worldwide (per GEMS/Food cluster) is shown in Table 8.

Glycidyl esters are formed in the processing of vegetable oils mainly during deodorization. The extent to which they are formed may depend on the oilseed or fruit being processed and the process and the type of equipment used (AOCS, 2016).

Reports of the analysis of foodstuffs in a number of countries indicate that refined vegetable oil is a major contributor to the levels of glycidyl esters found in food (EFSA, 2016). There is little evidence that glycidyl esters are formed in food during processing or cooking, and there is a reasonable correlation between the levels of glycidyl esters in the oils used and the amounts in which they were used.

Palm oil (and its products) is a major fat in South-east Asia, whereas soybean oil is dominant in North and South America and rapeseed and sunflowerseed oils are more common in Europe. The pattern of consumption of individual oils complicates the determination of the source of oil in any finished food. A mixture of oils is often used to give a food a particular texture or structure.

Glycidyl ester content was also tested in more than 100 different edible fats, oils and related products containing fats/oils such as cookies and cooking sprays in Canada (Becalski et al., 2015a). Most virgin/unprocessed/unrefined oils did not contain detectable levels of glycidyl esters, but their levels were highly variable in processed oils/fats, reaching 11 mg/g (expressed as glycidol equivalents).

Table 8
Percentage of individual oils consumed per GEMS/Food cluster diets

	% of total oil consumption per GEMS/Food cluster diet																
Vegetable oil	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Coconut oil	0.5	0.1	2.6	1.6	1.9	1.1	6.2	1.0	5.5	1.2	7.7	13.7	1.1	71.3	1.4	0.0	35.7
Cottonseed oil	13.5	2.4	3.2	1.5	7.8	7.9	2.3	0.4	4.4	1.9	0.0	–	4.8	0.0	0.0	2.9	–
Groundnut oil	1.3	0.1	12.6	0.4	8.3	0.8	1.0	0.3	7.6	0.8	9.4	–	20.4	1.5	0.2	2.3	0.6
Maize germ oil	1.6	1.2	1.1	16.0	1.4	4.3	2.6	1.3	1.4	5.9	5.2	4.3	1.7	0.5	1.9	0.0	–
Olive oil	4.4	0.9	0.4	4.9	0.6	9.7	7.2	24.2	0.3	6.5	6.2	4.6	0.1	0.3	8.9	0.2	0.9
Palm oil	41.3	0.3	64.7	31.0	17.4	7.7	7.0	10.4	30.0	2.1	28.0	–	44.7	1.8	12.7	51.1	39.8
Palm kernel oil	0.4	0.0	3.8	1.5	0.9	2.7	0.8	0.7	4.6	0.6	0.0	–	14.6	3.1	0.1	0.6	14.2
Rapeseed and mustard oil	6.1	3.9	0.1	5.7	14.2	3.5	32.9	18.8	14.5	14.0	7.5	–	0.7	0.7	22.1	0.1	–
Rice bran oil	0.1	–	–	–	3.0	–	–	0.0	1.0	0.6	–	–	–	11.1	–	–	–
Sesame seed oil	0.6	0.0	1.2	2.2	0.7	1.8	0.4	0.3	2.0	0.2	0.0	0.2	1.7	1.0	0.1	8.9	0.0
Soybean oil	17.7	3.4	8.9	21.8	34.5	30.3	19.3	21.1	27.1	52.7	28.6	26.5	4.7	7.5	11.1	8.9	8.3
Sunflower seed oil	12.5	87.9	1.5	13.4	9.4	30.1	20.4	21.5	1.7	13.5	7.6	50.8	5.6	1.3	41.7	25.1	0.6

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme

8. Food consumption and dietary exposure estimates

8.1 Introduction

An evaluation of the dietary exposure to glycidyl esters was undertaken by the Committee for the first time. As outlined in section 7, the highest concentrations of glycidyl esters and the free form glycidol are in plant oils and foods that contain these oils. Animal fats such as butter and lard may also have high concentrations. Glycidyl esters and glycidol may also be present in infant formulas as these usually contain plant-based oils as a fat source. Therefore, estimates of dietary exposure were evaluated based on concentrations in these key food groups (plant and animal fats and oils, and in infant formula) and other foods where information was available.

Both national and international assessments of dietary exposure were evaluated by the Committee as were exposures for infants from consumption of infant formula. The assessments included those sourced from the literature, information provided to the Committee and estimates calculated by the Committee.

Based on the outcomes of the toxicological assessment by the Committee, only chronic (long-term) dietary exposure assessments were required for the evaluation. The toxicological data were based on the glycidol form of the contaminant. As a result, estimated dietary exposures to glycidol were required in order to allow a direct comparison between the estimates of dietary exposures and the toxicological evaluation, for risk characterization purposes.

8.2 Methods for evaluating dietary exposures to glycidyl esters

8.2.1 Data sources

(a) Concentration data

Glycidyl ester concentrations in foods were sourced from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database. Concentration data in fats and oils were available from Brazil, Canada, Japan, Singapore and the USA. Glycidyl ester concentrations in infant and follow-on formulas were submitted by Brazil, Canada, Japan and the USA. The Canadian data also included concentration data for some snacks and desserts. The Committee reviewed and summarized these data to conduct both national and international estimates of dietary exposure.

(b) Consumption data

Food consumption data for individual countries used by the Committee for estimating national dietary exposure were sourced from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss). Consumption data from this source are based on 2 or more days of food consumption data for individuals collected from population survey data (for further details, see http://www.who.int/foodsafety/databases/en/). Food consumption data are presented according to the CIFOCOss food classification system, which, for most food groups, is based on the Codex raw commodity classification system, as well as the classification system in the Codex General Standard for Food Additives for some processed commodities. Food consumption data for a number of different age groups were represented in the CIFOCOss dataset based on the data collected in the countries' national surveys. Consumption by the general population and children were used for exposure estimates. The data were not split by sex.

The WHO GEMS/Food cluster consumption database was used by the Committee to calculate international estimates of dietary exposure. This dataset includes mean per capita consumption data for 17 GEMS/Food cluster diets. The consumption values are for raw commodities and some processed foods based on FAO food balance sheet data. The clusters group countries that are culturally and economically comparable (Sy et al., 2013). The countries in each cluster are shown in the GEMS/Food cluster diets database.[1] A food classification system is also used to present food consumption amounts; the Level 2 codes are major food groups and the Level 3 codes provide more detailed consumption data about specific types of foods in the group. The Level 3 consumption data (Table A2 in Appendix 1) were used for the dietary exposure calculations.

Consumption data for infant and follow-on formulas were available from previous Joint FAO/WHO Expert Committee on Food Additives (JECFA) assessments (Annex 1, reference *221*). These consumption data were used to estimate dietary exposure for infants (see section 8.3.3).

(c) Literature review

The scientific literature was reviewed to identify any national or regional studies that estimated dietary exposure to glycidyl esters or glycidol. Medline and EBSCO databases were searched using terms such as "glycidyl ester", "glycidol", "dietary exposure", "dietary intake" and "consumption". These terms were also used in a general internet search to capture grey literature. The EFSA (2016) review

[1] https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/GEMS_cluster_diets_2012&userid=G7_ro&password=inetsoft123

included dietary exposure estimates from a number of European countries and therefore was used to obtain estimates relevant to this assessment.

8.2.2 Overview of methods for the glycidyl ester/glycidol dietary exposure assessment

The Committee determined national and international dietary exposures deterministically by multiplying mean concentrations of glycidol in a food by mean consumption of that food. Exposures from all foods were summed to obtain an estimate of total dietary exposure. A common approach of multiplying mean exposures by a factor to obtain an approximate high-percentile exposure was used and is outlined below. The national and international estimates of dietary exposure, although both based on data from the GEMS/Food contaminants database, are not directly comparable because of the different types of food consumption data.

The Committee calculated national glycidol exposure estimates using consumption data from the CIFOCOss dataset with the concentration data from the GEMS/Food contaminants database when both datasets were available for a country (i.e. only Japan and the USA for this evaluation). The mean consumption amount for all respondents (consumers and nonconsumers), in g/kg bw per day for each food, was used to allow summing the exposure across food groups in order to estimate chronic (long-term) dietary exposure from the whole diet, or from as much of the diet as captured by the concentration data. By using the consumption data on a g/kg bw basis, the relevant body weights from survey respondents in each country were taken into account. As the summary high-percentile consumption data provided in the CIFOCOss dataset could not be used to sum exposures across food groups, a factor of 2 was applied to the mean per person exposure estimate to approximate potential high-percentile exposures. This factor approximates the 90th percentile (FAO/WHO, 1985, 2009).

For the international estimates of dietary exposure conducted by the Committee, per capita consumption amounts for foods in g/day from the GEMS/Food cluster diets were combined with mean concentrations for cluster diet food groups (Level 3 classification) to estimate mean dietary exposures. The exposures in μg/day were divided by 60 kg body weight to express the results per kg bw. The international dietary exposure estimates provide information on the variation of exposures at a global level as well as the key foods contributing to dietary exposure in different regions. The estimates also provide information about possible levels of dietary exposure for countries included in each of the 17 clusters for which no national data exist. A factor of 2 was applied to the mean per capita exposure estimate to approximate potential high-percentile exposures. This factor approximates the 90th percentile (FAO/WHO, 1985, 2009).

The estimates of dietary exposure found in the literature or published reports were reviewed for robustness and summarized for the current evaluation.

Major contributing foods to dietary exposures were also summarized for each assessment where possible.

Dietary exposures for infants (up to 12 months of age) included infants fed infant formula. Some exposure estimates for formula-fed infants were conducted by the Committee using information on formula consumption previously used by the Committee and concentrations from the GEMS/Food contaminants database or concentrations from the literature. Further details on this methodology are provided in section 8.2.6. There were no CIFOCOss consumption data for infant formula to enable calculating national estimates. Estimates of infants' dietary exposure from EFSA (2016) were also reviewed.

8.2.3 Data in the GEMS/Food contaminants database

Concentrations of glycidyl esters and glycidol from the GEMS/Food contaminants database used in the dietary exposure assessment were from Brazil, Canada, Japan, Singapore and the USA. Data were available for foods sampled from the years 2010–2015. Characteristics of the concentration data from the GEMS/Food contaminants database are summarized in Table 9. Analytical methods were either the direct method measuring fatty acid esters (Canada and the USA) or the indirect method where esters were chemically converted to the free form glycidol (Japan). Analytical methods used by Brazil and Singapore were not specified. Concentrations determined by the direct method were determined with greater sensitivity (i.e. had lower LODs or LOQs).

The concentration data from the GEMS/Food contaminants database were used by the Committee to calculate estimates of dietary exposure, with edits and exclusions made where required. Aggregated data were excluded because they could not be combined and summarized according to the food groupings needed for the dietary exposure calculations. Aggregated data were only provided by Japan, which also provided individual data for the same foods. Therefore, the individual data were used and compiled as required for the assessments. After excluding the aggregated data, there were 746 data points for glycidyl esters and glycidol in the dataset (Brazil, $n = 40$; Canada, $n = 136$; Japan, $n = 389$; Singapore, $n = 10$; USA, $n = 171$), before further edits and exclusions were made for the dietary exposure assessment calculations. There was no information as to whether samples were single purchase units or composite samples.

Data were available for foods sampled for the years 2010–2015, which would include more recent and more reliable analytical methods. Only 8% of the data were collected prior to 2012, all from Canada. Because of this, and as

Table 9
Characteristics of the data from the GEMS/Food contaminants database for glycidyl esters and glycidol by country

Country	Analytical method	Food categories with occurrence data	Reported as	Sampling period	LOD (µg/kg)	LOQ (µg/kg)	Use in exposure assessments		
							National estimate	International estimate	Infant estimate
Brazil	Not specified	Follow-on infant formula	Glycidyl ester[a]	2015	100	200	×	×	✓
Canada[b]	Direct	Infant formula Animal and vegetable fats and oils Other foods (cookies, spreads, mayonnaise)	Glycidol equivalents	2010–2013	1–9	2–10	×	×	✓
Japan	Indirect	Infant formula Animal and vegetable fats and oils	Glycidol	2012–2015	30–80	60–300	✓	✓	✓
Singapore[b]	Not specified	Margarines and spreads	Glycidyl ester[a]	2014	1 000	3 000	×	×	×
USA	Direct	Animal and vegetable fats and oils	Glycidol	2013–2015	5	14	✓	✓	×

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LOD: limit of detection; LOQ: limit of quantification; USA: United States of America; ✓ = used in exposure estimate; × = not used in exposure estimate

[a] This was the contaminant name given in the GEMS/Food contaminants database; however, the method of analysis was not specified and no further information was provided to determine the form of the contaminant for which the results were reported.

[b] Data from Singapore and Canada were not used in the national estimates of dietary exposure because food consumption data were not available for these countries in the CIFOCOss dataset.

the dataset was already quite small, no data were excluded based on the year of sampling.

Analytical results below the LOD (nondetects or left-censored data) made up 22% of the data points (excluding aggregated results). To enable summarizing the concentration data, a numerical value was assigned to the nondetects. For the dietary exposure calculations, two scenarios were defined: (1) nondetects were assigned a value of zero (lower bound [LB]); and (2) nondetects were assigned the value of the LOQ (upper bound [UB]). The actual concentration falls somewhere between the LB and the UB. Substituting the value of the LOQ for nondetects could lead to a very conservative estimate of the UB of dietary exposure. Where an actual concentration that was between the LOD and LOQ was given, this value was retained as is in the dataset.

Due to the small number of data points available, no data were excluded due to the level of the LOQ. This could mean the UB concentrations and therefore

the UB estimates of exposure were overestimated. This would be the case for data from Singapore with an LOD of 1000 µg/kg. However, no national estimates of dietary exposure were calculated for Singapore as there were no consumption data in the CIFOCOss data and Singapore was not included in the international estimates as it is not assigned to any of the clusters.

All data points were converted to the same concentration units (µg/kg).

Of the results in the concentration dataset (excluding aggregated data), 50% were for the edible portion of the food and 50% reported for edible plus inedible portions. No conversions were applied to concentrations given for edible plus inedible to be comparable to those concentrations based on the edible portions. The reason for this was that concentrations based on "edible plus inedible" were all fats, oils or foods for infants where it can be assumed that there were no inedible portions.

For butter and margarine, concentrations were based on the fat content of the food (i.e. reported as µg/kg of fat). To make them consistent with the food consumption data, these amounts were converted to µg/kg of the whole food by multiplying by 0.8 based on the requirement in Codex[2] that these foods contain a minimum milk fat content of 80% mass/mass (m/m). This is a worst-case scenario if this value is applied to spreads also (which are commonly <80% fat) where no per cent value for fat in the sample was provided.

The majority of data points (93%) were indicated to be from "targeted" sampling. The remainder (7%) were "random" samples. There could be many different reasons for targeting samples, including investigating a contamination incident, targeting analysis of specific foods in which the food chemical in question is known to occur or sampling from specific stores. The reason for targeting is not identified in the GEMS/Food contaminants database. Results from both sampling protocols were therefore included in the analysis.

No weightings were applied to the concentrations, as there was insufficient information about the available data to allow this.

No concentrations for any fats or oils were carried over to other foods such as fat-based mixed dishes. This was because either there were no consumption data for these types of fat- or oil-based mixed foods in the dataset (e.g. for CIFOCOss) for the relevant countries or the consumption data were all on a raw commodity basis (e.g. in both CIFOCOss and the GEMS/Food cluster diets). Therefore, it is assumed that this includes consumption of fats and oils from all sources.

Concentration data also needed some country-specific modifications to make them consistent and in a usable format for summarizing and using in the dietary exposure assessment (both national and international exposure

[2] Codex STAN 279-1971 and Codex STAN 32-1981.

estimates). Modifications are described separately below for Japan (section 8.2.4(a)) and the USA (section 8.2.4(b)), the countries for which national dietary exposure estimates could be calculated.

Where the method of analysis was not specified or the form of the result not described (as the ester or glycidol), the value was assumed to be glycidol.

8.2.4 National assessments of dietary exposure using concentration data from the GEMS/Food contaminants database

For national estimates of dietary exposure, each food in the GEMS/Food contaminants database was assigned a food classification at the most specific level of the CIFOCOss classification structure (Level 3). This enabled the data to be summarized and the mean concentrations derived for each classification to be used in the dietary exposure calculations.

(a) Concentration data from Japan

Specific assignments of the CIFOCOss classification codes made for the Japanese data are as follows:

- No data for specific types of oils were included in CIFOCOss. Therefore, the concentrations from the GEMS/Food contaminants database for all types of oils (codes OR0305, OR0696, OR0541, OR0665, OR0702, OR0645, OR0699, OR0495, OR0700) were assigned OR0172 – "Vegetable oils, nes (not elsewhere specified)" for which there were consumption data. Table A1 (in Appendix 1) shows a breakdown of the concentration data according to each specific type of oil classified under OR0172. Rice bran oil had the highest concentration with a mean of 2925 µg/kg (n = 24, 0% not detected), followed by grapeseed oil with a mean of 1238 µg/kg (n = 4, 0% not detected) and palm oil with a mean of 1224 µg/kg (n = 5, 0% not detected). Canola and soybean oils are the most commonly consumed/available in Japan (FAO, 2016); therefore, combining the concentration data into one group with oils with higher concentrations may lead to an overestimate of dietary exposure.
- No consumption data were listed for FA0818 (pig lard), so the concentration data from the GEMS/Food contaminants database for this food were coded to MF0100 – "Mammalian fats (except milk fats) and skin, nes".
- Concentration data from the GEMS/Food contaminants database for Japan included "fish oil supplements", which were excluded from the exposure estimate since national food consumption survey data in the CIFOCOss did not include dietary supplements.

- The CIFOCOss consumption data for Japan listed the code 02.1 as "Animal or vegetable fats, nes". It was assumed that the code 02.0.1 was the correct code for this food since it matches the description of the food group in the table of CIFOCOss classification codes.

Concentration data for glycidol from Japan are shown in Table 10, except for the data on infant formula, which are presented in Table 14.

(b) Concentration data from the USA

Data in the GEMS/Food contaminants database included samples of fats and oils collected from both the retail level (n = 116) and infant formula manufacturers (n = 55). All data were included when summarizing concentrations per fat/oil for the national and international estimates of dietary exposure on the assumption that concentrations by type of oil, regardless of sampling site, would be representative of concentrations in general purpose foods consumed by the population.

The concentration data from the GEMS/Food contaminants database for the USA included glycidol concentrations in butter. However, because an appropriate food classification code could not be identified in the CIFOCOss consumption data, butter is not included in the exposure estimate for the USA. This is unlikely to substantially impact the exposure estimate, as the reported concentration of glycidol in butter in the USA is low compared with other fats and oils.

Concentration data for glycidol from the USA are shown in Table 11, except for the data on infant formula, which are presented in Table 14.

8.2.5 International assessments of dietary exposure using concentration data from the GEMS/Food contaminants database

Each data point in the GEMS/Food contaminants database was assigned a cluster number according to the cluster for the country from which the data originated. Pooling occurrence data for the countries according to each cluster number was then possible. Foods in the GEMS/Food contaminants database were also assigned a food classification code (Level 3 GEMS/Food cluster diet) to enable means for each food group to be derived in order to calculate the dietary exposure for each cluster.

Concentration data for foods, other than infant formula, were only available for cluster G10. These data were from Japan, the USA and Canada (which, while not used for a national dietary exposure assessment and only combined with cluster G10 data, are summarized for information in Table 12). The LB (nondetects assigned a value of 0) and UB (nondetects assigned a value the same as the LOQ) mean concentrations for cluster G10 were derived for each

Table 10
Japan: Summary of glycidol concentration data used in the national estimates of dietary exposure

Food	CIFOCOss classification	No. of samples	No. of nondetects (<LOD)	% nondetects	Mean concentration (µg/kg)[a]	
					LB	UB
Animal or vegetable fats, nes	02.0.1	8	0	0	1 075	1 075
Vegetable fats (excluding oil), nes	02.1.2	98	5	5	583	592
Butter	02.2.1	25	21	84	6	46
Mammalian fats (except milk fats) and skin, nes	MF0100	23	23	21	209	209
Vegetable oils, nes	OR0172	111	0	0	931	969

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; UB: upper bound
[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ.

Table 11
USA: Summary of glycidol concentration data used in the national estimates of dietary exposure

Food	CIFOCOss classification	No. of samples	No. of nondetects (<LOD)	% nondetects	Mean concentration (µg/kg)[a]	
					LB	UB
Animal or vegetable fats, nes	02.0.1	6	2	33	170	170
Vegetable fats (excluding oil), nes	02.1.2	5	0	0	460	460
Butter	02.2.1	4	2	50	13	20
Vegetable oils, nes	OR 0172	29	6	21	830	833
Olive oil	OR 0305	10	5	50	239	246
Palm oil	OR 0696	26	2	8	3 542	3 543
Soybean oil	OR 0541	19	0	0	319	319
Coconut oil	OR 0665	21	4	19	355	358
Sunflower seed oil	OR 0702	8	1	13	275	277
Maize oil	OR 0645	10	0	0	653	653
Safflower seed oil	OR 0699	15	0	0	378	378
Rapeseed oil (including canola)	OR 0495	8	0	0	279	279
Sesame seed oil	OR 0700	3	1	33	77	82
Peanut oil and butter	OR 0697	5	2	40	291	297
Cottonseed oil	OR 0691	2	0	0	500	500

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; No.: number; UB: upper bound
[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ.

food group classification (see Table 13). The highest glycidol concentrations were found in rice bran oil (LB: 2894 µg/kg) and palm oil (LB: 3239 µg/kg).

Table 12
Canada: Summary of glycidol concentration data[a]

Food	No. of samples	No. of nondetects (<LOD)	% nondetects	Mean concentration (µg/kg)[a] LB	UB
Food for infants and small children, nes (excluding infant formula)	2	0	0	208	208
Snacks – potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	12	2	17	579	57
Vegetable fats (excluding oil), nes	12	3	25	216	218
Vegetable oils, nes	28	2	7	662	663
Olive oil	20	14	70	129	136
Palm oil	1	1	100	0	10
Coconut oil	4	1	25	579	582
Sunflower seed oil	6	2	33	209	212
Maize oil	2	0	0	140	140
Rape seed oil (including canola)	7	2	29	222	225
Sesame seed oil	5	0	0	335	335
Lard (of pigs)	2	0	0	38	38
Peanut oil and butter	4	2	50	281	286

LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; No.: number; UB: upper bound
[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ.

Table 13
Summary of glycidol concentration data in each food group in GEMS/Food cluster diet G10

Food	Level 3 code food classification	No. of samples	No. of nondetects (<LOD)	% nondetects	Mean concentration (µg/kg)[a] LB[b]	UB
Rice bran oil	36	26	0	0	2 894	2 894
Maize oil	60	24	1	4	764	767
Infant food (excludes infant formula)	109	2	0	0	208	208
Soybean oil	237	22	2	9	284	312
Groundnut oil	244	9	4	44	287	292
Palm oil	257	30	3	10	3 239	3 241
Palm kernel oil	258	2	0	0	516	516
Olive oil, virgin	261	40	26	65	175	209
Sunflower seed oil	268	21	6	29	219	241
Rapeseed oil	271	23	6	26	214	247
Safflower seed oil	281	19	1	5	335	351
Sesame seed oil	290	38	5	13	177	196
Cottonseed oil	331	2	0	0	500	500
Butter, cow milk	886	29	23	79	7	43
Oil fish, marine mammals	1 223	34	5	15	728	737

Table 13 (continued)

Food	Level 3 code food classification	No. of samples	No. of nondetects (<LOD)	% nondetects	Mean concentration (µg/kg)[a]	
					LB[b]	UB
Food prep, nes	1 232	12	2	17	57	57
Fat prep, nes	1 243	34	0	0	898	898
Coconut (copra) oil	9 017	28	5	18	365	367
Oil of vegetable origin, nes	9 019	73	10	14	674	682
Lard and lard stearin oil	9 035	25	0	0	195	195
Margarine (liquid and short)	9 041	82	7	9	420	428

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; No.: number; UB: upper bound

[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ.

[b] Per cent contribution of food groups (where at least one cluster had a contribution) to the dietary exposure to glycidol from GEMS/Food cluster diets are shown in Table A2 (in Appendix 1).

As there were no data for the other 16 clusters, the concentration data for cluster G10 were used for all other clusters to estimate dietary exposure. This assumes that food is traded in a global market and that concentrations from commodities grown in one area of the world are representative of other areas of the world. This may not in fact be the case for some fats and oils (e.g. palm oil). Therefore, the small number of data is a limitation of this assessment.

Concentration data for infant and follow-on formula and fish oil supplements were excluded from the cluster diet exposure calculations.

8.2.6 Assessment for infants fed infant formula or follow-on formula

(a) Concentration data

Glycidyl esters are found in infant and follow-on formulas, including powders, liquids and concentrates, from the oils that are used in the manufacture of the products.

A summary of the concentration data for glycidol in infant formula and/or follow-on formula is shown in Table 14. Some of these data were from the GEMS/Food contaminants database from Brazil, Canada, Japan and the USA. There were 230 data points in total and these included samples described as infant formula and follow-up/follow-on formula. Data were available for different forms of formula expressed as concentrates, powder and ready-to-drink. Data were also available for formula for special medical purposes. EFSA (2016) presented occurrence data for infant formula, as did a paper from Germany (Weisshaar, 2011). Except for the EFSA data, which EFSA used to conduct their own estimates of exposure and which JECFA reviewed for this evaluation, the Committee used all these data to estimate dietary exposure via infant formula.

Table 14
Summary of glycidol concentration data for infant formula products

Country / Region	Food	Sampling period	No. of samples	No. of nondetects (<LOD)	% nondetects	Concentration (µg/L)[a,b] LB mean	UB mean	P95	Reference / Source
Brazil	Follow-on formulas, powder	2015	40	13	33	27	35	NC	GEMS/Food contaminants database
Canada	Infant formulas (includes powder, concentrated and ready-to-drink forms)	2012–2013	31	20	65	13	20	NC	GEMS/Food contaminants database
Japan	Infant formulas, powder	2013–2014	23	8	35	5	8	NC	GEMS/Food contaminants database
	Follow-on formulas, powder		18	6	33	4	7	NC	GEMS/Food contaminants database
	Formula for special medical purposes		20	6	30	6	9	NC	GEMS/Food contaminants database
USA	Infant formula	2013–2016	89	1	1	13	13	NC	GEMS/Food contaminants database
	Follow-on formula		9	0	0	5	5	NC	GEMS/Food contaminants database
Germany	Infant and follow-on formula	2009–2010	40	NS	NS	April 2009: 63 October 2009: 42 May 2010: 17[c]		NS	Weisshaar (2011)
Europe	Infant formula, milk-based, powder	Not provided	70	NS specifically for this food[d]	26	11	13	30	EFSA (2016)

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; NC: not calculated; No.: number; NS: not specified; P95: 95th percentile; UB: upper bound

[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ. For Brazil, the LOQ was 200 µg/kg, for Canada 10 µg/kg and for Japan 60 or 200 µg/kg.

[b] Concentrations expressed as ready to consume.

[c] Not stated how the means were derived, or if they were LB or UB. It was also not stated if there were any nondetect results.

[d] The descriptor of the year of sampling for a number of foods, including infant formula, showed that samples were collected between 2012 and 2015, with most collected in 2014. However, the exact year the infant formula data were collected cannot be determined from the information provided.

Estimates of dietary exposure were calculated for different ages if the dataset provided enough details to permit occurrence data to be separated for infant formula (0–6 months of age) and follow-on formula (6–12 months of age).

A number of conversions were required to adjust the concentration data to make all data points in the same form (i.e. infant formula as consumed) to calculate dietary exposure. This was the case for data from the GEMS/Food contaminants database and the data from Germany. Concentrations in

formula powders were converted by multiplying the concentration by 0.13 as a reconstitution factor.[3] To convert concentrations provided on a fat basis to an as-consumed formula basis, values were multiplied by a factor of 0.04 based on the minimum fat content for infant formula in the Codex Standard,[4] which is 40% m/m. As consumption of formula for the dietary exposure calculations was on an as-consumed liquid basis (millilitres or litres), a density of 1.05 (FSANZ, 2013) was used to convert the concentrations from per kilogram to per litre. For data provided for infant formula concentrates, a dilution factor of 2 was applied (i.e. concentration × 0.5) to convert the concentrations to an as-consumed value.

Some data from Japan (n = 23) were for formula for special medical purposes and, while the summary of these data is shown in Table 14, they were excluded from the dietary exposure assessment as they are not used by the general population and were considered not relevant.

For the occurrence data from Germany, Weisshaar (2011) noted that the concentration of glycidol in infant formula products decreased over the course of three surveys between April 2009 and May 2010, from a mean of 1.5 to 0.4 mg/kg fat. For this reason, only the May 2010 results were used for the dietary exposure calculations.

(b) Methods for estimating dietary exposure to glycidol in infant formula

The Committee has previously published estimates of dietary exposure for infants to various food chemicals (e.g. food additives) in infant formula (Annex 1, reference *221*). This assessment also used this approach to estimate dietary exposures to glycidol in infant formula in the following way.

A number of sources of infant formula consumption data were used to estimate both the mean and a high exposure and to estimate exposure across the range of ages up to 12 months. Using a range of consumption data also takes into account, in an indirect way, variations in energy requirements, infant body weights and formula consumption amounts. Three sources of infant formula consumption data were combined with the occurrence data in Table 14 to estimate dietary exposures.

Median infant formula consumption estimates were derived from estimated energy requirements (EERs) for fully formula-fed infants. Standard body weights and EERs for male and female infants aged 1, 3 and 6 months were taken from daily human energy requirements defined in the *Human Energy*

[3] The dilution factor was derived from the reconstitution instructions on several labels of powdered infant formulas sold in Australia. The number calculated was consistent with that used in the EFSA (2016) assessment for glycidyl esters (inverse of 7.7 dilution factor) and other dietary exposure assessments involving analysis of powdered infant formula (Health Canada, 2008) (powdered = 0.124).

[4] Codex STAN 72-1981 requires minimum fat content to be 1.05 g per 100 kJ. Using the conversion 1 g fat = 31 kJ, this equates to approximately 40% m/m.

Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation (FAO/WHO/UNU, 2004). It should be noted that the EERs of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. EERs for formula-fed infants are used here. Table 15 summarizes the data and consumption amounts.

Another dietary exposure scenario used high (95th percentile) daily energy intakes reported by Fomon (1993) for formula-fed infants. Formula-fed males and females aged 1 month have EERs of 122 and 117 kcal/kg bw per day, respectively (FAO/WHO/UNU, 2004). Fomon (1993) reported maximum 95th percentile energy intakes for male and female infants at 14–27 days of age of 148.7 and 146.0 kcal/kg bw per day, respectively. For all dietary exposure estimates, a common formula energy density of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily. This resulted in a formula consumption amount of 222 mL/kg bw per day for males and 218 mL/kg bw per day for females. These amounts were used to estimate dietary exposures.

The German DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study measured actual consumption of infant formula at 3, 6, 9 and 12 months (Kersting et al., 1998). The study also reported high (95th percentile) consumption amounts of infant formula at each of the four ages mentioned. Intakes were reported in g/kg of dry powdered infant formula. Using typical preparation instructions for infant formula (13 g of powdered infant formula to yield 100 mL of ready-to-feed formula), the 95th percentile formula intakes at 3, 6, 9 and 12 months were 188, 122, 82 and 68 mL/kg bw per day. These high-percentile infant formula intakes are lower than those estimated using Fomon (1993) data and confirm that the use of high-percentile infant formula intake for infants 14–27 days old provides a suitable high-exposure scenario. Estimates of dietary exposure were still calculated based on data from the DONALD study as it included formula intake up to 12 months of age. The DONALD study estimated that, on average, 26.1% of infant energy intake at 12 months was from infant or follow-on formula consumption.

The DONALD study included infants aged 3, 6, 9 and 12 months and was used for estimates of dietary exposures for countries that had occurrence data for infant and follow-on formulas; FAO/WHO/UNU (2004) and Fomon (1993) only had data for up to 6 months of age and therefore could be used for calculations based on occurrence in infant formula only. Where the type of formula was not specified or where infant and follow-on formula data were combined, all three data sources for consumption were used for exposure estimate calculations.

EFSA estimated a mean exposure (based on mean occurrence level) and a high exposure (based on 95th percentile concentrations) to represent a brand loyal consumer or consumer of a contaminated bulk buy batch. The consumption data used were based on recommended volumes from the labels of seven products.

Table 15
Estimated infant formula consumption amounts for infants up to 6 months of age

Sex	Age (months)	Median body weight (kg)	EERs (kcal/day)[a]	Consumption (mL/day)[b]
Male	0–1	4.6	560	836
	2–3	6.3	629	939
	5–6	7.9	662	988
Female	0–1	4.4	509	760
	2–3	5.8	585	873
	5–6	7.3	626	934

EER: estimated energy requirement; FAO: Food and Agriculture Organization of the United Nations; UNU: United Nations University; WHO: World Health Organization
[a] Median body weights and EERs reported according to the Joint FAO/WHO/UNU expert report on human energy requirements (FAO/WHO/UNU, 2004).
[b] Volume of ingested formula based on a formula energy density of 67 kcal per 100 mL to meet an infant's energy requirements in full.

For a 1- to 4-month-old infant, the consumption value used was 170 g/kg bw per day, with WHO growth standards used to determine mean body weight.

8.3 Assessments of dietary exposure

8.3.1 National estimates

National estimates of dietary exposure to glycidyl esters, expressed as glycidol, are summarized in Table 16 and explained for each country in this section.

One estimate of dietary exposure in Germany, found in the literature, was reviewed (Weisshaar, 2011).

The Committee derived national estimates of dietary exposure using concentration data from the GEMS/Food contaminants database and consumption data from CIFOCOss for countries where both sets of data were available (i.e. Japan and the USA). Data for infant formula were excluded as separate dietary exposure assessments were conducted for this population group. High-percentile dietary exposure was calculated by applying a factor of 2 to the mean dietary exposure for the population to estimate the 90th percentile of exposure. Where the Committee calculated estimates of dietary exposure, the lower-bound scenario (zero concentration assigned to any result below the LOD) was used to determine the major contributors in order to not exaggerate the contribution of food groups that had nondetectable levels of glycidyl esters or glycidol.

(a) Japan

Although occurrence data for different types of oils were available, there was only one consumption line for oils for Japan. As a result, all of the occurrence data were pooled for the exposure calculation. Palm oil is known for high glycidol

Table 16
National dietary exposure estimates of glycidyl esters[a]

Country	Population[c]	Mean exposure (µg/kg bw per day)		High exposure[b] (µg/kg bw per day)	
		LB[d]	UB[d]	LB[d]	UB[d]
Japan	General population	0.16	0.17	0.33	0.34
	Children (age not specified)	0.38	0.40	0.76	0.79
USA	General population	0.18	0.18	0.37	0.37
	Children (age <6 years)	0.37	0.37	0.74	0.74

bw: body weight; CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound
[a] Expressed as glycidol, from the calculations conducted by the Committee.
[b] High exposure = mean exposure × 2, which approximates the 90th percentile.
[c] Age groups as reported in the CIFOCOss dataset.
[d] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where nondetect results (<LOD) were assigned a value equivalent to the LOQ.

concentrations, but these were still lower than the "not elsewhere specified" (nes) oils in the Japanese dataset, so exposure results were not necessarily skewed by combining oil concentrations into one category.

Estimated dietary exposures to glycidol for the general population for Japan (LB–UB µg/kg bw per day) ranged between 0.16–0.17 at the mean and 0.33–0.34 at the high percentile. For children, dietary exposures were about twice as high, at 0.38–0.40 at the mean and 0.76–0.79 at the high percentile.

The major contributor to estimated dietary exposures to glycidol for Japan (based on the lower-bound scenario) was "vegetable oils, nes" (OR0172) at 92% for both the general population and children. This was the classification for which all oils were coded as no specific consumption data were available. Therefore, it is not possible to determine what each type of oil in fact would contribute. The next highest contributor was "vegetable fats (excluding oil), nes" (classification code 02.1.2), at 8% for the general population and 7% for children. All other food groups ("animal or vegetable fats, nes"; "butter"; "mammalian fats (except milk fats)"; and "skin, nes") contributed less than 1%.

(b) USA

Estimated dietary exposures to glycidol (µg/kg bw per day) for the USA for the general population were 0.18 at the mean and at 0.37 for the high percentile. For children, dietary exposures were approximately twice as high, at 0.37 at the mean and 0.74 at the high percentile.

The major contributor to estimated dietary exposures to glycidol for the USA was soybean oil (68% for the general population; 67% for children). Other contributors included peanut oil and peanut butter (9% for the general

population; 12% for children), cotton seed oil and maize oil (7% each for each of the population groups assessed), rapeseed oil (4% for the general population; 3% for children) and palm oil (3% for the general population; 3% for children). All other foods contributed 1% or less. In relation to palm oil, Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) data (FAO, 2016) for the USA show consumption of palm kernel oil, not palm oil; therefore, this may be an overestimate of the contribution.

(c) Germany

One estimate of dietary exposure to glycidol in Germany was reviewed (Weisshaar, 2011). This estimate, found as a result of a literature search, was based on concentrations of glycidol in oils from 200 samples collected in 2009/2010. Samples included palm oil (median concentration 2.5 mg/kg), soybean oil (500 µg/kg), rapeseed oil (100 µg/kg), sunflower seed oil (400 µg/kg), palm kernel oil (500 µg/kg), corn oil (600 µg/kg) and coconut oil (500 µg/kg). Dietary exposures were based on tonnage of oil available for Germany assuming 50% was used for human food consumption, and per capita exposure estimated at 51.8 µg/person per day (or 0.9 µg/kg bw per day assuming a 60 kg body weight). Palm oil was the highest contributor to the glycidol exposure, at 73%, followed by soybean oil at 9% and all other oils at 5% or less. Because this calculation was based on oil available for consumption, it would be similar to those estimated using food balance sheet data and therefore may overestimate actual dietary exposures based on reported food consumption amounts by individuals.

(d) Europe

EFSA (2016) estimated dietary exposure to glycidol from esters for a number of European countries and presented a summary of the estimates (Table 17). Estimated dietary exposures were calculated using consumption data from the EFSA Comprehensive European Food Consumption Database, which included consumption data from 17 countries collected from individuals using one or more 24-hour recalls or 3- to 7-day dietary records.

Concentrations of glycidol from esters were from fats and oils (n = 4754) and other foods (n = 1719) including infant formula powder, cereal-based products (bread, breakfast cereals, cookies, pastries), meat and fish (fried or baked), smoked meat or fish and potato snacks. The data used were sampled between 2012 and 2015. The highest concentrations were in palm oil (mean middle bound of 3955 µg/kg; middle bound where there were nondetect results were assigned a value equivalent to the LOD/2 or LOQ/2).

The main contributor to middle bound dietary exposure in infants (<12 months) consuming a mixed diet was infant formula (50%). For toddlers (≥12

Table 17
Summary of EFSA estimates of dietary exposure to glycidol from esters

Population group (age)	Range of exposures[a] (µg/kg bw per day)	
	Mean	95th percentile
Infants (<12 months)[b]	0.3–0.8	1.2–2.2
Toddlers (≥12 to <36 months)	0.4–0.9	1.0–2.1
Other children (≥36 months to <10 years)	0.3–1.0	0.8–1.7
Adolescents (≥10 to <18 years)	0.2–0.5	0.4–1.1
Adults (≥18 to <65 years)	0.1–0.3	0.3–0.7
Elderly adults (≥65 to <75 years)	0.1–0.3	0.2–0.6
Very elderly adults (≥75 years)	0.1–0.3	0.2–0.8

bw: body weight; EFSA: European Food Safety Authority; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound
[a] Includes LB (nondetect results assigned the value of 0) and UB estimates (nondetect results assigned the LOD or LOQ).
[b] Before and after weaning.
Source: EFSA (2016)

to <36 months), the major contributor was vegetable fats and oils. For other children (≥36 months to <10 years), adolescents (≥10 years to <18 years), adults (≥18 years to <65 years), elderly adults (≥65 years to <75 years) and very elderly adults (≥75 years), the main contributor was margarines.

8.3.2 International exposure estimates

The estimated dietary exposures to glycidyl esters, expressed as glycidol, across all 17 clusters (Table 18) ranged from 0.16 to 1.02 µg/kg bw per day at the mean LB and from 0.16 to 1.03 µg/kg bw per day for the UB mean, and for the high percentile between 0.32 and 2.03 µg/kg bw per day at the LB and from 0.32 to 2.05 µg/kg bw per day at the UB.

The major contributors to dietary exposures (based on the LB scenario) differed depending on the cluster. The major contributor for 11 of the clusters was palm oil, with contributions of up to 88% of glycidol exposure. Margarine was the highest contributor for five of the clusters (with up to 40% contribution to glycidol exposure).

The highest exposures (>0.5 µg/kg bw per day at the mean) were calculated for clusters G03, G04 and G11. For clusters G03 and G04, the higher estimated dietary exposures (LB–UB mean of 0.60–0.60 and 1.02–1.03 µg/kg bw per day, respectively) were likely due to high consumption of palm oil (9.8 and 14.8 g/day for clusters G03 and G04, respectively, with all other clusters consuming 6.7 g/day or lower). Palm oil also contained the highest glycidol concentration (LB–UB range: 3239–3240 µg/kg) out of all the foods included. For cluster G11, the relatively high estimated dietary exposure (mean of 0.6 µg/kg bw per day) was likely due to high consumption of margarine (28.3 g/day, whereas all other

Table 18
Estimated dietary exposures to glycidyl esters for the GEMS/Food cluster diets

	Estimated exposures to glycidyl esters (µg/kg bw per day)[a,b]			
	Mean		High percentile[c]	
Cluster diet	LB	UB	LB	UB
G01	0.44	0.45	0.88	0.9
G02	0.2	0.21	0.39	0.41
G03	0.6	0.6	1.2	1.2
G04	1.02	1.03	2.03	2.05
G05	0.38	0.38	0.75	0.76
G06	0.35	0.36	0.7	0.72
G07	0.43	0.46	0.87	0.92
G08	0.31	0.33	0.61	0.67
G09	0.34	0.34	0.67	0.68
G10	0.4	0.43	0.81	0.85
G11	0.6	0.62	1.21	1.24
G12	0.29	0.29	0.58	0.58
G13	0.46	0.46	0.93	0.93
G14	0.16	0.16	0.32	0.32
G15	0.31	0.32	0.61	0.65
G16	0.29	0.29	0.57	0.58
G17	0.48	0.48	0.96	0.97

bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; UB: upper bound
[a] Expressed as glycidol.
[b] Based on average adult body weight of 60 kg.
[c] The high percentile was calculated by applying a factor of 2 to the mean dietary exposure to estimate the 90th percentile of exposure. Exposures as shown may not always appear as a factor of 2 due to rounding.

clusters consumed 18.2 g/day or less). Margarine had a moderately high glycidol concentration (LB–UB range: 420–428 µg/kg).

8.3.3 Estimated dietary exposures for infants

Estimated dietary exposures from infant formula were calculated for infants aged 0–12 months. This population includes those fed solely formula and those also eating solid foods (Table 19).

Estimates of dietary exposure to glycidol from infant formula (depending on the country, type of formula and method used) at the mean ranged between 0.1 and 3.6 µg/kg bw per day. High dietary exposures (which are based on either 95th percentile consumption or on 95th percentile concentrations, depending on the assessment) ranged between 0.3 and 4.9 µg/kg bw per day.

Where countries had data for both infant and follow-on formula (Japan and the USA), estimated dietary exposures were lower for follow-on formula.

Table 19
Summary of estimates of dietary exposure to glycidyl esters[a] for infants from infant formula

Country/ Region	Food	Data source	Dietary exposure[a,b] (µg/kg bw per day)			
			Mean LB	Mean UB	P95 LB	P95 UB
Brazil	Follow-on formula	DONALD	1.0–1.1	1.2–1.4	1.8–2.2	2.4–2.9
Canada	Infant formula	FAO/WHO/UNU (2004)	1.6–2.4	2.5–3.6	NC	NC
		Fomon (1993)	NC	NC	2.8–2.9	4.4
		DONALD	0.5–1.7	0.7–2.6	0.9–2.4	1.4–3.8
Europe	Infant formula	EFSA (2016)	1.8	2.1	4.9	0.76
Germany	Infant and follow-on formula	FAO/WHO/UNU (2004)	2.1–3.1	2.1–3.1	NC	NC
		Fomon (1993)	NC	NC	3.7–3.8	3.7–3.8
		DONALD	0.6–2.2	0.6–2.2	1.2–3.2	1.2–3.2
Japan	Infant formula	FAO/WHO/UNU (2004)	0.6–0.9	1.0–1.5	NC	NC
		Fomon (1993)	NC	NC	1.1–1.1	1.7–1.8
		DONALD	0.3–0.7	0.5–1.1	0.6–0.9	1.0–1.5
	Follow-on formula	DONALD	0.1–0.2	0.2–0.3	0.3–0.3	0.5–0.6
USA	Infant formula	FAO/WHO/UNU (2004)	1.6–2.4	1.6–2.4	NC	NC
		Fomon (1993)	NC	NC	2.8–2.9	2.8–2.9
		DONALD	0.9–1.7	0.9–1.7	1.6–2.4	1.6–2.4
	Follow-on formula	DONALD	0.2–0.2	0.2–0.2	0.3–0.4	0.3–0.4

bw: body weight; DONALD: DOrtmund Nutritional and Anthropometric Longitudinally Designed Study; EFSA: European Food Safety Authority; FAO: Food and Agriculture Organization of the United Nations; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; NC: not calculated; P95: 95th percentile; UB: upper bound; UNU: United Nations University; WHO: World Health Organization

[a] Expressed as glycidol.

[b] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ. Ranges are based on multiple age + sex groups for the one type of formula.

This is likely due to a number of reasons, including the lower concentration of glycidol in follow-on formula based on the data available, a higher body weight for these infants, lower consumption of formula due to a smaller proportion of energy intake coming from formula due to the consumption of other foods, and lower formula consumption per kilogram of body weight.

8.4 Limitations and uncertainties in the dietary exposure assessment

Overall, there is a limited amount of information currently available for glycidyl esters or glycidol in terms of occurrence and estimates of dietary exposure. Data are available from Europe and North America and, to a lesser extent, from Asia, but there are none for most other regions. This could be important when making risk assessment conclusions from estimates of dietary exposure that do not include countries with a higher consumption of palm oil and other oil sources with high concentrations of glycidyl esters. In order to make more definitive and holistic conclusions regarding global dietary exposures to glycidyl esters, it would

be useful to have more information on occurrence and dietary exposure from more countries from a variety of regions. This would also mean that international dietary exposure estimates for a broader range of clusters could be determined more reliably.

The number of national estimates of dietary exposure that can be conducted at present is limited by the number of countries for which both consumption data in the CIFOCOss dataset and occurrence data are available. Countries with relevant consumption data are encouraged to submit these to WHO for inclusion in the CIFOCOss dataset.

Where occurrence data for glycidyl esters are submitted to the GEMS/Food contaminants database, it is important to note the method of analysis used to determine the concentrations and the form of the chemical for which the results apply (e.g. esters or glycidol). This would limit the uncertainty around interpreting the occurrence data and the need for making assumptions about the form of the data.

The LOD and LOQ for analytical methods need to be sufficiently low to enable the most realistic estimates of dietary exposure possible when there are nondetected values for samples in the dataset, particularly not to exaggerate mean UB concentrations and estimates of dietary exposure. Some of the LODs/LOQs in the current dataset were high. However, due to the small number of samples in the total dataset, no data points were excluded based on a high LOD/LOQ. This may exaggerate UB estimates of dietary exposure, and therefore could be considered a worst-case scenario.

In relation to occurrence data, the majority of the data for glycidyl esters or glycidol are currently for fats and oils. Where consumption data are expressed on a raw commodity basis and therefore capture all uses of fats and oils in the diet (e.g. in mixed dishes, for frying, in processed foods), it is less likely that this would result in an underestimate of dietary exposure. However, if fat or oil consumption data do not include all sources, more occurrence data in other foods, particularly those that are fat or oil based, are required to estimate total dietary exposures more reliably.

In the occurrence dataset, there were often very small numbers of samples for specific types of fats/oils. These were not excluded from the estimates of dietary exposure given the small total dataset to begin with. In addition, excluding foods with small sample numbers would have left very few data points to use in the exposure calculations. Therefore, additional samples for certain types of fats and oils would reduce the uncertainty around the mean concentrations derived for these foods for use in the exposure assessment calculations.

Concentrations of glycidyl esters vary widely in oils as they are highly dependent on the oil processing conditions and methods and the processing plant. This means that there is some uncertainty in the dietary exposure estimates,

particularly if they are based on only certain oils. In addition, because of the small number of occurrence data, the high degree of variation may not have been captured. This is another reason why a larger number of occurrence data would be useful.

9. Dose–response analysis and estimation of toxic/carcinogenic risk

9.1 Identification of key data for risk assessment

9.1.1 Pivotal data from biochemical and toxicological studies

Complete hydrolysis of glycidyl esters to glycidol in the gastrointestinal tract was assumed for the present evaluation based on in vivo (Wakabayashi et al., 2012; Appel et al., 2013) and in vitro (Frank et al., 2013) experimental data. Glycidol is genotoxic and carcinogenic in various organs in male and female rats and mice. The oral carcinogenicity studies with mice and rats by NTP (1990) are considered the pivotal studies for risk assessment. Fertility in male rats was also impaired at a similar dose based on significantly decreased sperm count at and above the lowest tested oral glycidol dose of 17.9 mg/kg bw per day for 13 weeks (NTP, 1990). Other effects on male fertility and on neurotoxicity in rats and mice were seen consistently in several limited studies and occurred generally at higher doses.

Previous risk assessments for glycidol by national and international bodies, for example, EFSA (2016), Food Safety Commission of Japan (FSCJ, 2015) and United States Environmental Protection Agency (USEPA, 2010), also considered the NTP carcinogenicity studies in rats and mice as pivotal studies in risk assessment.

9.2 General modelling considerations

9.2.1 Selection of data

The Committee determined that the two oral long-term NTP (1990) studies of toxicity and carcinogenicity of glycidol administered to rats and mice were suitable for dose–response analysis.

9.2.2 Measure of exposure

In both studies, glycidol was administered by gavage for 2 years to mice and rats of both sexes. See Table 4 and Table 5 for additional details and tabular results from both studies.

9.2.3 Measure of response

Statistically significantly increased tumour incidences were seen in various organs in mice and rats of both sexes. Benchmark doses were calculated for all tumour end-points with a clear dose–response, and the lowest lower 95% confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) was chosen as the point of departure.

This approach is different from that of EFSA (2016), which calculated the response using the T25 as the point of departure (Sanner et al., 2001). (The T25 is defined as the chronic dose rate that will give 25% of the test animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard lifetime of that species.) EFSA used the T25 in that analysis as there were only two dose groups (and a control group), and it was thought that there was not enough information to adequately model the dose–response. For the present analysis, the Committee determined that there was enough information to model the dose–response and the benchmark dose for a 10% inhibition (BMD_{10}) as well as the $BMDL_{10}$.

9.2.4 Selection of mathematical model

The current analysis used USEPA Benchmark Dose Software (BMDS) version 2.6.1 (USEPA, 2016) using the following eight restricted models: quantal linear, multistage (2 degree), Weibull, gamma, logistic, log logistic, probit and log probit. All models with a Pearson chi-squared goodness-of-fit test statistic above 0.1 were considered adequate. Fully saturated models, that is, models that perfectly interpolate the data points with zero degrees of freedom, were excluded. BMD_{10} and $BMDL_{10}$ values for neoplasms in mice and rats are shown in Table 20 and Table 21, respectively.

Table 22 and Fig. 4 describe the fit of these eight models to the mesotheliomas in the tunica vaginalis/peritoneum in male rats, which had the lowest $BMDL_{10}$, of 2.38 mg/kg bw per day (dose adjusted from 5 to 7 days/week) of all tumour end-points.

From the acceptable models, the lowest $BMDL_{10}$ was computed using the quantal linear model. Fig. 4 gives the predicted curve of the quantal linear model, which allows a visual inspection of this model from the given data. The gamma, Weibull and multistage (2 degree) models also provided the exact same BMD_{10}/$BMDL_{10}$. This is caused by their parameters hitting the bounds and degenerating into the quantal linear model.

Table 20
Ranges of BMD_{10} and $BMDL_{10}$ values for neoplasms in mice

End-point	BMD_{10} [a] (mg/kg bw per day)	$BMDL_{10}$ [a] (mg/kg bw per day)
Male		
Harderian gland (adenoma or carcinoma)	8.2–11.5	5.3–8.9
Forestomach (squamous cell papilloma or carcinoma)	24.3–27.8	14.8–22.8
Skin (squamous cell papilloma or carcinoma)	36.0–68.6	31.6–33.4
Liver (adenoma or carcinoma)	5.4–7.9	2.6–5.2
Lung (alveolar/bronchiolar adenoma or carcinoma)	18.6–33.9	9.2–13.6
Female		
Harderian gland (adenoma or carcinoma)	15.7–17.1	13.9–15.0
Mammary gland (adenoma, fibroadenoma or adenocarcinoma)	13.7–20.3	9.1–16.4
Uterus (carcinoma or adenocarcinoma)	45.0–46.3	24.6–31.4
Subcutaneous tissue (sarcoma or fibrosarcoma)	21.9–28.6	7.6–23.7
Skin (squamous cell papilloma or carcinoma)	36.7–139.3	35.6–52.5

BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
[a] Calculated for doses administered orally to mice for 5 days/week for 2 years (NTP, 1990).

Table 21
Ranges of BMD_{10} and $BMDL_{10}$ values for neoplasms in rats

End-point	BMD_{10} [a] (mg/kg bw per day)	$BMDL_{10}$ [a] (mg/kg bw per day)
Male		
Tunica vaginalis/peritoneum (mesothelioma)	2.97–7.98	2.38–6.33
Mammary gland (fibroadenoma)	14.5–22.2	8.9–16.6
Brain (glioma)	26.0–38.1	16.4–30.2
Forestomach (papilloma or carcinoma)	42.9–43.1	23.6–33.9
Intestine (adenomatous polyp or adenocarcinoma)	51.6–67.5	35.0–43.3
Zymbal gland (carcinoma)	50.2–53.8	28.9–38.6
Thyroid gland (follicular cell adenoma or carcinoma)	22.4–31.8	13.2–24.5
Female		
Mammary gland (fibroadenoma)	5.9–10.2	4.2–7.2
Mammary gland (adenocarcinoma)	13.6–20.6	9.8–16.0
Brain (glioma)	32.4–44.6	19.1–33.7
Oral mucosa (papilloma or carcinoma)	35.1–34.7	14.8–26.9
Forestomach (papilloma or carcinoma)	15.9–32.0	10.6–25.2
Clitoral gland (adenoma, adenocarcinoma or carcinoma)	27.7–35.4	15.3–23.3
Thyroid gland (follicular cell adenoma or carcinoma)	55.9–73.7	35.8–44.4
Haematopoietic system (leukaemia)	17.5–30.9	9.7–13.6

BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
[a] Calculated for doses administered orally to rats for 5 days/week for 2 years (NTP, 1990).

Table 22

Dose–response modelling of mesothelioma in the tunica vaginalis/peritoneum in male rats

Model	BMD_{10} (mg/kg bw per day)[a]	$BMDL_{10}$ (mg/kg bw per day)[a]	*P* value	AIC	Accepted
Restricted models					
Gamma	2.97	2.38	0.388	132.88	Yes
Logistic	7.98	6.33	0.0030	141.02	No
Log logistic[b]	2.59	0.960	N/A	134.14	No
Probit	7.75	6.31	0.0027	141.38	No
Log probit	5.36	4.19	0.508	132.57	Yes
Weibull	2.97	2.38	0.388	132.88	Yes
Multistage	2.97	2.38	0.388	132.88	Yes
Quantal linear	2.97	2.38	0.388	132.88	Yes

AIC: Akaike information criterion; BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight; N/A: not applicable

[a] Calculated for doses administered for 5 days/week.

[b] Model was fully saturated/perfectly fit the data with zero degrees of freedom.

Fig. 4

Fit of the quantal linear model to the observed mesotheliomas in the tunica vaginalis/peritoneum in male rats

BMD: benchmark dose; BMDL: lower 95% confidence limit on the benchmark dose; BMR: benchmark response

10. Comments

10.1 Biochemical aspects

10.1.1 Glycidyl esters

Seven glycidyl esters (glycidyl laurate, myristate, palmitate, stearate, oleate, linoleate and linolenate) were shown to be rapidly (within 15 minutes) and fully hydrolysed by lipase from *A. niger* in a static in vitro system with gastric electrolyte solution at pH 4.8. In a dynamic gastrointestinal tract model simulating the different gastrointestinal compartments, the efficient degradation of the seven different glycidyl esters was confirmed using milk as a food matrix. It was shown for deuterated glycidyl oleate and glycidyl palmitate that the major hydrolysis product was glycidol (92%). The chain length of the fatty acids (12–18 carbons) did not have a significant impact on kinetics (Frank et al., 2013).

Following dosing of male rats with [2-^{14}C]glycidyl palmitate by gavage at a single dose of 209.4 mg/kg bw, tissue distribution and excretion were investigated. Seven days after dosing, 41.3% of the ^{14}C activity had been excreted in urine, 32.9% in expired air and 21.6% in faeces; 9.1% remained in tissues and organs. The highest concentrations of retained radiolabel were in liver, skeletal muscle, bone and erythrocytes 24 hours and 7 days after administration (Appel et al., 2013).

Male rats that had received a single equimolar dose of glycidol (50 mg/kg bw) or glycidyl palmitate (209.4 mg/kg bw) by gavage showed similar concentrations of the glycidol-derived haemoglobin adduct diHOPrVal in blood. Although the same steady-state level of haemoglobin adducts was seen for both substances, the level was reached for glycidyl palmitate with a delay of approximately 4–8 hours compared with glycidol. Concentrations of 2,3-dihydroxypropyl mercapturic acid excreted in urine were also similar for both substances at the three sampling time points (0–8 hours, 8–24 hours, 24–48 hours), reaching a recovery of approximately 14% of the dose as 2,3-dihydroxypropyl mercapturic acid 48 hours after administration. Based on the measurements of these two biomarkers, the authors concluded that glycidyl palmitate was rapidly and efficiently hydrolysed to glycidol (Appel et al., 2013).

Male rats and male cynomolgus monkeys were administered a single gavage or intravenous dose of glycidol (75 mg/kg bw) or a single equimolar gavage dose of glycidyl linoleate (341 mg/kg bw), and glycidol concentrations in plasma were monitored (for 24 hours in rats, 96 hours in cynomolgus monkeys). Glycidyl linoleate was not detectable in either species at any sampling time point. In the rat, maximum glycidol concentrations in plasma were reached 15–30 minutes after oral dosing, and concentrations were nondetectable after 24 hours for both

substances. Similar blood kinetics for glycidol were observed for the administered glycidyl linoleate compared with glycidol in rats. In cynomolgus monkeys, the AUC for glycidol in plasma was only 56% following orally administered glycidyl linoleate compared with glycidol. Times to reach maximum blood values were 4 times longer in cynomolgus monkeys than in rats, whereas C_{max} values after oral dosing of glycidol and glycidyl linoleate were also significantly reduced for cynomolgus monkeys compared with rats (factor of 4 for glycidol and factor of 17 for glycidyl linoleate). Although only a small number of cynomolgus monkeys were available at each sampling point ($n = 3$) and only "free" glycidol was measured in blood, the authors suggest that the pharmacokinetic differences between rats and cynomolgus monkeys might be attributable to differences in lingual or gastric lipase activity, stomach pH or epoxide metabolism (Wakabayashi et al., 2012).

In conclusion, glycidyl esters are efficiently hydrolysed in rats following oral dosing, resulting in the release of free glycidol. For cynomolgus monkeys, hydrolysis of glycidyl esters in the gastrointestinal tract is also evident, but to a lesser extent compared with rats. There are no human studies currently available describing the hydrolysis of glycidyl esters. Based on the results from in vitro gastrointestinal tract simulation models and in vivo evidence in rats, the Committee concluded that substantial hydrolysis of glycidyl esters to glycidol is likely to occur in the gastrointestinal tract. For the purpose of the current assessment, complete hydrolysis of the glycidyl esters is assumed.

10.1.2 Glycidol

Approximately 87–92% of orally administered [1,3-^{14}C]glycidol was absorbed from the gastrointestinal tract of male rats administered a single oral dose (gavage) of 37.5 or 75 mg/kg bw. Similar disposition kinetics were observed for oral and intravenous dosing, with 40–48% of the radioactivity excreted in urine, 5–12% in faeces, 26–32% exhaled as carbon dioxide and 7–8% retained in tissues within 72 hours (9–12% within 24 hours) after administration; highest concentrations of radioactivity were in blood cells, thyroid, liver, kidney and spleen. Fifteen different metabolites were detected in urine, but not further identified (Nomeir et al., 1995).

In the previously described study by Wakabayashi et al. (2012), absolute systemic bioavailability of glycidol was estimated as 69% in rats and 34% in cynomolgus monkeys, respectively.

Glycidol has been shown to be conjugated with glutathione and excreted in urine of rats and mice as *S*-(2,3-dihydroxypropyl)cysteine and 2,3-dihydroxypropyl mercapturic acid (Jones, 1975). In vitro investigations with rat liver and pulmonary microsomes have demonstrated that glycidol can be conjugated with glutathione or hydrolysed to form glycerol. The formation

of glycerol from glycidol is catalysed by epoxide hydrolases (Jones, 1975; Patel, Wood & Leibman, 1980).

Human and rat blood samples were incubated with varying concentrations of glycidol for 1 hour at 37 °C, and a dose-dependent formation of diHOPrVal–haemoglobin adducts was observed, with no significant species differences (Honda et al., 2014).

Rats received a single oral dose of glycidol (4.92–75 mg/kg bw), and whole blood was analysed for diHOPrVal–haemoglobin adducts 24 hours after dosing. A dose-dependent increase in diHOPrVal levels was observed, which was statistically significantly different from control levels in all dose groups (Honda et al., 2014).

10.2 Toxicological studies

10.2.1 Glycidyl esters

No oral repeated-dose toxicity studies in rodents administered glycidyl esters were identified. The only available study is on various genotoxicity end-points using glycidyl linoleate in comparison with glycidol, which indicates that this ester is less genotoxic than glycidol. Although glycidol was positive in vitro in all tested bacterial strains (*S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA, with and without metabolic activation), glycidyl linoleate tested positive only in strains TA100 and TA1535 with and without metabolic activation and in *E. coli* WP2uvrA with metabolic activation. In the in vitro chromosomal aberration test with Chinese hamster lung cells, glycidol induced structural aberrations but no numerical aberrations, whereas glycidyl linoleate was negative for both end-points. When tested in vivo, neither substance induced micronuclei in the bone marrow of mice (Ikeda et al., 2012).

10.2.2 Glycidol

The oral LD_{50} of glycidol is 450 mg/kg bw in mice and 420–850 mg/kg bw in rats (Hine et al., 1956; Thompson & Hiles, 1981; Thompson & Gibson, 1984).

In oral short-term toxicity studies with mice and rats, significantly reduced sperm count and reduced sperm motility were observed at doses of 53.6 mg/kg bw per day and higher in mice and at doses at and above the lowest tested dose of 17.9 mg/kg bw per day in rats (doses adjusted to 7 days/week dosing). Effects at higher doses (generally above 100 or 200 mg/kg bw per day; doses adjusted to 7 days/week dosing) included effects on kidney (tubule degeneration) and brain (demyelination in medulla and thalamus and/or necrosis in the cerebellum) in both species, as well as testicular atrophy in rats (NTP, 1990).

In oral long-term studies on toxicity and carcinogenicity in mice and rats, glycidol induced tumours in various tissues in both sexes at doses equal to and greater than the lowest tested doses of 17.9 and 26.8 mg/kg bw per day for mice and rats, respectively (doses adjusted to 7 days/week dosing) (NTP, 1990). The NTP (1990) concluded that there was "clear evidence for carcinogenic activity" in male mice based on increased incidences of neoplasms of the Harderian gland, forestomach, skin, liver and lung, and in female mice based on increased incidences of neoplasms of the Harderian gland, mammary gland, uterus, subcutaneous tissue and skin. In rats, the NTP (1990) concluded that there was "clear evidence of carcinogenic activity" in male rats based on increased incidences of mesotheliomas of the tunica vaginalis, fibroadenomas of the mammary gland, gliomas of the brain and neoplasms of the forestomach, intestine, skin, Zymbal gland and thyroid gland, and in female rats for increased incidences of fibroadenomas and adenosarcomas (now termed carcinosarcoma) of the mammary gland, gliomas of the brain, neoplasms of the oral mucosa, forestomach, clitoral gland and thyroid gland and leukaemia. Glycidol administered orally (17.9–142.9 mg/kg bw per day, doses adjusted to 7 days/week dosing) for 40 weeks to transgenic $p16^{Ink4a}/p19^{Arf}$ mice induced increased incidences of histiocytic sarcomas and alveolar/bronchiolar adenoma (NTP, 2007), in contrast to another transgenic mouse strain ($p53^{+/-}$ mice), in which no tumours were reported after 6 months of oral administration of glycidol at 25–50 mg/kg bw per day (Tennant et al., 1999, cited in NTP, 2007).

Glycidol was clearly genotoxic in vitro in many bacterial and mammalian cell assays on mutagenicity with and without an exogenous metabolic system and induced sex-linked recessive lethal mutations and heritable translocations in *D. melanogaster*, sister chromatid exchanges and chromosomal aberrations in Chinese hamster cells, DNA strand breaks in CHO cells, rat kidney epithelial cells and human embryonic kidney cells as well as sister chromatid exchanges and chromosomal aberrations in human lymphocytes (IARC, 2000; El Ramy et al., 2007; Ikeda et al., 2012; Aasa et al., 2016; Ozcagli et al., 2016). Glycidol also tested mostly positive in vivo, including DNA strand breaks in rat liver and urinary bladder (Wada et al., 2014), induction of micronuclei (NTP, 1990) and chromosomal aberrations and sister chromatid exchanges in mouse bone marrow (Sinsheimer et al., 1993).

In addition, the reactivity of the epoxy moiety adds evidence for the mutagenicity of glycidol via direct DNA interaction without need of prior metabolic activation.

Several studies on effects on reproduction and fertility were identified; however, none of them meets modern standards as a result of unconventional experimental design. Oral administration of glycidol at 200 (but not at 100) mg/kg bw per day for 5 days was shown to induce temporary/reversible infertility

and spermatocoeles in male rats (Cooper, Jones & Jackson, 1974). Intraperitoneal administration of glycidol at approximately 3.5 mg/kg bw per day for 14 days was shown to reduce sperm motility in male rats without affecting fertility, in contrast to equimolar 3-MCPD, which produced complete infertility (Brown-Woodman, White & Ridley, 1979).

In an oral 14-day study on immunotoxicity in mice, glycidol doses of 125–250 mg/kg bw per day were shown to significantly alter several immune modulatory end-points compared with controls, whereas two bacterial host susceptibility tests were negative, indicating no effects on cell-mediated immunity functions; the NOAEL for immunotoxicity was 25 mg/kg bw per day (Guo et al., 2000).

In an oral 4-week study on neurotoxicity in rats, decreased relative brain weight, abnormal gait and axonopathy in the central and peripheral nervous systems were observed at glycidol doses of 200 mg/kg bw per day; the NOAEL was 30 mg/kg bw per day (Akane et al., 2014a).

In an oral developmental neurotoxicity study in which pregnant rats were dosed from gestation day 6 until postnatal day 21, abnormal gait and axon injury in the central and peripheral nervous systems were observed at glycidol doses of 108.8 mg/kg bw per day in dams. No changes in reproductive end-points were seen in dams at any dose. The maternal toxicity NOAEL was 48.8 mg/kg bw per day. The NOAEL for offspring toxicity was 18.5 mg/kg bw per day, based on reduced body weight gain at higher doses. At 108.8 mg/kg bw per day, histopathological changes in the brain were observed in offspring, whereas gait was unaffected at any dose (Akane et al., 2013).

10.3 Observations in humans

No clinical or epidemiological studies were identified.

10.4 Analytical methods

During studies related to indirect 3-MCPD ester analysis, artificially elevated concentrations of 3-MCPD were obtained when chlorinated compounds were used in the analytical procedure, relative to results from methods that used other reagents (e.g. sodium bromide, ammonium sulfate) (Weisshaar & Perz, 2010; Kuhlmann, 2011). This indicated that structurally related compounds were present in the oils or fats, and conversion to 3-MCPD was a function of the analytical procedure (Weisshaar & Perz, 2010).

Both indirect and direct methods of analysis are used for the determination of glycidyl ester concentrations in edible oils and foods. The

indirect methods, which are used most frequently, require hydrolytic cleavage of the esters from the glycidol moiety prior to analysis, similar to indirect methods for the measurement of MCPD esters (Crews et al., 2013). Using direct methods, the analysis of intact glycidyl esters is performed without cleavage of the fatty acid esters from the glycidol moiety. Glycidyl ester analysis generally includes the addition of isotopically labelled (deuterated or ^{13}C) MCPD, MCPD esters or glycidyl esters prior to extraction of samples to allow for correction of any losses or conversion that may occur during sample preparation, followed by extraction with solvent.

The presence of the glycidol epoxide ring necessitates the development of stable intermediates through a reaction of the glycidol moiety with a nucleophilic agent (e.g. sodium chloride or bromide), which may be performed either before or after hydrolysis of the ester group. The reaction to cleave the fatty acid esters from the glycidyl esters has been successfully performed using acidic or alkaline conditions or via enzymatic cleavage in a low pH environment (pH 5). Following completion of the cleavage of fatty acid methyl esters from the glycidyl esters, the reaction is stopped by balancing the pH in the reaction vessel. Addition of sodium chloride or sodium bromide results in the formation of MCPD or monobromopropanediol, with analysis following derivatization, which is most frequently reported using phenylboronic acid (Crews et al., 2013).

Indirect methods that involve the comparison of results obtained for MCPD alone or MCPD concentrations based on the sum of MCPD plus the MCPD formed through the conversion of glycidol originating from glycidyl esters require application of a conversion factor (0.67) to correct for the molecular weight difference between MCPD (110.54 g/mol) and glycidol (74.08 g/mol) (Weisshaar & Perz, 2010). The results obtained using this approach are based on the assumption that complete conversion of glycidol to MCPD has occurred and that the only source of the additional MCPD is from glycidyl esters (Kuhlmann, 2011).

Three indirect official methods pertaining to the analysis of glycidyl esters exclusively in oils and fats have been developed by the AOCS (AOCS, 2013a,b,c). One of the AOCS official methods quantifies glycidol by the difference in the 3-MCPD concentrations measured in paired samples, where the glycidol in one sample of the pair has been converted to MCPD while the other sample is not subject to the conversion, and therefore the result is based on MCPD concentrations alone. AOCS Official Method Cd 29c-13 (AOCS, 2013c) is harmonized with the international standard method in fats and oils (ISO 18363-1) (British Standards Institution, 2015).

Direct analysis of intact glycidyl esters, where glycidyl esters are isolated from samples and analysed without cleavage of esters, is also performed in some laboratories. Analysis of extracted samples is generally performed following a two-

step cleanup procedure followed by LC-MS (Becalski et al., 2012; MacMahon et al., 2013). Reporting of results using direct methods tends to be as a total glycidol concentration based on five or seven glycidyl esters, to address the dominant fatty acids (palmitic acid, linolenic acid, linoleic acid, oleic acid, stearic acid plus lauric acid and myristic acid) present in oils.

Additionally, a method for the direct detection of glycidyl esters in edible oil has been developed jointly by the AOCS and the JOCS. Similar to other methods reported in the literature, the method requires two stages of SPE cleanup followed by LC-MS analysis (AOCS Official Method Cd 28-10) (AOCS & JOCS, 2012).

10.5 Sampling protocols

Although the Codex Alimentarius Commission has not established specific sampling protocols for glycidyl esters, general guidelines on sampling have been developed (FAO/WHO, 2004). Best practices have been established for numerous contaminants and include the collection of samples by qualified individuals using containers that are clean and non-reactive and that protect samples from contamination or damage during transport and storage. Sampling of commercial food products must ensure that samples collected are representative of the lot. Therefore, collection of multiple samples (incremental samples) from within the lot is recommended and may be used to form an aggregate sample from which laboratory samples may be analysed. Prior to the subsampling for laboratory analysis, homogenization of the aggregate sample should be performed, consistent with GLP. Sample collection must be focused on food commodities that are relevant to glycidyl esters, such as fats, oils and products containing fats and oils.

10.6 Effects of processing

Glycidyl esters are present in processed oils, fats and food prepared using these products. These compounds are, however, not generally present in crude or unrefined oils at elevated levels. As a result, they are considered to be food processing–induced compounds, where the processing of oils or fats leads to the production of glycidyl esters. The steps required for refining or purification of oils or fats may contribute to the formation (e.g. deodorization) or mitigation (e.g. degumming) of these food contaminants.

Preparation of refined oils from the crude form involves several steps, which include degumming, neutralization, bleaching and deodorization (Pudel et al., 2011). Degumming of oils removes phospholipids and is generally performed

at relatively low temperatures (80–120 °C) (Pudel et al., 2011; Šmidrkal et al., 2011). Neutralization of oils involves interaction of the oil with sodium carbonate or bicarbonate to lower the acid value (increase the pH), prior to deodorization (Freudenstein, Weking & Matthäus, 2013). The bleaching process involves exposure of oils to bleaching clays to remove phospholipids from the oils (Hrncirik & van Duijn, 2011). The final stage of oil refining is known as deodorization, where, in addition to acid treatment, oils are heated at elevated temperatures (>200 °C) (Pudel et al., 2011). The refining process has been investigated to determine the impact on formation of glycidyl esters, although the majority of research has focused on the MCPD esters owing to their earlier discovery.

It has been established by several researchers that deodorization, where oils are exposed to elevated temperatures, is the critical step in the formation of glycidyl esters (Pudel et al., 2011).

Finished palm oil products have been shown to have higher glycidyl ester levels than other oils when subjected to similar refining conditions. Processing of palm oil has resulted in elevated glycidyl ester and MCPD ester production, relative to other oil types, indicating that oil constituents have an impact on the effect of processing (Destaillats et al., 2012a). Glycidyl ester values were significantly higher after deodorization of non-degummed palm oil samples, suggesting that the removal of the precursors during the degumming step is beneficial.

10.7 Prevention and control

The formation of glycidyl esters is a function of the composition of the oil and the processing conditions used to refine crude oils. The type of oil processed has an impact on the glycidyl ester formation capacity, with palm oil producing greater amounts of glycidyl esters compared with other oil types owing to the high levels of diacylglycerols in the oil (Weisshaar & Perz, 2010). Oils high in diacylglycerols, which are known to be glycidyl ester precursors, are expected to produce higher levels of glycidyl esters, although other compounds, such as glycerol and phospholipids, may also contribute to the formation of glycidyl esters (Destaillats et al., 2012a; Shimizu, Vosmann & Matthäus, 2012). Chlorinated compounds are not anticipated to have an impact on glycidyl ester formation, whereas they are required for the formation of 3-MCPD esters (Nagy et al., 2011).

Strategies to prevent and control the formation of glycidyl esters in final oil products include:

- selection of raw material with low precursor content;
- removal of precursors using chemical treatment at mid-range temperatures;

- deodorization performed at neutral pH below 240 °C;
- adoption of dual deodorization protocols;
- utilization of adsorbents to remove glycidyl esters in post-treatment.

The Committee noted the commitment of the European fats and oils industry trade organization (FEDIOL) to continue to reduce the levels of glycidyl esters in refined vegetable oils and encouraged the organization to continue to reduce the levels of these contaminants.

10.8 Levels and patterns of contamination in food commodities

Globally, oils and fats are regional in their production and may be consumed in higher proportions in the production area than in importing countries. The variety of oils consumed in any given region varies depending on the source (FAOSTAT database: http://www.fao.org/faostat/en). Palm oil and its products are a major fat source in South-east Asia, but less dominant in Europe and North America, whereas soybean oil is the predominant vegetable oil in North and South America, and rapeseed and sunflower seed oils are more common in Europe. The determination of the types of oil in a finished food is often complicated by the use of mixtures to give a food a particular texture or structure, and the oils used may reflect the availability of suitable ingredients.

It is apparent that glycidyl esters are formed in the processing of vegetable oils mainly during the deodorization stage. The extent to which they are formed depends on the oilseed or fruit being processed, the process being used and the type of equipment installed. Hence, the refined oil obtained from any oil source may vary in glycidyl ester content. From reports of the analysis of foodstuffs in a number of countries, it appears that refined vegetable oil is a major contributor to the levels of glycidyl esters found in food (EFSA, 2016). There appears to be little evidence that glycidyl esters are formed in food during processing or cooking, and there is a reasonable correlation between the levels of glycidyl esters in the oils used and the amounts of the oils that were used in the food. A search using PubMed did not yield any publications showing the occurrence of free glycidol in food.

Glycidyl ester content was tested in more than 100 different edible fats, oils and related products containing fats or oils, such as cookies and cooking sprays, in Canada (Becalski et al., 2015a). Most virgin/unprocessed/unrefined oils did not contain detectable levels of glycidyl esters. However, glycidyl ester levels were highly variable in refined oils and fats, reaching 11 μg/g, expressed as glycidol equivalents.

It should be noted that methods for the analysis of glycidyl esters in foods, other than for fats and oils, have not been subjected to full collaborative study, and it is not clear if the same samples were analysed by any of the laboratories involved in the provision of the majority of the results received from the USA, Canada and the European Union in response to the call for data. Although these methods themselves might exhibit reasonable precision for different food types, their accuracy has not been evaluated under rigorous conditions. Recently, a collaborative study has been organized for the analysis of contaminants in high-fat foods (margarines and mayonnaise), but no international work on other food types has been initiated.

10.9 Food consumption and dietary exposure assessment

Estimates of dietary exposure to glycidyl esters, expressed as glycidol, were calculated by the Committee using concentration data from the GEMS/Food contaminants database. Occurrence data were available for five countries (Brazil, Canada, Japan, Singapore, USA), primarily for fats and oils and infant formula. The occurrence data from Japan and the USA were used to estimate national dietary exposures, as consumption data were also available for these countries in the CIFOCOss consumption database.

For the international estimates of dietary exposure to glycidol, occurrence data were available for only one of the 17 clusters (G10), and those data were therefore used for all cluster estimates.

The literature was also reviewed to identify estimates of dietary exposure. Only one estimate of dietary exposure was found (from Germany; Weisshaar, 2011), in addition to the EFSA (2016) assessment.

A summary of the range of estimated dietary exposures to glycidyl esters, expressed as glycidol, is shown in Table 23. These include national estimates of dietary exposure (both those estimated by the Committee and those from the literature), international dietary exposure estimates and estimated dietary exposures for infants from consumption of infant formula.

Overall, national estimates of dietary exposure to glycidyl esters, expressed as glycidol, from all sources at the mean for adults ranged between 0.1 and 0.3 μg/kg bw per day and for high-percentile exposures between 0.2 and 0.8 μg/kg bw per day. The estimates for children and adolescents were higher and at the mean ranged between 0.2 and 1.0 μg/kg bw per day and for high-percentile exposures between 0.4 and 2.1 μg/kg bw per day. Depending on the country and population group, palm oil, margarine or soybean oil was the main contributor to exposure, and infant formula for infants consuming mixed diets.

Table 23
Summary of estimated dietary exposures to glycidyl esters, expressed as glycidol

		Range of estimated dietary exposures (µg/kg bw per day)[a]	
Exposure assessment	**Source of estimate**	**Mean**	**High percentile[b]**
National	Committee, literature	Adults: 0.1–0.3	Adults: 0.2–0.8
		Children/adolescents: 0.2–1.0	Children/adolescents: 0.4–2.1
International[c]	Committee	0.2–1.0	0.3–2.1
Infants[d]	Committee[e] and literature	0.1–3.6	0.3–4.9

bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
[a] Includes lower-bound and upper-bound estimates.
[b] Ranges of high-percentile exposures are given, including 90th and 95th, depending on the assessment.
[c] Estimates are per capita based on a mean body weight of 60 kg for adults.
[d] Includes all estimates for infants from infant and follow-on formula and from mixed diets for 0–12 months of age.
[e] Based on concentration data for infant formula from the GEMS/Food contaminants database and the literature.

International estimates of mean dietary exposure to glycidyl esters, expressed as glycidol, were in the same range of between 0.2 and 1.0 µg/kg bw per day for adults, and high exposures (90th percentile) were between 0.3 and 2.1 µg/kg bw per day for adults. Palm oil was the highest contributor to overall dietary glycidol exposure for 11 of the clusters, with margarine being the highest contributor for five clusters.

Estimated dietary exposures to glycidyl esters, expressed as glycidol, for infants 0–12 months of age were broader in range than estimates for children, adults or the general population, particularly around the high end of the ranges. Mean exposures ranged between 0.1 and 3.6 µg/kg bw per day, and high-percentile exposures between 0.3 and 4.9 µg/kg bw per day.

10.10 Dose–response analysis

Complete hydrolysis of glycidyl esters to glycidol was assumed for the present evaluation. Glycidol is genotoxic and is carcinogenic in various tissues of rats and mice of both sexes, and the NTP carcinogenicity studies with mice and rats (NTP, 1990) are considered the pivotal studies for risk assessment. To find the most sensitive target organ, the USEPA's Benchmark Dose Software (BMDS version 2.6.1) models were fitted to data reported in the NTP studies. For this analysis, doses were adjusted by a factor of 5/7 to account for the fact that the animals were dosed only 5 out of 7 days of the week. All models in the BMDS software were fitted to the data using its default settings for restricted models. For the male rat, mesothelioma in the tunica vaginalis/peritoneum provided the lowest BMDLs. In mice, hepatocellular adenoma/carcinoma in males provided the lowest BMDLs. Results for the female rats and mice were significantly higher.

Table 24
Results of benchmark dose modelling from the NTP (1990) study in rats and mice

Species / study type (route of administration)	Doses (mg/kg bw per day)[a]	Critical end-point	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)
Mouse				
Two-year study of toxicity and carcinogenicity (gavage)	0, 17.9, 35.7	Hepatocellular adenoma/ carcinoma (males)	5.4	2.6
Rat				
Two-year study of toxicity and carcinogenicity (gavage)	0, 26.8, 53.6	Mesothelioma of tunica vaginalis/peritoneum (males)	3.0	2.4

BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight; NTP: National Toxicology Program
[a] Doses adjusted to 7 days/week dosing.

Overall, mesothelioma in the tunica vaginalis/peritoneum in the male rat from the NTP (1990) study was the most sensitive end-point, with a benchmark dose for a 10% response (BMD_{10}) of 3.0 mg/kg bw per day and a $BMDL_{10}$ of 2.4 mg/kg bw per day, obtained using the quantal linear model (Table 24).

11. Evaluation

Experimental evidence indicates that glycidyl esters are substantially hydrolysed to glycidol in the gastrointestinal tract and elicit toxicity as glycidol. The Committee therefore based its evaluation on the conservative assumption of complete hydrolysis of glycidyl esters to glycidol. Whereas the experimental data supporting substantial hydrolysis are derived from studies with post-weaning animals, the Committee concluded that the capacity of the neonate to hydrolyse fatty acids in the gut is efficient, and therefore the same assumption of substantial hydrolysis could be extended to this age group.

The Committee concluded that glycidol is a genotoxic compound and considered its carcinogenicity as the most sensitive end-point on which to base a point of departure. The lowest $BMDL_{10}$ was 2.4 mg/kg bw per day for mesotheliomas in the tunica vaginalis/peritoneum in male rats observed in the NTP (1990) carcinogenicity study (doses adjusted for non-continuous dosing).

The Committee noted that there are no published collaboratively studied methods for the determination of glycidyl esters in complex foods, in contrast to the situation with fats and oils; therefore, caution should be applied when interpreting analytical data from complex foods.

The Committee further noted that there was uncertainty in comparing the reported levels in the same foods from different regions because of the lack of interlaboratory comparisons and the absence of data arising from proficiency testing schemes.

As it is not appropriate to establish a health-based guidance value for substances that are both genotoxic and carcinogenic, the margin of exposure (MOE) approach is chosen.

National estimates of dietary exposure were used for determining the MOEs. This was because they were considered to be the most representative of dietary exposure, as they are based on consumption data from national dietary surveys. The majority of the surveys used include 2 or more days of data, which better estimate chronic dietary exposure.

The national dietary exposures are considered to be reliable estimates, as they are based on a range of foods in the diet and include the key foods in which glycidol contamination is known to occur – namely, fats and oils. The concentrations in specific foods in the majority of cases have been able to be matched directly with consumption data for the same foods.

The Committee considered that the lower ends of the ranges of the MOEs for infants, children and adults (Table 25) were low for a compound that is genotoxic and carcinogenic and that they may indicate a human health concern.

11.1 Recommendations

The Committee recommends that appropriate efforts to reduce concentrations of glycidyl esters and glycidol in fats and oils, in particular when used in infant formula, should continue to be implemented.

The Committee recommends the development of better exposure biomarkers to facilitate measurements in humans consuming glycidyl esters in food in support of risk assessment.

The Committee recommends that additional international collaborative studies should be undertaken on methods of analysis for glycidyl esters in relevant fat- or oil-containing foods in order to remove the uncertainty surrounding the accuracy of the data submitted to the GEMS/Food contaminants database used in future evaluations.

It is recommended that more data be submitted to the GEMS/Food contaminants database, including the form (the ester form or not) and the analytical methods used, in particular for fats and oils, where a high degree of variability in concentration is observed.

Table 25
Dietary exposures and MOEs compared with the $BMDL_{10}$

Population group	Range of estimated dietary exposures to glycidol (µg/kg bw per day)[a]		MOEs[b]	
	Mean	High percentile	Mean	High percentile
Adults	0.1–0.3	0.2–0.8	8 000–24 000	3 000–12 000
Children	0.2–1.0	0.4–2.1	2 400–12 000	1 100–6 000
Infants	0.1–3.6	0.3–4.9	670–24 000	490–8 000

$BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight; LB: lower bound; MOE: margin of exposure; UB: upper bound
[a] Includes LB and UB estimates from a range of national estimates of dietary exposure.
[b] Compared with a $BMDL_{10}$ of 2.4 mg/kg bw per day. MOEs are expressed as a range; the lower end of the range relates to UB mean and high-percentile exposures, and the higher end of the range relates to LB mean and high-percentile exposures.

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Appendix 1

Table A1

Glycidol concentration data for specific fats and oils for Japan coded to CIFOCOss Level 3 classification code OR0172 – "Vegetable oils, nes" for the assessment of dietary exposure

				Mean concentration (µg/kg)[a]	
Oil name	**No. of samples**	**No. of nondetects (<LOD)**	**% nondetects**	**LB**	**UB**
Canola	8	4	50	143	233
Coconut oil	3	0	0	150	150
Corn oil	12	1	8	961	966
Flaxseed oil	1	1	100	0	300
Grapeseed oil	4	0	0	1 238	1 238
Olive	10	7	70	205	319
Palm oil	5	0	0	1 224	1 224
Rice bran oil	24	0	0	2 925	2 925
Safflower seed oil	4	1	25	175	250
Sesame seed oil	30	4	13	160	184
Soybean oil	3	2	67	67	267
Sunflower seed	7	3	43	164	224

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; No.: number; UB: upper bound

[a] LB concentrations where samples with nondetect results (<LOD) were assigned a value of 0 and UB concentrations where samples with nondetect results (<LOD) were assigned a value equivalent to the LOQ.

Table A2

Per cent contribution (from the LB scenario) of food groups (where at least one cluster had a contribution) to the dietary exposure to glycidol from GEMS/Food cluster diets

Level 3 classification code	Level 3 food name	% contribution per GEMS/Food cluster diet																
		G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
36	Rice bran oil	<1	0	0	0	5	0	0	0	2	1	0	0	0	18	0	0	0
60	Maize oil	3	5	<1	7	1	8	3	2	<1	9	4	2	<1	<1	3	<1	0
109	Infant food	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	0	3	<1	<1	<1	<1	<1
9035	Lard and lard stearin oil	<1	5	<1	<1	<1	<1	2	12	2	2	6	6	<1	<1	14	<1	<1
1223	Oil fish, marine mammal	<1	0	0	<1	0	<1	0	0	<1	<1	0	0	0	0	0	0	0
237	Soybean oil	4	3	2	4	10	10	6	14	8	22	10	3	1	3	8	4	2
244	Groundnut oil	<1	<1	2	<1	3	<1	1	<1	3	<1	4	<1	5	<1	<1	<1	0
257	Palm oil	63	0	88	79	64	49	24	0	72	12	18	0	78	44	4	80	58
258	Palm kernel oil	<1	<1	2	1	<1	<1	0	<1	2	<1	0	0	6	4	0	<1	5
261	Olive oil, virgin	1	<1	<1	<1	<1	2	2	8	<1	3	1	<1	<1	<1	2	<1	<1
268	Sunflower seed oil	2	27	<1	1	2	6	8	14	<1	5	2	4	<1	<1	15	5	<1
271	Rapeseed oil	<1	<1	<1	<1	3	<1	10	9	3	5	0	0	<1	<1	5	<1	0
281	Safflower seed oil	<1	<1	0	<1	<1	<1	<1	<1	<1	<1	0	0	<1	0	0	0	0
290	Sesame seed oil	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	0	<1	<1	<1	<1	2	0
331	Cottonseed oil	6	5	1	<1	2	7	2	<1	3	1	0	0	2	<1	0	1	0
886	Butter (cow milk)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
9017	Coconut (copra) oil	<1	<1	<1	<1	<1	<1	<1	0	2	<1	0	4	<1	18	<1	<1	11
9019	Oil of vegetable origin, nes	<1	1	1	<1	<1	<1	<1	<1	<1	<1	0	1	2	<1	<1	6	4
9041	Margarine (liquid and short)	18	40	<1	3	7	14	22	34	<1	32	33	14	2	9	38	<1	4
1243	Fat preparations, nes	<1	10	<1	1	<1	<1	13	5	<1	4	22	51	<1	<1	7	<1	11
1232	Food preparations, nes	<1	2	<1	1	<1	<1	4	<1	<1	<1	0	12	<1	<1	2	<1	4

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; nes: not elsewhere specified

3-Monochloro-1,2-propanediol esters and 3-monochloro-1,2-propanediol

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1. Explanation 667
2. Biological data 668
2.1 Biochemical aspects of 3-MCPD esters 668
2.1.1 Absorption, distribution and excretion of 3-MCPD esters 668
2.2 Biochemical aspects of 3-MCPD 672
2.2.1 Absorption, distribution and excretion of 3-MCPD 672
2.3 Toxicological studies of 3-MCPD esters 673
2.3.1 Acute toxicity 673
2.3.2 Short-term studies of toxicity 673
(a) Mice 673
(b) Rats 673
2.3.3 Long-term studies of toxicity and carcinogenicity 676
2.3.4 Genotoxicity 676
2.3.5 Reproductive and developmental toxicity 676
(a) Multigeneration reproductive toxicity 677
(b) Developmental toxicity 677
2.3.6 Special studies 677
(a) Effects on lipid metabolism in mice 677
2.4 Toxicological studies of 3-MCPD 678
2.4.1 Acute toxicity 679
2.4.2 Short-term studies of toxicity 679
(a) Mice 679
(b) Rats 679
2.4.3 Long-term studies of toxicity and carcinogenicity 680
(a) Mice 680
(b) Rats 681

2.4.4 Genotoxicity 683
2.5 Observations in humans 685
2.5.1 Biomarkers of exposure 685
3. Analytical methods 686
3.1 Chemistry 686
3.1.1 Formation 687
3.2 Description of analytical methods 689
3.2.1 Introduction 689
3.2.2 Screening tests 690
(a) Methods for free 3-MCPD 690
(b) Methods for 3-MCPD esters 690
3.2.3 Quantitative methods 693
3.2.4 Reference methods 694
3.2.5 Quality assurance considerations 695
4. Sampling protocols 696
5. Effects of processing 697
5.1 Degumming/washing 698
5.2 Neutralization 698
5.3 Bleaching 698
5.4 Deodorization 699
6. Prevention and control 699
6.1 Harvest and storage conditions 699
6.2 Reduction of precursors – optimization of refining processes 700
6.3 Deodorization conditions 701
7. Levels and patterns of contamination in food commodities 702
8. Food consumption and dietary exposure estimates 703
8.1 Concentrations in food used in the dietary exposure estimates 704
8.2 Food consumption data used in the dietary exposure estimates 707
8.3 Assessments of dietary exposure 707
8.3.1 National estimates 707
(a) Brazil 725
(b) China 728
(c) Europe (total 3-MCPD) 730
(d) Japan 731
(e) USA 732
(f) Multiple countries – infant formula 732
8.3.2 International estimates 736
(a) 3-MCPD esters 736
(b) Free 3-MCPD 737
(c) Total 3-MCPD 740
8.4 Limitations of assessment of dietary exposure to 3-MCPD 740
9. Dose–response analysis and estimation of toxic/carcinogenic risk 742
9.1 Identification of key data for risk assessment 742
9.1.1 Pivotal data from biochemical and toxicological studies 742
9.2 General modelling considerations 743
9.2.1 Selection of data 743
9.2.2 Measure of exposure 743
9.2.3 Measure of response 743
9.2.4 Selection of mathematical model 743

9.2.5 Benchmark dose estimates 743
10. Comments 744
10.1 Biochemical aspects 744
10.1.1 3-MCPD esters 744
10.1.2 3-MCPD 748
10.2 Toxicological studies 748
10.2.1 3-MCPD esters 748
10.2.2 3-MCPD 749
10.3 Observations in humans 750
10.4 Analytical methods 751
10.5 Sampling protocols 752
10.6 Effects of processing 752
10.7 Prevention and control 753
10.8 Levels and patterns of contamination in food commodities 754
10.9 Food consumption and dietary exposure assessment 756
10.9.1 National estimates 757
10.9.2 International estimates 758
10.10 Dose–response analysis 758
11. Evaluation 759
11.1 Recommendations 760
12. References 761

1. Explanation

3-MCPD is a synonym for 3-(mono)chloro-1,2-propanediol and α-(mono)-chlorohydrin, a chiral molecule that exists as (*S*)-(+)-enantiomer (Chemical Abstracts Service (CAS) No. 60827-45-4) and (*R*)-(−)-enantiomer (CAS No. 57090-45-6). Although there are some differences in toxicity based on limited data, the majority of toxicity studies have been conducted with the racemic mixture (CAS No. 96-24-02).

3-MCPD was classified by the International Agency for Research on Cancer (IARC) (2012) as possibly carcinogenic to humans (Group 2B) based on sufficient evidence for carcinogenicity in experimental animals (ILSI, 2009; Bakhiya et al., 2011; Habermeyer, Guth & Eisenbrand, 2011; MacMahon, 2014).

3-MCPD esters are processing-induced contaminants found in various refined oils and fats. They are formed from acylglycerols in the presence of chlorinated compounds during deodorization at high temperature. "3-MCPD esters" is a general term for 3-MCPD esterified with one (sn-1- and sn-2-monoesters) or two identical or different fatty acids (diesters) (Fig. 1). Depending on the fatty acid composition of the oil or fat, a variety of different 3-MCPD esters can be formed during processing. In foods that contain refined vegetable oils or

fats, mainly diesters are found. Concentrations of 3-MCPD esters in refined oils increase incrementally as follows: rapeseed oil < soybean oil < sunflower oil < safflower oil < walnut oil < palm oil (Weisshaar, 2008a).

3-MCPD esters have not been previously evaluated by the Committee. The present evaluation was conducted in response to a request from the Codex Committee on Contaminants in Foods. 3-MCPD was evaluated at the forty-first, fifty-seventh and sixty-seventh meetings of JECFA (Annex 1, references *107*, *154* and *184*). At the sixty-seventh meeting, the Committee reaffirmed a provisional maximum tolerable daily intake (PMTDI) for 3-MCPD of 2 µg/kg body weight (bw) based on a lowest-observed-effect level (LOEL) of 1.1 mg/kg bw per day for tubular hyperplasia in the kidney seen in a long-term carcinogenicity study in rats (Sunahara, Perrin & Marchesini, 1993). An uncertainty factor of 500 was applied to allow for the absence of a clear no-observed-effect level (NOEL) and to account for the effects on male fertility and inadequacies in the studies of reproductive toxicity. The Committee at that time noted that it had been reported that fatty acid esters of 3-MCPD are present in foods, but there were insufficient data to allow the evaluation of either their intake or their toxicological significance.

A literature search of publicly available peer-reviewed literature in PubMed was conducted for toxicity data on 3-MCPD esters (no time restrictions, full data set) as well as for 3-MCPD since the last JECFA evaluation (2005–2016, with a focus on biochemical aspects, oral repeated-dose toxicity studies and genotoxicity studies). The recent opinion by the European Food Safety Authority (EFSA) (2016) was taken into consideration, as well as secondary literature (reports and reviews). Only recent occurrence data obtained from a literature search on Scopus (2012–2016) were evaluated as there have been considerable improvements in the analysis of 3-MCPD esters and as changes in oil processing have led to a decrease in the levels of 3-MCPD esters in the finished oils. Data generated prior to this date were few in number and considered less reliable.

2. Biological data

2.1 Biochemical aspects of 3-MCPD esters

2.1.1 Absorption, distribution and excretion of 3-MCPD esters

A key aspect for risk assessment is to elucidate to what extent 3-MCPD esters are hydrolysed to free 3-MCPD in order to conduct combined or separate risk assessments. Most absorption, distribution, metabolism and excretion (ADME) studies therefore focused on this aspect.

Fig. 1

A) 3-MCPD diester, B) sn-2 3-MCPD and C) sn-1 3-MCPD monoester, with examples of fatty acid chains i) myristic acid, ii) palmitic acid and iii) oleic acid

Examples of possible R groups (fatty acid chains)

In an in vitro model with porcine pancreatic lipase and pancreatin, simulating conditions of the small intestine, 3-MCPD-1-monoesters (monostearate, monooleate, monopalmitate and monomyristate; fatty acid ester in position 1) were rapidly and almost completely hydrolysed to 3-MCPD (>95% within 1 minute of incubation at 37 °C). The release from 3-MCPD palmitate-oleate was slower, releasing about 45%, 65% and 95% of 3-MCPD after 1, 5 and 90 minutes of incubation (Seefelder et al., 2008).

In another in vitro model, approximately 40% of 3-MCPD dioleate was hydrolysed mainly to 3-MCPD-sn2-monooleate, releasing only small amounts of free 3-MCPD, after a 3-hour incubation with porcine pancreatic lipase at 37 °C. In contrast, hydrolysis of 3-MCPD-sn1-monooleate to free 3-MCPD was almost complete within 3 hours of incubation. The same results were obtained with pancreatin from porcine pancreas, indicating that hydrolysis at the sn-2-position is a slow process (only 15% hydrolysed within 3 hours). When using human Caco-2 cells, only 3-MCPD monooleates were hydrolysed to free 3-MCPD, but not 3-MCPD dioleates. The released 3-MCPD was able to permeate the Caco-2 monolayer, likely by diffusion (Kaze et al., 2016).

The 3-MCPD-1-monoesters, 3-MCPD-1-monolaureate and 3-MCPD-1-monooleate, underwent extensive hydrolysis in vitro by human intestinal Caco-2 cells, releasing free 3-MCPD in the cell culture supernatant. Spontaneous

hydrolysis of either monoester (in the absence of Caco-2 cells) was not observed. In contrast, no release of 3-MCPD was observed when the cells were incubated with 3-MCPD dipalmitate. However, the diester 3-MCPD dipalmitate was shown to be taken up and metabolized by the cells (the diester disappeared from the medium, and no free 3-MCPD was released) (Buhrke, Weisshaar & Lampen, 2011).

To quantify the release of 3-MCPD from 3-MCPD esters in vivo, a single equimolar dose of 3-MCPD (10 mg/kg bw; 98% purity) or 3-MCPD dipalmitate (53.2 mg/kg bw) was administered in corn oil by gavage to fasted (16 hours) male Wistar rats (8–9 weeks old). Time courses of concentrations of 3-MCPD and its dipalmitate were measured in blood (0–48 hours), some tissues (kidney, liver, fat, small and large intestine), urine and faeces (from metabolic cages). 3-MCPD was rapidly absorbed with a C_{max} of 4850 ng/mL in plasma at 0.37 hours (T_{max}). When administered as 3-MCPD dipalmitate, 3-MCPD peak concentrations in plasma were approximately 5 times lower and appeared later (949 ng/mL at 3 hours). Based on the area under the curve (AUC) for 3-MCPD, approximately 86% of 3-MCPD dipalmitate was released as free 3-MCPD (AUC_{24} for 3-MCPD in plasma of 9030 and 7760 ng·h/mL within 24 hours of administration of 3-MCPD and its dipalmitate, respectively). The kinetic parameters k, k_a and k_e of the one-compartment model were determined as 6930 ng/L, 7.03 L/h and 0.692 L/h for 3-MCPD and as 18 300 ng/mL, 0.356 L/h and 0.309 L/h for 3-MCPD dipalmitate, respectively. On the basis of the elimination rate constants (k_e), the half-lives for 3-MCPD were estimated as 1.0 for administered 3-MCPD and 2.2 hours for administered 3-MCPD dipalmitate.

Concentrations of 3-MCPD in liver and kidney were similar to those in the blood and lower than those in fat tissue at all sampling time points and for either compound. After administration of 3-MCPD dipalmitate, no unchanged diester was detected (limit of detection [LOD] of 0.25 μg/g) in blood, liver, kidney or fat at any time point. High amounts of 3-MCPD dipalmitate (62–72% of the administered dose) were recovered in the small and subsequently the large intestine (including intraluminal contents) during the first hours after administration, but dropped to 0.07% of the dose 24 hours after administration. In comparison, only 4.1% of the administered 3-MCPD dose was still detected in the small and large intestine 30 minutes after dosing. 3-MCPD dipalmitate was excreted in low amounts as unchanged diester (1.1% of the applied dose within 48 hours) in faeces. Excretion of free 3-MCPD in urine (within 72 hours) and faeces (within 48 hours) was investigated and was similar for administered 3-MCPD and its dipalmitate, with 2.0% and 2.4% of the dose as 3-MCPD in urine and 0.5% and 1.4% in faeces, respectively.

The authors concluded that a significant amount of 3-MCPD dipalmitate was hydrolysed to 3-MCPD in the gastrointestinal tract based on the similarities

in urinary and faecal excretion, blood levels and tissue distributions of free 3-MCPD. The delayed peak concentrations of 3-MCPD in blood following administration of the dipalmitate may be explained by delayed absorption and enzymatic hydrolysis in the gastrointestinal tract as compared with the free 3-MCPD (Abraham et al., 2013).

Fasted male F344 rats (n = 3, 172–218 g) received a single dose of the vehicle (olive oil), 3-MCPD, 3-MCPD dipalmitate, 3-MCPD monopalmitate or 3-MCPD dioleate in olive oil at equimolar doses of 360 µmol/kg bw (40 mg/kg bw for 3-MCPD) by gavage. All chemicals tested were reported as 98% pure. In all treatment groups, 3-MCPD was detected in the serum at the only tested time point of 30 minutes after administration, resulting in mean 3-MCPD serum concentrations of less than the lower limit of quantification (LLOQ; <0.45 nmol/mL), 87, 16, 63 and 14 nmol/mL for control, 3-MCPD, 3-MCPD dipalmitate, 3-MCPD monopalmitate and 3-MCPD dioleate, respectively. Concentrations of 3-MCPD esters in serum were below or nearly below the LLOQ (<0.03 nmol/mL for 3-MCPD dipalmitate and 3-MCPD dioleate and <0.06 nmol/mL for 3-MCPD monopalmitate).

This study shows that 3-MCPD monopalmitate is efficiently hydrolysed to 3-MCPD within 30 minutes, whereas less 3-MCPD was released from the diesters 3-MCPD dipalmitate and 3-MCPD dioleate within this time period, indicating a slower release (Onami et al., 2015).

Adult Wistar rats (200–220 g; n = 10) were dosed with 3-MCPD dipalmitate (>98% purity) by oral gavage with 0 (corn oil), 9.78, 39.19 or 156.75 mg/kg bw per day for 90 days. Prior to scheduled termination, 24-hour urine samples were collected and analysed for a variety of 3-MCPD metabolites. In general, a higher percentage of the dose was excreted as 3-MCPD mercapturate (or 2,3-dihydroxypropyl mercapturic acid, DHPMA) (6.9–20.4%) than as 3-MCPD (1.8–9.2%); no/only trace concentrations of β-chlorolactic acid were detected. While relatively similar amounts, on a per cent dose basis, of 3-MCPD mercapturate and 3-MCPD were excreted in the high- and mid-dose groups, approximately 50% greater amounts of 3-MCPD mercapturate were excreted in males than in females in the low-dose group. To compare metabolism and to assess bioavailability, equimolar doses of 3-MCPD (1.84, 7.37 or 29.5 mg/kg bw per day) were also tested.

According to the authors, urinary metabolites (3-MCPD and 3-MCPD mercapturate) were lower by approximately 30% when 3-MCPD dipalmitate was administered compared with 3-MCPD.

Following administration of a single gavage dose of deuterated 3-MCPD-d_5 dipalmitate (156.7 mg/kg bw, in corn oil) to Wistar rats (2 males, 2 females), 3-MCPD-d_5 and *S*-DHPMA-d_5 were detected in urine, with highest concentrations within 24 hours after administration. In contrast, 3-chloro-

2-propanolglucuronide-d_5, 3-chloro-2-propanolsulfate-d_5 and β-chlorolactic acid-d_3 were below the LODs. One week prior to this experiment, the same animals had received a single equimolar dose of 3-MCPD-d_5 (20–29 mg/kg bw) by gavage, and a similar urinary metabolite pattern was seen except for 2–3 times higher urinary 3-MCPD-d_5 and *S*-DHPMA-d_5 concentrations compared with 3-MCPD-d_5 dipalmitate administration (Barocelli et al., 2011).

Gut content samples from stomach, duodenum and caecum of untreated F344 *gpt* delta rats (triplicates from nine males) were incubated in vitro with equimolar concentrations of 3-MCPD, 3-MCPD dipalmitate, 3-MCPD monopalmitate or 3-MCPD dioleate (7.5–38 μmol/mL, depending on the experiment) for 30 minutes at 37 °C. In all gut content samples, the unchanged parent compound was found in quantities nearly equal to the original dose except for 3-MCPD dipalmitate where concentrations after 30 minutes' incubation were approximately 3–4 times lower than that initially added. Low amounts of newly produced 3-MCPD were found in all gut content samples incubated with the 3-MCPD esters (versus <LLOQ without incubation). Of note, glycidol was less than the LLOQ (2.7 nmol/L) in all samples incubated with 3-MCPD or any ester (Onami et al., 2015).

2.2 Biochemical aspects of 3-MCPD

ADME studies since the previous JECFA evaluations are summarized below.

2.2.1 Absorption, distribution and excretion of 3-MCPD

In the study by Barocelli et al. (2011) (described above), groups of 5–10 male and female Wistar rats were administered 3-MCPD (98% purity) by gavage at doses of 0 (corn oil), 1.84, 7.37 or 29.5 mg/kg bw per day for 90 days. For 24 hours prior to scheduled termination, urine was collected and analysed for a variety of 3-MCPD metabolites. Of the daily dose, 5–28% was excreted as DHPMA, 2–10% as unchanged 3-MCPD and only trace amounts (geometric mean <0.01–0.15 mg per 24 hours; <1% of dose) as β-chlorolactic acid (Barocelli et al., 2011).

A single gavage dose of deuterated 3-MCPD-d_5 given to rats (29.5 mg/kg bw to two males, and 20 mg/kg bw to two females; strain not stated but probably Wistar rat, as in the main study) resulted in a recovery of 3.8–22.3% of the dose as 3-MCPD-d_5 and 1–5.2% of the dose as DHPMA-d_5 in the urine, while β-chlorolactic acid-d_3 was below the LOD (only stated for nondeuterated β-chlorolactic acid, as 9 μg/L) (Barocelli et al., 2011).

These new data fit the previously postulated metabolic pathway in which 3-MCPD is considered to be either detoxified by glutathione conjugation and excreted as DHPMA in urine, or enzymatically oxidized to oxalic acid via

β-chlorolactaldehyde and β-chlorolactic acid (see Fig. 2 of the glycidyl esters monograph, page 584; Annex 1, reference *155*). These results may indicate a more important role of the glutathione pathway than was previously considered.

2.3 Toxicological studies of 3-MCPD esters

2.3.1 Acute toxicity

In rats and mice, orally administered 3-MCPD sn-1-mono- and diesters are acutely less toxic than 3-MCPD (Table 1). 3-MCPD dipalmitate and 3-MCPD-1-monopalmitate showed acute kidney and testis toxicities, including necrosis and protein casts in renal tubules and decreased spermatids in seminiferous tubules (Liu et al., 2012). Another study found acute oral toxicity in kidney, testis, brain, thymus and lung (Liu et al., 2017).

2.3.2 Short-term studies of toxicity

(a) Mice

No data were available.

(b) Rats

In an oral 4-week study comparing the toxicity of 3-MCPD to 3-MCPD dipalmitate, groups of six male Wistar rats (aged 7–8 weeks) received 3-MCPD (10 mg/kg bw per day, 98% purity) or 3-MCPD dipalmitate (13.3 and 53 mg/kg bw per day, the higher dose being equimolar to the 3-MCPD dose) or the corn oil vehicle by gavage for 28 days. Body weight and organ weights were measured and histopathology and proteomics of kidney, liver and testis were assessed. No overt compound-related toxicity was observed except for interstitial mononuclear cell infiltration in kidney tissue in two males at the high 3-MCPD dipalmitate dose and severe diffuse testicular tubular atrophy in two males in the 3-MCPD group. Slight hepatocellular necrosis in one male at the high (equimolar) 3-MCPD dipalmitate dose and in one male in the 3-MCPD group were also mentioned as being possibly compound-related. Other findings were seen in single rats only (Braeuning et al., 2015; Sawada et al., 2015, 2016). The authors reported a broad overlap of proteomic changes induced by 3-MCPD and 3-MCPD-dipalmitate in kidney tissues that may indicate a common mechanism of toxicity (Sawada et al., 2016).

Groups of 10–12 male Wistar rats (age not stated; 180–220 g) were administered 3-MCPD dipalmitate (>95% purity) in feed at doses of 0, 12.3 or 267 mg/kg bw per day for 13 weeks. Body weight, clinical chemistry and necropsy of all animals and weight and histopathology of testis, liver, brain, spleen and kidney were assessed. Significantly increased organ weights were seen for kidney,

Table 1
Acute oral toxicity of 3-MCPD and 3-MCPD esters

Substance	Species	Group size / sex	Route	LD_{50} (mg/kg bw)[a]	Reference
3-MCPD					
(*R/S*)-racemate	ICR mice	NS	Gavage	191	Qian et al. (2007)
(*R*)-enantiomer	ICR mice	NS	Gavage	291	Qian et al. (2007)
(*S*)-enantiomer	ICR mice	NS	Gavage	118	Qian et al. (2007)
3-MCPD	Rats	M	Gavage	152	Ericsson & Baker (1970)
Diester					
3-MCPD 1-linoleate / 2-palmitate	Swiss mice	5 M, 5 F	Gavage	5 000 (904)	Liu et al. (2017)
3-MCPD 1-palmitate / 2-linoleate	Swiss mice	5 M, 5 F	Gavage	>5 000 (>904)	Liu et al. (2017)
3-MCPD dipalmitate	Swiss mice	10 M, 10 F	Gavage	>5 000 (>941)	Liu et al. (2012)
3-MCPD dipalmitate	Wistar rats	2 M, 2 F	Gavage	1 780 (332)	Li et al. (2013)
Monoester					
3-MCPD 1-monopalmitate	Swiss mice	5 M, 5 F	Gavage	2 677 (848)	Liu et al. (2012)
3-MCPD 1-monostearate	Swiss mice	5 M, 5 F	Gavage	2 974 (872)	Liu et al. (2017)
3-MCPD 1-monooleate	Swiss mice	5 M, 5 F	Gavage	2 081 (614)	Liu et al. (2017)
3-MCPD 1-monolinoleate	Swiss mice	5 M, 5 F	Gavage	2 016 (598)	Liu et al. (2017)

bw: body weight; F: female; LD_{50}: median lethal dose; M: male; 3-MCPD: 3-(mono)chloro-1,2-propanediol; NS: not stated in the abstract (article in Chinese)
[a] Expressed as 3-MCPD.

liver and testis at 267 mg/kg bw per day and for spleen at both doses (body-weight gain was lower at the highest dose, but not in a significant manner). The only significant histopathologic finding was increased renal tubular epithelium cell degeneration and accumulation of tubular hyaline casts in kidney at 267 mg/kg bw per day (Li et al., 2013).

In an oral 13-week study comparing 3-MCPD to 3-MCPD dipalmitate, 3-MCPD-1-monopalmitate and 3-MCPD dioleate, groups of 10 male and 10 female F344 rats (aged 7 weeks) received water (control), olive oil (vehicle control), 3-MCPD (40 mg/kg bw per day), 3-MCPD dipalmitate (14, 55 or 220 mg/kg bw per day), 3-MCPD monopalmitate (8, 32 or 130 mg/kg bw per day) or 3-MCPD dioleate (15, 60 or 240 mg/kg bw per day) in olive oil by gavage 5 days a week for 13 weeks. All substances were of 98% purity, and the highest dose of each ester was equimolar to 40 mg/kg bw per day 3-MCPD (which was comparable to the highest dose in the rat carcinogenicity study; see section 2.4.3(b); Cho et al., 2008a). Doses after correction for noncontinuous dosing were 28.6 mg/kg bw per day for 3-MCPD; 10, 39.3 and 157.1 mg/kg bw per day for 3-MCPD dipalmitate; 5.7, 22.9 and 92.9 mg/kg bw per day for 3-MCPD monopalmitate; and 10.7, 42.9 and 171.4 mg/kg bw per day for 3-MCPD dioleate. Body weight and organ weights were measured, clinical signs and mortality were recorded, and serum biochemistry measures, haematological examination and gross and

histopathologic examination of tissues were performed. Five of the 10 females that received 3-MCPD died or were euthanized due to acute renal tubular necrosis within 4 weeks of treatment, while mortality in the corresponding males and all groups treated with up to equimolar doses of esters was low and not dose-related. No significant difference in body weight was seen between treated and control groups. Absolute and relative kidney weights were significantly increased in rats treated with 3-MCPD and all 3-MCPD esters at medium and high doses. Equimolar doses of the 3-MCPD esters produced similar relative kidney weight increases compared with 3-MCPD. Relative liver weights were also significantly increased in rats treated with 3-MCPD and in all high-dose 3-MCPD ester groups except for females administered 3-MCPD monopalmitate. Incidences of ductus epididymis with apoptotic cells in the initial segment of the epididymis were significantly increased in males treated with 3-MCPD and in all male high-dose 3-MCPD ester groups. Significantly decreased haemoglobin was seen with 3-MCPD and in most groups administered the high doses of 3-MCPD esters, with a tendency for dose–response.

According to the authors, the no-observed-adverse-effect levels (NOAELs) of 3-MCPD dipalmitate, 3-MCPD monopalmitate and 3-MCPD dioleate were the lowest tested doses of 10, 5.7 and 10.7 mg/kg bw per day, respectively (corrected for noncontinuous dosing; corresponding to 1.8 mg/kg bw per day 3-MCPD). Overall, the highest tested doses of 3-MCPD esters were toxic to kidney, liver and epididymis with a potency similar to equimolar 3-MCPD, except for the higher acute renal toxicity in female rats administered 3-MCPD (Onami et al., 2014a).

Groups of 5–10 male and 5–10 female young (age not stated; 200–220 g) Wistar rats were administered 3-MCPD dipalmitate (0, 9.78, 39.19 or 156.75 mg/kg bw per day; >98% purity) or equimolar doses of 3-MCPD (0, 1.84, 7.37 or 29.5 mg/kg bw per day; 98% purity) by gavage in corn oil for 90 days. Assessed end-points included mortality, body weight, haematology and clinical chemistry as well as organ weights and histopathology upon necropsy of all animals at study end. Scores for the severity of histopathological effects in kidney and testis were calculated for individual end-points and as summary scores in order to compare the toxicities of 3-MCPD with 3-MCPD dipalmitate.

3-MCPD dipalmitate was mainly toxic to kidney in both sexes, and to testis in males; the effects were mainly at the highest dose. Renal effects included histopathological changes (e.g. tubular epithelial hyperplasia, glomerular lesions and accumulation of hyaline casts; chronic progressive nephropathy was not reported) and increased kidney weight (dose-related according to the authors; however, the tabulated results indicate an effect at the highest dose only compared with control). Effects on testis included degeneration of seminiferous tubules, testicular atrophy and necrosis of germ cells and Sertoli cells. Increased relative

liver weight, mild anaemia and altered serum parameters were also mainly reported at the highest dose.

The effects of 3-MCPD dipalmitate on kidney and testis were described by the study authors as "similar but milder" than those of equimolar 3-MCPD. In particular, the acute renal failure in females administered the high 3-MCPD dose was not seen with 3-MCPD dipalmitate (section 2.4.2(b); Barocelli et al., 2011). The Committee noted that there were deficiencies in the reporting of this study.

2.3.3 Long-term studies of toxicity and carcinogenicity

No information was available for 3-MCPD esters.

2.3.4 Genotoxicity

To examine in vivo genotoxicity, equimolar doses of 3-MCPD, 3-MCPD dipalmitate, 3-MCPD-1-monopalmitate or 3-MCPD dioleate were administered by gavage (in olive oil) to groups of six male F344 *gpt* delta rats carrying a transporter transgene lambda EG10 (Onami et al., 2014b). All substances were 98% pure and were administered at 40 (3-MCPD), 220 (3-MCPD dipalmitate), 130 (3-MCPD monopalmitate) or 240 (3-MCPD dioleate) mg/kg bw per day 5 days a week for 4 weeks. The 3-MCPD dose was selected according to the highest dose administered to rats in the Cho et al. (2008a) carcinogenicity study and accounted for 28% of the oral median lethal dose (LD_{50}). No positive control was used. Tissues and blood for the genotoxicity tests were sampled 24 hours after the last dose. Absolute and relative kidney weights were significantly increased in all treatment groups compared with vehicle control as were relative spleen weights for 3-MCPD and 3-MCPD dioleate.

The in vivo genotoxicity tests were negative for all tested substances: chromosomal aberrations in the micronucleus assay with bone marrow, *Pig-a* mutation assay with red blood cells and the *gpt* assay for mutant frequencies of *gpt* and *red/gam* (*Spi*$^-$) genes in kidney and testis. However, the micronucleus test was negative in the absence of signs of bone marrow toxicity (assessed as percentage of reticulocytes among total erythrocytes). It is therefore not clear whether the substances reached the bone marrow. Other limitations of all tests are the absence of a positive control and the use of only one dose level (Onami et al., 2014b). The results are summarized in Table 2.

2.3.5 Reproductive and developmental toxicity

3-MCPD dipalmitate (purity not stated) was administered to mature male Wistar rats (n = 6) by oral gavage at doses of 0 (trioctanoin solvent control), 100 or 200 mg/kg bw per day for 5 consecutive days. Fertility was assessed for up to 5 weeks post-dosing by weekly consecutive serial matings with females

Table 2
In vivo genotoxicity testing for 3-MCPD esters

End-point	Species / strain	Compound	Dose (mg/kg bw per day)[a]	Result	Comments
Micronucleus test – bone marrow	Male F344 *gpt* delta rats	CDP CMP CDO	220 130 240	Negative Negative Negative	In absence of signs of bone marrow toxicity; only 1 dose level tested; no positive control
Pig-a mutation assay – red blood cells	Male F344 *gpt* delta rats	CDP CMP CDO	220 130 240	Negative Negative Negative	Only 1 dose level tested; no positive control
Gpt gene mutation assay – kidney, testis	Male F344 *gpt* delta rats	CDP CMP CDO	220 130 240	Negative Negative Negative	Only 1 dose level tested; no positive control
Spi$^-$ gene mutation assay – kidney, testis	Male F344 *gpt* delta rats	CDP CMP CDO	220 130 240	Negative Negative Negative	Only 1 dose level tested; no positive control

bw: body weight; CDO: 3-MCPD dioleate; CDP: 3-MCPD dipalmitate; CMP: 3-MCPD-1-monopalmitate; 3-MCPD: 3-(mono)chloro-1,2-propanediol
[a] Dosing: one dose level was administered by gavage 5 days/week for 4 weeks (98% purity, $n = 6$ males). Doses were equimolar to 3-MCPD (40 mg/kg bw per day), which also tested negative in these test systems. Doses in this table have not been corrected for dosing schedule.

of established fertility. Rats in both dose groups were infertile (no offspring produced) for the first week after dosing with partial recovery of fertility in the low-dose group by week 2 and in the high-dose group by week 3. A 0.17 mmol/kg bw dose of 3-MCPD dipalmitate (100 mg/kg bw per day) produced a similar degree of infertility as 0.09 mmol/kg bw of 3-MCPD (10 mg/kg bw per day). On a molar basis, the results for 3-MCPD dipalmitate were described as comparable to 3-MCPD (Rooney & Jackson, 1980).

Effects on testis or epididymis were also seen in the oral repeated-dose toxicity studies with 3-MCPD esters (with mainly 3-MCPD dipalmitate tested) in rats (Barocelli et al., 2011; Li et al., 2013; Onami et al., 2014a; Sawada et al., 2015).

Oral repeated-dose toxicity studies with 3-MCPD esters are summarized in Table 3.

(a) Multigeneration reproductive toxicity

No information was available.

(b) Developmental toxicity

No information was available.

2.3.6 Special studies

(a) Effects on lipid metabolism in mice

Effects on lipid metabolism were examined in male mice after 4-week oral administration of four different 3-MCPD diesters. Groups of 3–14 adult (age not

Table 3
Summary of oral repeated-dose toxicity studies with 3-MCPD esters

Species	Compound	Study duration/ Route	Dosing (mg/kg bw per day)	Effect	NOAEL/LOAEL (mg/ kg bw per day)	References
Wistar rat	CDP	13 weeks Feed	0, 12.3, 267 (n = 10–12 males)	Increased kidney, liver, testis and spleen weight, renal histopathological findings (tubular epithelium cell degeneration, accumulation of tubular hyaline casts)	NOAEL: 12.3 (2.2)[a] LOAEL: 267 (48.1)[a]	Li et al. (2013)
F344 rat	CDP	13 weeks Gavage	0, 10, 39.3, 157.1[b] (n = 10/sex)	Increased kidney weight (both sexes)	NOAEL: 10 (1.8)[a] LOAEL: 39.3 (7.2)	Onami et al. (2014a)
F344 rat	CMP	13 weeks Gavage	0, 5.7, 22.9, 92.9[b] (n = 10/sex)	Increased kidney weight (both sexes)	NOAEL: 5.7 (1.8)[a] LOAEL: 22.9 (7.2)[a]	Onami et al. (2014a)
F344 rat	CDO	13 weeks Gavage	0, 10.7, 42.9, 171.4[b] (n = 10/sex)	Increased kidney weight (both sexes)	NOAEL: 10.7 (1.8)[a] LOAEL: 42.9 (7.2)[a]	Onami et al. (2014a)
Wistar rat	CDP	13 weeks Gavage	0, 9.78, 39.19, 156.75 (n = 5–10/sex)	Renal and testicular histopathological changes	Not applicable due to deficiencies in reporting	Barocelli et al. (2011)

bw: body weight; CDO: 3-MCPD dioleate; CDP: 3-MCPD dipalmitate; CMP: 3-MCPD monopalmitate; LOAEL: lowest-observed-adverse-effect level; 3-MCPD: 3-(mono)-chloro-1,2-propanediol; NOAEL: no-observed-adverse-effect level

[a] Expressed as 3-MCPD.

[b] Equimolar doses of CDP, CMP or CDO were administered 5 days/week for 13 weeks; tabulated doses have been corrected for dosing schedule, i.e. are average doses over 7 days. The highest dose level was equivalent to the only tested dose level of 3-MCPD at 28.6 mg/kg bw per day.

stated) male C57BL/6J mice (18–22 g) received saline (control), corn oil (vehicle control) or equimolar doses of the diesters (98% purity) 3-MCPD dipalmitate (3, 5 or 10 mg/kg bw per day), 3-MCPD distearate (3, 6 or 11 mg/kg bw per day), 3-MCPD dioleate (3, 6 or 11 mg/kg bw per day) or 3-MCPD dilinoleate (3, 6 or 11 mg/kg bw per day) in corn oil by gavage for 28 days. The highest dose levels corresponded to 16.5 µmol/kg bw per day. Only body weight, liver weight, serum and liver lipid profiles and serum liver enzymes were assessed. No significant differences in body weight and relative liver weight were observed. Significant changes seen included lipid accumulation in liver (increased triglyceride and total cholesterol levels, lipid droplets) and increased aspartate aminotransferase activity, total cholesterol and low density lipoprotein levels in serum, mainly at the highest dose levels of all the tested 3-MCPD diesters. The observed effects were therefore of similar magnitude for all tested diesters (Lu et al., 2015).

2.4 Toxicological studies of 3-MCPD

Toxicological studies since the previous JECFA evaluation at the sixty-seventh meeting (Annex 1, reference *184*) are summarized below.

2.4.1 Acute toxicity

The oral LD_{50} values of the (*R*/*S*)-, (*R*)- and (*S*)-enantiomers of 3-MCPD in ICR mice (sex not stated) were 191, 291 and 118 mg/kg bw, respectively (Qian et al., 2007) (Table 1).

2.4.2 Short-term studies of toxicity

(a) Mice

3-MCPD (CAS No. 96-24-02; 98% purity) was administered for 13 weeks to groups of 10 male and 10 female B6C3F1 mice (6 weeks old) in drinking-water at concentrations of 0, 5, 25, 100, 200 or 400 mg/L (equal to doses of 0, 0.94, 4.59, 18.05, 36.97 and 76.79 mg/kg bw per day for males and 0, 0.79, 3.94, 15.02, 30.23 and 61.34 mg/kg bw per day for females, respectively). Body weight, water and feed consumption, sperm motility, vaginal cytology evaluation and, at study-end necropsy, haematology, blood chemistry, organ weights and histopathology were assessed.

There were no deaths. Body weight was statistically significantly decreased compared with control at the highest dose in males from week 12 and in females from week 8 (7% and 14%, respectively, at study end). Relative (not absolute) kidney weight was increased in both sexes at 200 mg/L and above. Relative (not absolute) liver weight was increased in females at high doses. (These relative organ weights are probably increased only because of a mistake in reported final body weights; therefore, they are not considered treatment related). No treatment-related histopathological findings in kidney and liver were seen. In males, dose-related degeneration of germinal epithelium was statistically significant at 200 mg/L and above, and sperm motility was significantly reduced at the highest dose. At 400 mg/L, the estrous cycle length increased as females spent more time (average 1.4 days) in diestrus compared with controls.

According to the authors, the NOAEL was 100 mg/L (equal to 18.05 mg/kg bw per day) for males and 100 mg/L (equal to 15.02 mg/kg bw per day) for females (Cho et al., 2008b). The Committee noted that the NOAEL for females was 200 mg/L (equal to 30.23 mg/kg bw per day).

(b) Rats

In the subchronic study described above (section 2.3.2(b), on 3-MCPD esters), Barocelli et al. (2011) administered 3-MCPD (CAS No. 96-24-02; 98% purity) at doses of 0, 1.84, 7.37 or 29.5 mg/kg bw per day (n = 5–10 of both sexes) in corn oil by gavage for 90 days to Wistar rats. 3-MCPD was mainly toxic to the kidney in both sexes and to the testis in males. Nephrotoxicity was particularly severe in females, resulting in death due to acute renal failure at 29.5 mg/kg bw per day in 35% of females (5/10 and 2/10 in two separate experiments) versus 1/10

females at the intermediate dose and no or sporadic cases in males. According to the authors, there was a dose-related increase in kidney weight in both sexes. However, tabulated results indicate increased kidney weight compared with control only at the highest dose. Effects observed in both sexes included various histopathological findings in the kidney (e.g. tubular epithelial hyperplasia, glomerular lesions and accumulation of hyaline casts), mainly at the middle and high doses. Chronic progressive nephropathy was not reported.

In the highest 3-MCPD dose group, 9/10 males showed total degeneration of seminiferous tubules and lymphomononuclear infiltrates in the epididymis versus no or mild degenerative effects in controls. At the intermediate 3-MCPD dose, a mild decrease of spermatids and atrophy of spermatogenic and supporting cells in the seminiferous tubules were observed. The lowest dose was associated with only minimal degenerative effects.

The authors also reported mild anaemia and significantly altered serum parameters, mainly at the highest doses (Barocelli et al., 2011). The Committee noted that there were deficiencies in the reporting of this study.

2.4.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In a 2-year carcinogenicity study, 3-MCPD (CAS No. 96-24-02; 98% purity) was administered to groups of 50 male and 50 female B6C3F1 mice, aged 6 weeks, in drinking-water at 0, 30, 100 or 300/200 mg/L for 104 weeks (the highest concentration was lowered on day 100 to 200 mg/L due to reduced body-weight gain and feed and water consumption). Doses were equal to 0, 4.2, 14.3 and 33.0 mg/kg bw per day for males and 0, 3.7, 12.2 and 31.0 mg/kg bw per day for females, respectively. (Dose reduction was not considered in the high doses; 300 mg/L is equal to approximately 55–90 mg/kg bw per day in both sexes for the first 100 days; the 200 mg/L dose thereafter is equal to 33 and 31 mg/kg bw per day in males and females, respectively). Mortality, body weight, feed and water consumption, urine analysis, haematology and blood chemistry were assessed. At study end, all animals were necropsied and organs were weighed. Tissues underwent macroscopic examination in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 451 for "carcinogenicity studies" and histopathological examination.

Survival was not affected by dosing with at least 72% survival in each group. Body-weight gain and feed and water consumption were significantly reduced throughout the study at the highest dose despite the decrease in concentration to 200 mg/L. At the highest dose, blood urea nitrogen, alkaline phosphatase activity and albumin were significantly increased in serum, and some haematological parameters were significantly altered. No statistically

significantly increased tumour incidences or histopathological changes were seen at any dose. Notably, no treatment-related histopathological changes in kidney, testis and liver were observed.

According to the authors, the NOAEL was 33 mg/kg bw per day for males and 31 mg/kg bw per day for females, the highest doses tested (Jeong et al., 2010).

To evaluate carcinogenicity in transgenic CB6F1 *ras*H2-Tg mice, 3-MCPD was administered by gavage (in distilled water) at doses of 0, 10, 20 or 40 mg/kg bw per day for 26 weeks to groups of 25 males and 25 females, aged 8 weeks. *N*-Methyl-*N*-nitrosourea was administered in a single intraperitoneal injection as a positive control. Mortality, body weight and clinical signs were assessed and haematological, biochemical and histopathological examinations as well as organ weights were assessed at study end, upon complete necropsy of all animals.

No information on body weight was reported. There were no significant increases in tumour incidences in treated mice (in contrast to the positive control). Non-neoplastic effects included significantly increased and dose-related incidences of tubular basophilia in kidney in males at 20 mg/kg bw per day and above. Absolute and relative kidney weights were increased in males from 10 mg/kg bw per day and in females at 40 mg/kg bw per day. Significantly increased incidences in germ cell degeneration in testis and degenerative germ cell debris in the epididymis were seen at 40 mg/kg bw per day. At 40 mg/kg bw per day, significant increases in vacuolation in brain (both sexes) and spinal cord (females) were observed (Lee et al., 2016).

Kidney, testis and brain had already been identified as main target organs in the dose-range-finding study in which 3-MCPD was administered by gavage to groups of five male and five female CB6F1-non-Tg *ras*H2 mice at 0, 25, 50 or 100 mg/kg bw per day for 28 days. From the lowest dose of 25 mg/kg bw per day, degenerative germ cells were seen in the lumen of the seminiferous tubule. From 50 mg/kg bw per day, vacuolation in the brain was seen in both sexes. At 100 mg/kg bw per day, three males and one female died, and tubular basophilia in the kidney, axonal degeneration of the sciatic nerve, atrophy in the thymus and cardiomyopathy were observed in both sexes.

According to the authors, the NOAEL was less than 25 mg/kg bw per day in males and 25 mg/kg bw per day in females (Lee et al., 2015).

(b) **Rats**

In a 2-year carcinogenicity study, 3-MCPD (CAS No. 96-24-02; 98% purity) was administered to groups of 50 male and 50 female Sprague Dawley (SD) rats (6 weeks old) in drinking-water at concentrations of 0, 25, 100 or 400 mg/L (equal to 0, 1.97, 8.27 and 29.50 mg/kg bw per day for males and 0, 2.68, 10.34 and 37.03

mg/kg bw per day for females, respectively). 3-MCPD was added to deionized water and concentrations were reported to be within 10% of the theoretical concentrations. Study duration was 104 weeks for females and 100 weeks for males as survival of male controls reached 28% at week 100, which led to study termination for all male groups. The study was conducted according to OECD Test Guideline 451 (1981) for carcinogenicity studies. At study end, all rats were necropsied and histopathology of all tissues was performed.

Survival rates of dosed animals were similar to those of controls (data not shown): 28%, 34%, 18% and 26% in males and 30%, 44%, 22% and 32% in females, respectively. Poor survival in all groups was mainly due to euthanasia as a result of the observed pituitary gland tumours (incidences given for males only, without information on time of occurrence). At the highest dose, body weight was significantly decreased and water consumption significantly reduced throughout the study compared with controls. In male rats, dose-related statistically significant histological changes in kidney (renal tubular hyperplasia and chronic progressive nephropathy) and testis (atrophy and arteritis/periarteritis) were even seen at the lowest dose (Table 4). 3-MCPD was less toxic to kidney in females than in males, with significantly increased chronic progressive nephropathy from 10.34 mg/kg bw per day and renal tubular hyperplasia at 37.03 mg/kg bw per day. Incidences of renal tubular adenoma or carcinoma (combined) in both sexes and Leydig cell tumours increased in males in a dose-related trend and was statistically significantly different from control at the highest dose.

A NOAEL was not identified as significant effects were observed at the lowest dose (Cho et al., 2008a).

As the 2-year carcinogenicity study (Sunahara, Perrin & Marchesini, 1993) was the pivotal study at previous JECFA evaluations (Annex 1, references *155* and *184*), the table of the treatment-related pathological, hyperplastic and neoplastic lesions (Table 5) is included for comparison with the more recent carcinogenicity studies. In this study, groups of 50 male and 50 female Fischer 344 rats received 3-MCPD (purity 98%) in drinking-water at nominal concentrations of 0, 20, 100 or 500 mg/L for 104 weeks. As the drinking-water contained 2.7 mg/L 3-MCPD, absolute concentrations were 2.7, 26.5, 105.9 and 502.8 mg/L, equivalent to mean daily intakes of 0.11, 1.1, 5.2 and 28.3 mg/kg bw per day for males and 0.14, 1.4, 7.0 and 35.3 mg/kg bw per day for females.

When evaluating the study at the fifty-seventh meeting, the Committee concluded that the kidney was the main target organ with renal tubular hyperplasia as the most sensitive end-point. The lowest dose was considered as the LOEL, which was close to a NOEL.

The Committee had also evaluated the dose-related increases in the incidence of hyperplasia and/or tumours in the kidney, mammary glands and preputial gland (Annex 1, reference *155*).

Table 4
Incidences of neoplastic and non-neoplastic lesions in kidney and male reproductive organs in a 2-year study of 3-MCPD in male and female SD rats

	No. (incidence) per concentration of 3-MCPD in drinking-water[a]							
	Males				Females			
	0 mg/L	25 mg/L	100 mg/L	400 mg/L	0 mg/L	25 mg/L	100 mg/L	400 mg/L
Number of animals examined	50	50	50	50	50	50	50	50
Kidney								
Chronic progressive nephropathy	15 (30)	27 (54)*	39 (78)*	41 (82)*	6 (12)	8 (16)	23 (46)*	42 (84)*
Tubular hyperplasia	1 (2)	11 (22)*	21 (42)*	36 (72)*	1 (2)	0 (0)	1 (2)	10 (20)*
Tubular adenoma	0 (0)	0 (0)	1 (2)	4 (8)	0 (0)	0 (0)	1 (2)	6 (12)*
Tubular carcinoma	0 (0)	0 (0)	0 (0)	5 (10)*	1 (2)	0 (0)	1 (2)	3 (6)
Tubular adenoma or carcinoma (combined)	0 (0)	0 (0)	1 (2)	7 (14)*	1 (2)	0 (0)	2 (4)	9 (18)*
Testis								
Atrophy	6 (12)	16 (32)*	13 (26)*	34 (68)*	–	–	–	–
Arteritis/periarteritis	3 (6)	15 (30)*	9 (18)*	11 (22)*	–	–	–	–
Leydig cell tumour	1 (2)	1 (2)	4 (8)	14 (28)*	–	–	–	–
Epididymis atrophy	0 (0)	0 (0)	0 (0)	5 (10)*	–	–	–	–
Seminal vesicle atrophy	36 (72)	41 (82)	49 (98)*	44 (88)	–	–	–	–
Pituitary gland								
Pars distalis adenoma	25 (50)	26 (52)	24 (48)	13 (26)*	NS	NS	NS	NS
Hyperplasia	5 (10)	3 (6)	4 (8)	2 (4)	7 (14)	1 (2)*	4 (8)	1 (2)*

bw: body weight; 3-MCPD: 3-(mono)chloro-1,2-propanediol; NS: not stated; *: $P < 0.05$ (poly-3 and Fisher exact pair-wise test between treatment and control groups)
[a] Number of animals with the finding and, in parentheses, incidence (number of animals with finding / number of animals examined expressed as a percentage).
Source: Cho et al. (2008a)

2.4.4 Genotoxicity

A new in vivo genotoxicity study with 3-MCPD became available since the last JECFA evaluation.

3-MCPD (98% purity) was administered by gavage (in olive oil) to male F344 *gpt* delta rats carrying a transporter transgene lambda EG10 ($n = 6$) at a dose of 40 mg/kg bw per day for 5 days a week for 4 weeks. The 3-MCPD dose was selected according to the highest dose in the Cho et al. (2008a) carcinogenicity study in rats and accounted for 26% of the oral LD_{50}. Tissues and blood for genotoxicity tests were sampled 24 hours after the last dose. No positive control was included.

The micronucleus assay with bone marrow was considered negative in the absence of signs of bone marrow toxicity when assessed as percentage of reticulocytes among total erythrocytes. For comparison, in the negative in vivo micronucleus test by Robjohns et al. (2003), 3-MCPD at 60 mg/kg bw administered by gavage for 2 days to Han Wistar rats induced toxicity in the

Table 5

Incidences of treatment-related pathological, hyperplastic and neoplastic lesions in a 2-year study of 3-MCPD in male and female F344 rats

	Incidence per concentration of 3-MCPD in drinking-water[a]			
	2.7 mg/L[b]	26.5 mg/L	105.9 mg/L	502.8 mg/L
Males				
Testis				
Leydig cell hyperplasia	39/50	27/50*	4/50***	0/50***
Leydig cell adenoma	38/50	43/50*	50/50***	47/50*
Leydig cell carcinoma	0/50	0/50	0/50	3/50
Mammary gland				
Glandular hyperplasia	2/45	6/48	24/47***	43/49***
Fibroadenoma	0/45	0/48	2/47	10/49**
Adenoma	0/45	0/48	1/47	1/49
Adenocarcinoma	0/45	0/48	1/47	1/49
Kidneys				
Nephropathy (CPN)	36/50	40/50	45/50*	49/50***
Tubular hyperplasia	3/50	6/50	15/50**	34/50***
Tubular adenoma	0/50	0/50	1/50	5/50*
Pancreas				
Islet cell hyperplasia	14/48	8/50	5/50*	1/48***
Islet cell adenoma	16/48	9/50	7/50*	0/48***
Islet cell carcinoma	8/48	0/50**	2/50*	0/48**
Mixed adenoma	0/48	1/50	0/50	1/48
Preputial glands[c]				
Adenoma	1/5	2/13	6/16	5/11
Carcinoma	0/5	0/13	1/16	2/11
Females				
Kidneys				
Nephropathy (CPN)	24/50	23/50	42/50***	48/50***
Tubular hyperplasia	2/50	4/50	20/50***	31/50***
Tubular adenoma	0/50	1/50	0/50	9/50**

bw: body weight; CPN: chronic progressive nephropathy; 3-MCPD: 3-(mono)chloro-1,2-propanediol; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (pair-wise Fisher test between treated and control groups)

[a] Shown as number of animals with the finding / number of animals examined.

[b] Control. Drinking-water contained a background concentration of 2.7 mg/L 3-MCPD, which is taken into account in the stated concentrations of control and treated groups.

[c] The preputial gland was not included in the protocol but either was found incidentally on skin sections or was collected at autopsy if it contained a visible nodule. As this organ was not examined in all animals, no meaningful statistical analysis of the tumour incidence could be conducted.

Source: Sunahara, Perrin & Marchesini (1993)

targeted bone marrow; this study was evaluated by JECFA (Annex 1, references *186* and *212*). The *Pig-a* mutation assay with red blood cells from the abdominal aorta and the *gpt* assay, which investigates mutant frequencies of *gpt* and *red/gam* (*Spi*$^-$) genes in kidney and testis, were also negative (Onami et al., 2014b).

Table 6
Oral repeated-dose toxicity studies with 3-MCPD conducted since the previous JECFA evaluation (2007)

Species	Study duration / no. tested	Dosing (mg/kg bw per day) / route of administration	Effect	NOAEL/LOAEL (mg/kg bw per day)	Reference
B6C3F1 mouse	13 weeks *n* = 10/sex	0, 0.94, 4.59, 18.05, 36.97, 76.79 (males) in drinking-water	Degeneration of germinal epithelium in testis	NOAEL: 18.05 LOAEL: 36.97	Cho et al. (2008b)
Transgenic CB6F1 *ras*H2-Tg mouse	26 weeks *n* = 25/sex	0, 10, 20, 40 by gavage	Increased absolute and relative kidney weights in males	LOAEL: 10 (lowest dose)	Lee et al. (2016)
B6C3F1 mouse	2 years *n* = 50/sex	0, 3.7, 12.2, 31.0 (females) in drinking-water	No effects	NOAEL: 31.0	Jeong et al. (2010)
Wistar rat	13 weeks *n* = 5–10	0, 1.84, 7.37, 29.5 by gavage	Renal and testicular histopathological changes	Not applicable due to reporting deficiencies	Barocelli et al. (2011)
SD rat	2 years *n* = 50/sex	0, 1.97, 8.27, 29.5 (males) in drinking-water	Kidney: chronic progressive nephropathy and tubular hyperplasia in males Testis: atrophy and arteritis/ periarteritis	LOAEL: 1.97 (lowest dose)	Cho et al. (2008a)
F344 rat[a]	2 years	0.11,[b] 1.1, 5.2, 28.3	Renal tubular hyperplasia	LOEL: 1.1 (lowest dose)	Sunahara, Perrin & Marchesini (1993)

bw: body weight; LOAEL: lowest-observed-adverse-effect level; LOEL: lowest-observed-effect level; 3-MCPD: 3-(mono)chloro-1,2-propanediol; NOAEL: no-observed-adverse-effect level; SD: Sprague Dawley
[a] The pivotal study at the fifty-seventh meeting (Annex 1, reference *155*) shown to compare with the newer studies.
[b] Drinking-water used as solvent contained a background concentration of 3-MCPD at 2.7 mg/L. This is taken into account in the stated concentrations of control and treated groups.

The oral repeated-dose toxicity studies with 3-MCPD conducted since the previous JECFA evaluation at the sixty-seventh meeting are summarized in Table 6.

2.5 Observations in humans

No clinical or epidemiological studies were available for 3-MCPD and its esters.

2.5.1 Biomarkers of exposure

3-MCPD esters (mainly diesters with lauric, palmitic and oleic acids and asymmetric palmitic acid/oleic acid) were detected in all of the 12 analysed human breast milk samples (100–2195 µg/kg milk fat, expressed as 3-MCPD) from Czech mothers while none contained 3-MCPD (<LOD of 3 µg/kg milk fat). The authors calculated a mean content of 3-MCPD esters of 35.5 µg/kg milk

(range: <11–76 µg/kg milk; expressed as 3-MCPD), taking into account the large variability of fat content in human milk (1.7–7.2%, mean 3.8%) (Zelinkova et al., 2008). Although the authors suggested that foods containing high concentrations of 3-MCPD esters may be the source of the esters detected in the breast milk samples, the Committee noted that there have been no confirming reports of 3-MCPD esters in any other human samples. In addition, in animal experiments with high oral exposure to various 3-MCPD esters, there has been no evidence demonstrating that esters are absorbed intact from the gastrointestinal tract or that free 3-MCPD undergoes re-esterification in situ.

Of spot urine samples from 255 healthy men (n = 114) and women (n = 141) from Italy, 67% contained 3-MCPD and 100% contained DHPMA, respectively. Concentrations were similar in both men and women, from less than 1.9 µg/L (the limit of quantification [LOQ]; median 2.52 µg/L) for 3-MCPD and from 233 to 388 (median 296) µg/g creatinine for DHPMA (Andreoli, Cirlini & Mutti, 2015). The authors proposed 3-MCPD and DHPMA as urinary biomarkers for human exposure to 3-MCPD and its esters. However, DHPMA is not a specific biomarker, as it was also shown to be a metabolite of glycidol and its esters in rodents (see Appel et al., 2013, and Jones, 1975), likely due to commonalities in the metabolic pathways.

DHPMA was also measured in spot urine of 54 nonsmokers and 44 smokers from northern Bavaria, Germany. The median concentration of creatinine was 206 (range: 114–369) µg/g in nonsmokers and 217 (range: 165–342) µg/g in smokers; these concentrations were not statistically significantly different. The source of DHPMA was unknown, and the authors suggested that besides 3-MCPD, glycidol and their esters, there was also an unknown endogenous source due to the strong correlation with creatinine and rather high concentrations of DHPMA (Eckert et al., 2010, 2011).

3. Analytical methods

3.1 Chemistry

MCPD esters were first described in goat milk fat by Cerbulis et al. (1984). Both mono- and diesters (Fig. 1) are present in thermally processed oils/fats and in food products containing processed oils and fats. MCPD esters are also found in products containing processed oils or fats that were subsequently baked or roasted at high temperature (e.g. breads, coffee, etc.) (Crews, Brereton & Davies, 2001; Dolezal et al., 2005; Zelinkova et al., 2006; Divinova, Dolezal & Velisek, 2007; Crews et al., 2013). The parent MCPDs are known as glycerol chlorohydrins

owing to the replacement of one hydroxyl group of the parent glycerol molecule with a chlorine atom. They may also be referred to as free 3-MCPD (Davidek et al., 1980; Hamlet et al., 2002; Crews et al., 2013). MCPD diesters, rather than monoesters, are the predominant form found in palm oil, palm kernel oil, palm olein, sunflower oil and vegetable fat mixes (Seefelder et al., 2008; Dubois et al., 2012).

The initial work in the area of 3-MCPD focused on the determination of the free 3-MCPD or unesterified 3-MCPD. In analysing for 3-MCPD, there appeared to be 3-MCPD-containing food categories that were unlikely to contain soy sauce or hydrolysed vegetable protein (HVP). Subsequent reports indicated that perhaps oil and fats could be a common precursor for this contaminant. Analysis of oils and fats led to the confirmation of the presence of 3-MCPD fatty acid esters.

Early studies were hampered by the lack of analytical standards for 3-MCPD esters. However, this situation was rectified fairly recently, allowing the development of both direct and indirect methods for the measurement of 3-MCPD esters. Consequently, the presence of 3-MCPD fatty acid esters was confirmed in refined oils and fats.

3.1.1 Formation

Triacylglycerols, comprising a glycerol backbone and fatty acid chains connected through ester linkages, have been identified as the principal source of MCPD esters (Dubois et al., 2012). MCPD esters are structurally related to triacylglycerols or diacylglycerols where one fatty acid ester moiety is replaced by a chlorine atom. It is the cleavage of a fatty acid chain from the glycerol backbone, followed by chlorine substitution, that creates MCPD esters. Elevated temperatures (>170 °C), such as those used during the deodorization process in refining edible oils, facilitate these reactions (Craft & Destaillats, 2014). Exposure to hydrochloric acid during food processing, even under mild conditions, also can result in MCPD ester formation (Haines et al., 2011). Non-esterified MCPDs (Fig. 1) were reported in HVPs (and soy sauces) before MCPD esters were found in food products (Velisek et al., 1978). The treatment of lipid material with hydrochloric acid was determined to cause the formation of 3- and 2-MCPD (Fig. 2) in these products (Velisek et al., 1979, 2002).

Reaction mechanism studies have identified two major pathways by which acylglycerols convert to MCPD esters. One route involves an acyloxonium cation intermediate prior to MCPD ester formation (Collier, Cromie & Davies, 1991; Rahn & Yaylayan, 2011a; Destaillats et al., 2012). Collier, Cromie & Davies (1991) determined that partial glycerols provide anchimeric assistance leading to the formation of the intermediate ion. Direct nucleophilic substitution with chlorine

Fig. 2
A) 3-MCPD and B) 2-MCPD

also has been proposed, although this route has been recently identified as a less important pathway (Collier, Cromie & Davies, 1991; Destaillats et al., 2012). These reactions result in the preferential formation of 3-MCPD esters relative to the 2-MCPD esters owing to less steric hindrance at the primary carbons, that is, at the sn-1 and sn-3 positions (Rahn & Yaylayan, 2011a). 3-MCPD esters exist as racemic mixtures of the 2(*R*)- and 2(*S*)-enantiomers (Velisek et al., 2002; Hamlet et al., 2011). Zhang et al. (2013) showed that at very high temperatures (>200–240 °C) and low moisture conditions, radical intermediates are involved in the formation of 3-MCPD diesters.

The conversion of acylglycerols to MCPD esters requires the presence of inorganic chloride ions and organochlorine compounds likely to be present in the lipids derived from plant materials (Nagy et al., 2011). Release of chloride during heating allows the formation of MCPD esters via nucleophilic substitution of the fatty acid chain (Nagy et al., 2011). Bakery products prepared using recipes with salt also provide a source of the necessary chloride ions (Hamlet, Sadd & Gray, 2004).

Triacylglycerols have been identified by some researchers as the major source of MCPD ester formation, although others have found that partial glycerols (e.g. diacylglycerols or monoglycerols) are the precursors of this reaction pathway (Hamlet & Sadd, 2004; Franke et al., 2009; Rahn & Yaylayan, 2011b; Smidrkal et al., 2011; Destaillats et al., 2012; Shimizu, Vosmann & Matthaus, 2012; Freudenstein, Weking & Matthaus, 2013). It has been proposed that enzymatic and chemical hydrolysis of triacylglycerols to diacylglycerols prior to acyloxonium ion formation occurs readily because reduced diacylglycerols cause greater steric hindrance than triacylglycerols, thereby allowing the reaction leading to the formation of MCPD esters to proceed more readily (Smidrkal et al., 2011).

How edible oils used in food preparation impact MCPD ester formation depends on the relative presence of each of the reaction precursors, with concentrations of 3-MCPD ester higher in palm oil than other oils (e.g. rapeseed, corn) (Franke et al., 2009; Matthaus et al., 2011; Craft & Destaillats, 2014; Li et al., 2016a). Concentrations of 3-MCPD esters in refined oils increase incrementally from rapeseed oil < soybean oil < sunflower seed oil < safflower seed oil <

walnut oil < palm oil (Weisshaar, 2008a). Lauric acid, myristic acid, palmitic acid, linolenic acid, linoleic acid, oleic acid and stearic acid are the dominant fatty acids in edible oils and are observed most frequently as esters of 3-MCPD (MacMahon, 2014). MCPD ester composition corresponds to fatty acid profiles of the precursor lipids (MacMahon, 2014).

Similar to acylglycerol precursors, phospholipids present in lipid-rich foods may undergo direct acid-catalysed nucleophilic substitution with chlorine at the phosphoryl group (Hamlet, Sadd & Gray, 2004). Cyclic cation formation with neighbouring groups also occurs with phospholipids, resulting in MCPD ester production (Rahn & Yaylayan, 2011a). Consistent with MCPD ester formation via acylglycerols, phospholipids preferentially form 3-MCPD esters over 2-MCPD esters (Davidek et al., 1980).

MCPD esters have been found in toasted cereals and baked goods. Their presence in breads has been associated with the addition of MCPD ester precursors, such as monoglycerols and diacylglycerols, which are added as dough improvers (Hamlet, Sadd & Gray, 2004). Recipes that include salt result in elevated levels of MCPD, indicating that wheat flour used in baked goods without salt may not have sufficient chlorine to facilitate significant formation of MCPD esters, which are precursors of MCPD (Hamlet, Sadd & Gray, 2004). Low moisture (≤20%) content and low pH promote the formation of MCPD esters in baked products (Svejkovska et al., 2004, 2006).

Although MCPD ester formation is generally associated with food processing, isolated occurrence of 3-MCPD esters in unprocessed food has been reported. Studies examining the lipid content of goat milk from the USA reported 3-MCPD diesters in milk that had not undergone further processing (Cerbulis et al., 1984). 3-MCPD esters have also been reported in human milk samples that were frozen before sample preparation for analysis (Zelinkova et al., 2008).

3.2 Description of analytical methods

3.2.1 Introduction

The two main approaches to MCPD ester analysis measure MCPD esters as free MCPD – indirect methods – or as intact MCPD esters – direct methods. 3-MCPD esters are frequently distinguished from 3-MCPD in the literature through reference to bound MCPD (intact 3-MCPD esters) and free 3-MCPD, respectively. The indirect methods have been used more frequently and generally employ gas chromatography coupled with mass spectrometry (GC-MS), necessitating the formation of derivatives prior to analysis. In contrast, direct measurement allows the analysis of MCPD esters without prior derivatization, and uses liquid chromatography–mass spectrometry.

Some of the early work to quantify 3-MCPD and its esters was performed by separating these analytes from other lipid components using thin layer chromatography (TLC) coated with silica gel and plate development with a variety of solvents (petroleum / diethyl ether / acetic acid) (Davidek et al., 1980; Gardner et al., 1983; Kuksis et al., 1986). Following isolation of the lipid fractions containing MCPD esters, analyses were completed using GC-MS (Velisek et al., 1978; Cerbulis et al., 1984).

3.2.2 Screening tests

(a) Methods for free 3-MCPD

The analysis of free 3-MCPD often requires extracting a subsample separate from that extracted for MCPD esters. The solvent chosen to extract free 3-MCPD has frequently been acetone:hexane (1:1) (Divinova et al., 2004; Zelinkova et al., 2006). Direct partitioning into sodium chloride solutions has also been successful (Hamlet, 1998). Analysis of 3-MCPD is performed following derivatization, similar to analysis of MCPD esters.

(b) Methods for 3-MCPD esters

Indirect methods of analysis describe the measurement of 3-MCPD esters after individual esters have been converted to 3-MCPD through cleavage of the fatty acid moieties and determination of 3-MCPD content.

MCPD esters are extracted from homogeneous food samples using solvent to extract the lipid. Extraction generally includes adding isotopically labelled 3-MCPD analogues to allow for recovery correction during sample preparation (Kuhlmann, 2011; Kusters et al., 2011; Jedrkiewicz et al., 2016). Solvent choices vary between method and matrix, but solvent mixtures of varying polarity or a single solvent with mid-level polarity may be used (Divinova, Dolezal & Velisek, 2007; Ermacora & Hrncirik, 2012; Jedrkiewicz et al., 2016; Li et al., 2016a).

Labelled standards reported most frequently in the literature are deuterated and may be MCPD esters themselves or free MCPD (Zelinkova, Dolezal & Velisek, 2009a; Haines et al., 2011; MacMahon et al., 2013). However, ^{13}C analogues of MCPD esters have been used (Destaillats et al., 2012; Dubois et al., 2012). An early limitation of the work in this area was related to obtaining suitable standards for these analyses. However, reference standards are increasingly available for both the parent MCPDs and many MCPD esters.

Following addition of the solvent, MCPD esters are generally extracted via homogenization by vortex mixing and separation of aqueous from organic phases through centrifugation (Hamlet & Asuncion, 2011). Other techniques used in extraction include Soxhlet extraction, accelerated solvent extractor system

at high temperatures and pressures for dry powdered samples and microwave extraction (Divinova, Dolezal & Velisek, 2007; Wöhrlin et al., 2015; Marc et al., 2016). When necessary, water is removed prior to cleavage of the fatty acids from the MCPD esters.

Hydrolysis to remove the fatty acid esters has been successfully performed under acidic conditions using sulfuric acid in methanol (Divinova et al., 2004; Hamlet & Asuncion, 2011; Ermacora & Hrncirik, 2013; Wöhrlin et al., 2015) and under alkaline conditions with sodium methoxide (sodium methanolate) (Weisshaar, 2008b; Kusters et al., 2011; Karl et al., 2016; Li et al., 2016b) or methanolic sodium hydroxide (Jedrkiewicz et al., 2016; Kuhlmann, 2016). In acidic conditions, 3-MCPD can potentially be created during ester cleavage of samples containing chloride ions; as a result, the alkaline hydrolysis method was developed (Weisshaar, 2008b). However, alkaline conditions can also lead to variable MCPD concentrations; therefore, the reaction time and conditions required for the alkaline hydrolysis are critical (Hrncirik et al., 2011; Kuhlmann, 2011). Transesterification under alkaline conditions at low temperatures (−22 °C to −25 °C) allows for improved recoveries, although this requires extended reaction times (Kuhlmann, 2011). Upon cleavage of the esters, the pH is adjusted to neutral to stop the reaction.

Another approach involves cleavage of the fatty acids from MCPDs using enzymatic hydrolysis (Chung & Chan, 2012; Miyazaki & Koyama, 2016).

Following cleavage of fatty acids from the MCPD esters, samples are treated with a salt solution and fatty acids are removed as methyl esters from the reaction mixture (Crews et al., 2013). Sodium chloride and sodium and ammonium sulfates are most frequently used for this purpose (Divinova et al., 2004; Fiebig, 2011; Ermacora & Hrncirik, 2012; Jedrkiewicz et al., 2016). The use of sodium chloride rather than ammonium sulfate was found to result in higher MCPD concentrations in palm and rapeseed oil (Hrncirik et al., 2011) because of a reaction between sodium chloride and compounds (e.g. glycidol) in the oil (Haines et al., 2011). Sodium bromide is used as a reactant instead of sodium chloride when glycidol is to be determined along with MCPD in the same analysis (Kuhlmann, 2011; Chung & Chan, 2012).

Given that MCPD is missing a suitable chromophore and has a single chlorine atom, following its release from fatty acid ester(s) MCPD is generally derivatized for successful analysis. Derivatization is frequently conducted using phenylboronic acid (PBA) (Weisshaar, 2008b; Hamlet & Asuncion, 2011; Jedrkiewicz et al., 2016; Karl et al., 2016). However, heptafluorobutyrylimidazole derivatives are also used for MCPD determination (Brereton et al., 2001; Hamlet & Asuncion, 2011; Vicente et al., 2015). MCPD derivatization with cyclohexanone using Nafion on a silica support as a catalyst has also been used (Becalski, Zhao & Sit, 2013).

Analysis is generally performed using stable isotope dilution analysis with GC-MS, although early research into the determination of MCPD also used gas chromatography with flame ionization detection and electron capture detection (Wenzl, Lachenmeier & Gokmen, 2007). Nonpolar capillary columns are routinely used to analyse MCPD derivatives, and oven temperature gradients vary from method to method for analyte separation. Although a split/splitless injector system is used most often, programmed temperature vaporization injectors are also used for these analyses (Kuhlmann, 2011; Karl et al., 2016). Mass spectrometry with single quadrupole instruments is most frequently reported, although tandem mass spectrometry has also been used for these analyses (Hamlet, 1998; Hamlet & Sadd, 2004; Hamlet & Asuncion, 2011; Kuhlmann, 2011; Miyazaki & Koyama, 2016). The ions used to confirm and measure MCPD concentrations most frequently are *m/z* 147 as the quantifying ion with 196 as a qualifier, although *m/z* 91, 146 and 198 are also used as qualifying ions, consistent with PBA derivatization of MCPD (Divinova et al., 2004; Zelinkova, Dolezal & Velisek, 2009a; Hrncirik et al., 2011; Kuhlmann, 2011; Wöhrlin et al., 2015; Jedrkiewicz et al., 2016; Karl et al., 2016; Miyazaki & Koyama, 2016). The transitions monitored when tandem mass spectrometry was used to detect heptafluorobutyrylimidazole derivatives of MCPD include *m/z* 289→253, 75 for native MCPD with *m/z* 294 →257, and 79 for the deuterated analogues (Hamlet, 1998; Hamlet & Sadd, 2004; Hamlet & Asuncion, 2011). Two-dimensional gas chromatography coupled with time-of-flight (GC-TOF) mass spectrometry has also been reported to confirm results (Zelinkova et al., 2008).

Some authors have adopted the approach taken by Divinova et al. (2004) that allowed both free MCPD and bound MCPD (MCPD esters) concentrations to be determined separately, using indirect methods (Divinova et al., 2004; Zelinkova et al., 2006). This strategy requires additional sample preparation and multiple instrumental injections. More recently, as part of a comparative study examining the different official methods of analysis, an aqueous salt solution was used to extract free MCPD from samples prior to the extraction of MCPD esters from fish tissue, thereby allowing for determination of both free and bound MCPDs from the same individual sample (Karl et al., 2016).

3-MCPD detection limits have decreased over time with some of the early limits estimated to be 5000 μg/kg when flame ionization detection was used, to more recent work where detection limits close to 1 μg/kg were obtained using mass spectrometry (Wenzl, Lachenmeier & Gokmen, 2007). Variability in detection limit is a result of the matrices measured and instrumentation utilized as well as instrument settings. Variability may be limited by residual levels of 3-MCPD in method blanks (Brereton et al., 2001; Kuhlmann, 2011; Kusters et al., 2011; Becalski et al., 2015a,b; Jedrkiewicz et al., 2016).

3.2.3 Quantitative methods

The lack of analytical standards for individual MCPD esters has impacted the ability to analyse the esters directly, which resulted in the need to convert MCPD esters to MCPD and the use of indirect methods (Li et al., 2016b). Only recently have standards of MCPD esters and their deuterated and ^{13}C-labelled analogues become available. This has allowed researchers to develop quantitative methods to measure the MCPD esters themselves in edible oils (Dubois et al., 2012; Yamazaki et al., 2013). Similar to indirect analysis, isotope dilution is used to correct for losses during sample preparation.

Initial methods developed to analyse MCPD esters employed liquid chromatography coupled with time-of-flight (LC-TOF) mass spectrometry systems using diluted samples without additional sample preparation (Haines et al., 2011). Although this allowed the MCPD esters to be directly measured, it was compromised by high detection limits and the need for extensive instrument maintenance.

More recent inclusion of cleanup steps using solid-phase extraction have allowed MCPD esters to be effectively separated from oil constituents (e.g. triglycerides) (Hori et al., 2012a; Moravcova et al., 2012). A two-stage cleanup protocol using silica and C-18 adsorbents successfully separated monoesters from diesters (Dubois et al., 2012; MacMahon, Begley & Diachenko, 2013a; Yamazaki et al., 2013). In addition, cleanup using aminopropyl cartridges to separate 3-MCPD monoesters, coupled with silica gel cleanup to isolate 3-MCPD diesters, has been demonstrated to be effective (Moravcova et al., 2012). Various solvents have been identified for elution from cartridges (e.g. diethyl ether/hexane, ethyl acetate/hexane, acetonitrile, ethanol/acetonitrile/methanol, methanol) (Hori et al., 2012a; Moravcova et al., 2012; MacMahon et al., 2013; Yamazaki et al., 2013; MacMahon, Ridge & Begley, 2014). In addition to cleanup with solid-phase extraction alone, liquid–liquid partitioning prior to solid-phase extraction cleanup and supercritical fluid extraction have been performed, and successful analysis of intact MCPD esters has been reported (Hori et al., 2012a,b).

In addition to the LC-TOF mass spectrometric analyses, MCPD esters have been analysed using liquid chromatography or ultra-high performance liquid chromatography (UHPLC) coupled with QTrap MS/MS, Orbitrap systems and triple quadrupole mass spectrometers. Instruments have consistently been operated using electrospray ionization (ESI) in the positive ion mode, with separation achieved using gradient elution on C-18 and C-8 columns (Hori et al., 2012a,b; Moravcova et al., 2012; MacMahon et al., 2013; Yamazaki et al., 2013; MacMahon, Ridge & Begley, 2014). Moravcova et al. (2012) also employed a direct analysis in real time (DART) atmospheric pressure ion source for some of their work.

Unlike indirect analyses, direct methods require a large number of analytical standards, with a corresponding increase in ions to be monitored. Lauric, linoleic, linolenic, myristic, oleic, palmitic and stearic acids of 3-MCPD are measured in current direct methods, consistent with availability of analytical standards and their deuterated analogues (Haines et al., 2011; Dubois et al., 2012; MacMahon, Begley & Diachenko, 2013a; Yamazaki et al., 2013). Owing to the diversity in fatty acid chain length, monitoring of a broad mass range (e.g. *m/z* 314.5–617.5) is required for direct analysis of MCPD esters (MacMahon, Begley & Diachenko, 2013a; Yamazaki et al., 2013). The determination of individual isomers continues to be a challenge, and attempts to separate all isomers are not always successful (Dubois et al., 2012).

Variable detection limits have been reported when direct MCPD ester determination has been performed, with elevated LODs (70–1690 µg/kg) observed in the earlier work where sodium adducts were formed (Haines et al., 2011). Ammonium adducts have been used more recently, and the reported detection limits have ranged from 2 to 71 µg/kg (Dubois et al., 2012; Hori et al., 2012b; Moravcova et al., 2012; Yamazaki et al., 2013). Higher detection limits were observed when the DART ion source was used (40–174 µg/kg) for individual MCPD esters (Moravcova et al., 2012).

Cleavage of fatty acid chains from MCPD esters occurs in vivo by lipases in the gastrointestinal tract. As a result, MCPDs are expected to be fully released from fatty acids in humans. Although analysis of intact MCPD esters provides information on the form present in food, toxicological impacts are known for MCPD rather than the MCPD esters (Crews et al., 2013). To compare results obtained using indirect (i.e. MCPD) and direct (MCPD ester) methods, conversion of MCPD ester concentrations to total MCPD equivalent concentrations is required (Dubois et al., 2012).

3.2.4 Reference methods

The Association for Official Analytical Chemists (AOAC) published a first action for an official method for the analysis of free 3-MCPD based on a collaborative study that focused on the determination of free 3-MCPD exclusively (and not MCPD esters) in a variety of foods (HVPs, malt extracts, soup powders, bread crumbs, salami and cheese) (Brereton et al., 2001; AOAC, 2005).

More recently, the American Oil Chemists' Society (AOCS) developed three official methods for the analysis of MCPD esters: AOCS Cd 29a-13, Cd 29b-13 and Cd 29c-13. In keeping with the focus of the work, each method has been developed for analyte determination exclusively in oils and fats. All three methods result in the determination of the 2- and 3-MCPD equivalent concentrations with a concurrent determination of glycidol equivalent. Method AOCS Cd 29a-

13 specifies an acidic hydrolysis of MCPD esters whereas method AOCS Cd 29b-13 specifies an alkaline hydrolysis of MCPD esters. The third method (Cd 29c-13) allows for the separate determinations of 3-MCPD equivalents and glycidol equivalents by difference (AOCS, 2013a,b,c).

A collaborative study compared the results obtained for MCPD esters in oils using the German Society for Fat Science (DGF; Weisshaar, 2008b) and the SGS Germany GmbH (Kuhlmann, 2011) methods. The conclusion of this study was that both methods would accurately determine 3-MCPD potential in fats and oils, following the assumption that glycidol was the only 3-MCPD forming substance in the oil (Fiebig, 2011).

International standard methods are available for the determination of MCPD esters in fats and oils (ISO 18363-1) and 3-MCPD in foodstuffs (BS EN 14573:2004). MCPD esters, or bound MCPD, are analysed using an indirect method with ester cleavage performed under alkaline conditions. Both methods are based on GC-MS detection and require derivatization, although the derivatizing reagent is different for each method (PBA–ISO 18363-1; HFBI–BS EN 14573:2004) (European Committee for Standardization, 2004; ISO, 2015). All methods in the ISO 18363 series are identical with and the collaborative trial data are taken from the respective AOCS method.

Although there are collaboratively studied methods for the determination of 3-MCPD esters in fats and oils, there are no published collaboratively studied methods for the determination of these contaminants in foods. Recent conference presentations (AOCS, 2016; Euro Fed Lipid, 2016) have detailed a collaborative study for the detection of 3-MCPD esters in mayonnaise and margarines. In view of the absence of collaborative studies on other food matrices, caution should be applied when interpreting analytical data arising from complex food analysis. Furthermore, there is a high degree of uncertainty in comparing the reported levels in the same foods from different regions because of the lack of interlaboratory comparisons and the absence of information on sample sharing or data arising from proficiency testing schemes.

3.2.5 Quality assurance considerations

Analyte loss during sample preparation is of concern to all analysts. Developing approaches to correct for losses is critical in the determination of accurate measurements. The most frequently adopted method to account for losses is the inclusion of surrogate standards, frequently made from stable isotope analogues of the compound(s) of interest, added prior to the initiation of sample preparation. Deuterated (e.g. d_5-) analogues of MCPD and its esters are routinely used for this purpose when MCPD and MCPD ester determination is undertaken (Brereton et al., 2001; Zelinkov, Dolezal & Velisek, 2009b; Crews

et al., 2013). Final derivatives of 2- and 3-MCPD for analysis are semi-volatile, and care should be exercised during final stages of evaporation. Additional approaches to ensure that analyses are of acceptable quality involve the inclusion of (1) reagent blanks to determine contributions to analyte concentrations from the laboratory or reagents used; (2) samples fortified with the analyte and treated as samples of unknown concentration to determine recovery through processing; (3) participation in proficiency testing programmes; and (4) measurement of analytes in reference materials (Wong, Cheong & Seah, 2006; Becalski, Zhao & Sit, 2013; Fry et al., 2013). Annual proficiency testing and quality control test materials are available only for MCPD and MCPD ester analysis in soy sauce and vegetable oil, respectively (Fera, 2015).

4. Sampling protocols

Although the Codex Alimentarius Commission has not established specific sampling protocols for MCPD or its esters, general guidelines on sampling have been developed (FAO/WHO, 2004). The European Union has been active in addressing methods of sampling for control of a number of chemical contaminants, initially including free 3-MCPD (European Commission, 2001, 2007, 2011, 2014). Although the European directives and regulations utilize principles established by Codex (FAO/WHO, 2004) for sample collection, etc., they have been updated and amended several times since being established, and currently include MCPD esters (European Commission, 2007, 2011, 2014).

Sampling protocols include the following:

- Sample collection must be performed by qualified individuals using containers that are clean and nonreactive and that protect samples from contamination or damage during transport and storage (European Commission, 2001).
- Sampling of commercial food products must ensure that the samples are representative of the lot. Therefore, collection of multiple samples (incremental samples) from within the lot is recommended. These may be used to form an aggregate sample from which subsamples are taken for analysis in the laboratory (FAO/WHO, 2004).
- Prior to the subsampling for laboratory analysis, the aggregate sample should be homogenized, consistent with good laboratory practices.
- Sample collection must be focused on food commodities that are relevant to MCPD and MCPD esters. Specific foods have been identified in the recent European regulations and include vegetable

oil/fats, specialized nutritional products (e.g. infant formulas), bakery products including bread and rolls, canned meat and fish, potato or cereal-based products and those foods containing or prepared with vegetable oils (European Commission, 2014).

5. Effects of processing

3-MCPD esters are generated during the refining process of crude oils and fats. Formation of 3-MCPD esters in oil has been associated with deodorization at high temperatures (Smidrkal et al., 2016). Unrefined oils contain a variety of compounds that contribute to the formation of 3-MCPD esters including acylglycerols, phospholipids, free fatty acids and chlorinated compounds (Franke et al., 2009; Nagy et al., 2011; Ramli et al., 2011). The refining practices performed prior to deodorization have an impact on 3-MCPD ester production, as does the temperature at which the deodorization is performed (Matthaus et al., 2011). The preliminary steps in the refining process include degumming or washing, neutralization, bleaching and deodorization (Pudel et al., 2011). The condition of fruit, etc., used for making oil will also have an impact on the levels of 3-MCPD esters, with bruised components contributing to higher levels of these contaminants (Gibon, De Greyt & Kellens, 2007).

Degumming of oil removes phospholipids and is generally performed at relatively low temperatures (80–120 °C) (Pudel et al., 2011; Smidrkal et al., 2011). Neutralization of oils involves interaction of the oil with sodium carbonate or bicarbonate to lower the acid value (increase the pH) prior to deodorization (Freudenstein, Weking & Matthaus, 2013). The bleaching process exposes the oils to bleaching clays to remove phospholipids (Hrncirik & van Duijn, 2011). During the final stage of oil refining, known as deodorization, in addition to acid treatment, oils are heated at elevated temperatures (Matthaus et al., 2011). The refining process has been investigated to determine the impact on formation of glycidyl esters and MCPD esters, although the majority of research has focused on the MCPD esters owing to their earlier discovery.

Palm oil has 3-MCPD ester concentrations higher than in soybean, rapeseed or sunflower seed oil (Kuhlmann, 2011; Weisshaar, 2011). Palm oil is known to contain low levels of phospholipids, so the degumming step is often skipped or else dry degumming (treatment with citric or phosphoric acid) is performed (Gibon, De Greyt & Kellens, 2007; Pudel et al., 2011). Because of the elevated 3-MCPD concentrations, palm oil has been the focus of much research into 3-MCPD ester formation and identification of mitigation strategies (Matthaus et al., 2011; Pudel et al., 2011; Strijowski et al., 2011). Other research

has examined seed oils (both virgin and refined) to investigate at what stage 3-MCPD formation takes place (Zelinkova et al., 2006).

Unlike the glycidyl esters, formation of 3-MCPD esters is not directly correlated with increased temperature, particularly above 240 °C (Zulkurnain et al., 2012). Rather, 3-MCPD ester formation does occur at temperatures corresponding to deodorization of oils (generally >200 °C) (Ozdikicierler, Yemiscioglu & Gumuşkesen, 2016).

5.1 Degumming/washing

Phospholipids, which have been identified as possible precursors of MCPD esters, are removed from oil using a combination of water or acid, which leads to their precipitation from the treated oil (Pudel et al., 2011). A combination of water and ethanol may also be used to remove phospholipids (Craft et al., 2012). Following washing with water and ethanol, Matthaus et al. (2011) observed a decrease in 3-MCPD ester concentrations relative to glycidyl ester concentrations. Different research groups have tested a variety of conditions related to degumming in model systems, including acid (e.g. citric acid, phosphoric acid) degumming, water degumming and no degumming, to assess effectiveness (Pudel et al., 2011; Ramli et al., 2011; Smidrkal et al., 2011; Zulkurnain et al., 2012, 2013).

5.2 Neutralization

Cleavage of fatty acids from acylglycerols under acidic conditions is known to occur and result in acidification of samples prior to analysis (Divinova et al., 2004). Adjusting the oil to a neutral pH may impact the 3-MCPD ester formation capacity during deodorization because this conversion is strongly pH dependent (Freudenstein, Weking & Matthaus, 2013). Differences in 3-MCPD ester concentrations have been observed when a variety of materials (e.g. calcium oxide, calcium carbonate) have been used for neutralization (Ramli et al., 2011).

5.3 Bleaching

Removal of pigments and phospholipids can also be effected by bleaching through the exposure to bleaching earth and moderate heat (60–90 °C) (Pudel et al., 2011). Bleaching has been tested at various pHs using both natural and sulfuric acid–activated clays (bentonite, attapugite) to determine which material reduces 3-MCPD ester concentrations most effectively (Ramli et al., 2011).

5.4 Deodorization

Exposure of oil to elevated temperatures during the deodorization step in the refining process is related to 3-MCPD ester formation, although no clear correlation between high temperature and ester formation has been established (Hrncirik & van Duijn, 2011). Maximum concentrations of 3-MCPD esters were obtained in neutralized, bleached palm oils deodorized at 180 °C for 1 hour, and reduced levels were observed when deodorization was performed at 180 °C for 5 hours. At 230 °C (for 1, 3 and 5 hours), the equivalent oil produced lower 3-MCPD concentrations than the batch treated at 180 °C for 1 hour (Hrncirik & van Duijn, 2011). Pudel et al. (2011) observed that 3-MCPD ester formation was highest after 90 minutes at multiple temperatures (240, 250 and 270 °C), but after this time period, 3-MCPD ester concentrations decreased. At deodorization temperatures (235 °C), 3-MCPD esters were formed in the presence of both organic and inorganic chlorine (Destaillats et al., 2012). Increased 3-MCPD ester concentrations in deodorized rapeseed oil were observed relative to the same oil following any other treatment (degumming, neutralization, etc.), although final concentrations remained below those obtained in deodorized virgin olive oils and prerefined olive oils (Franke et al., 2009).

6. Prevention and control

Similar to the glycidyl esters, formation of 3-MCPD esters is a function of the chemicals present in oils coupled with the processing conditions used to refine the crude oil. Unrefined or virgin oils generally have nondetectable concentrations of these contaminants, and they are usually observed in refined products (Franke et al., 2009; Strijowski et al., 2011). Higher 3-MCPD ester concentrations are observed in refined palm oils than other edible oils (Destaillats et al., 2012). As a result, much of the research related to 3-MCPD formation has focused on palm oil and the presence of critical precursors to 3-MCPD ester formation (e.g. triacylglycerols, chlorinated compounds) (Nagy et al., 2011; Destaillats et al., 2012).

A number of approaches can be taken to help mitigate 3-MCPD esters in final oil products.

6.1 Harvest and storage conditions

Growth and storage conditions of the oil-producing crops have an impact on the presence of precursors and, in some cases, may be beyond the control of

producers (because of, for example, climate, fertilizer usage, harvest conditions, etc.) (Matthaus et al., 2011).

Implementation of appropriate postharvest measures to reduce the bruising of fruit, which results in formation of acylglycerols and free fatty acids, may improve the quality of the fruit being used to prepare the oil and may ultimately result in lowered 3-MCPD ester concentrations in the final oil (Poku, 2002; Gibon, De Greyt & Kellens, 2007). Enzymatic activity in fruit increases with time, leading to over-ripened or damaged fruit having a higher mono- or diacylglycerol content when processed (Gibon, De Greyt & Kellens, 2007; Craft et al., 2012). This suggests that rapid processing of undamaged fruit into oil would lead to better oil quality with a lower potential for 3-MCPD ester formation.

6.2 Reduction of precursors – optimization of refining processes

Processing steps focused on the removal of the 3-MCPD ester precursors prior to oil deodorization contribute to the reduction of these contaminants in processed oils. Degumming or washing palm oils with water or acid lower 3-MCPD concentrations in the final product, although washing with 5% water with no acid was found to be most effective (Pudel et al., 2011). However, Pudel et al. (2011) indicated that the impact of removal of phospholipids does not correlate directly to final 3-MCPD ester concentrations. Success in reducing 3-MCPD diester levels has been observed when washing crude palm oil with a 1:1 mixture of ethanol and water prior to bench-top deodorization (Craft et al., 2012). Degumming of palm oil using phosphoric acid when combined with activated clays lowered 3-MCPD ester levels (Ramli et al., 2011). Reduced 3-MCPD ester concentrations were observed in previously refined and crude palm oils that had been degummed prior to deodorization, while rapeseed oil had 3-MCPD ester concentrations below the LOD (<0.4 mg/kg) in the similarly degummed samples (Franke et al., 2009). Washing of oil deodorized at 240 °C with aluminium nitrate did not reduce the 3-MCPD ester concentrations in oil as effectively as using water alone.

Neutralization of oil has also been investigated by some researchers. Using potassium hydroxide resulted in greater reduction in 3-MCPD ester formation (45%) than using sodium hydroxide (35%) (Pudel et al., 2011). Ramli et al. (2015) reported that oil acidity was an important contributor to 3-MCPD ester formation, indicating that neutralization would be beneficial in terms of reduction to 3-MCPD ester formation.

Treatment of oil using different adsorbents can impact 3-MCPD ester formation. Treatment with magnesium silicate was found to result in lower 3-MCPD ester concentrations in the final oil, relative to activated clay < natural

clay < activated carbon (Zulkurnain et al., 2012). The choice of adsorbent impacts oil impurities (e.g. carotene content, etc.) and colour in the final oil (Zulkurnain et al., 2012). Given that these are important characteristics, it may influence which adsorbent producers choose. Strijowski et al. (2011) also tested a variety of adsorbents for use in the bleaching of oils. Of nine adsorbents tested, only calcinated zeolite (<1% water) and a sodium aluminium silicate were effective in reducing 3-MCPD ester concentrations. Although the calcinated zeolite also reduced glycidyl ester concentrations, sodium aluminium silicate was not effective. Implementing additional treatment steps with alternative adsorbents provides a rapid means to reduce 3-MCPD esters and their precursors.

6.3 Deodorization conditions

Deodorization is a critical factor in the formation of 3-MCPD esters and has been the focus of much of the research aimed at reducing MCPD ester concentrations. Both elevated temperatures and duration impact 3-MCPD ester content in refined oils (Hrncirik & van Duijn, 2011). Although lowering the deodorization temperature may reduce the 3-MCPD ester content of oil, doing so may lead to a reduction in the quality of the final product, a critical consideration when choosing deodorization conditions.

Unlike the effect on glycidyl ester concentrations, inclusion of formic acid during deodorization did not reduce 3-MCPD ester content in the final product (Matthaus et al., 2011).

Dual deodorization has been proposed as a way to improve the quality of the oil. Deodorization first takes place at a high temperature (250–270 °C) for a short time and is followed by a second heat process at a lower temperature (200 °C) for a longer time.

Another way to reduce 3-MCPD esters is by using high pressure thermal sterilization (HPTS), which combines moderate temperatures (e.g. 90–121 °C) with high pressure (600 MPa) (Sevenich et al., 2013). Use of HPTS is still at the experimental stage.

Strategies to prevent and control 3-MCPD esters in final oil products include:

- Selection of raw material with low precursor content;
- Removal of precursors using chemical treatment at mid-range temperatures;
- Deodorization at neutral pH and at temperatures <240 °C;
- Adoption of dual deodorization protocols; and
- Utilization of adsorbents to remove 3-MCPD esters in post-treatment.

The Committee noted the commitment of the European Union Vegetable Oil and Proteinmeal Industry Union (FEDIOL) to continue to reduce the levels of 3-MCPD esters in refined vegetable oils and encouraged them to continue to reduce these contaminants using reasonable approaches to mitigation (FEDIOL, 2016).

7. Levels and patterns of contamination in food commodities

Globally, oils and fats are regional in their production and are likely to be consumed in higher proportions in the production area than in importing countries. As such, palm oil and its products are a major fat in South-east Asia, but are less dominant in Europe and North America, whereas soybean oil is common in North and South America, and rapeseed and sunflower seed oils in Europe. In addition, a mixture of oils is often used to give a food a particular texture or structure. Thus, the pattern of consumption of individual oils complicates determining the source of oil in any finished food.

3-MCPD esters are formed during the processing of vegetable oils, mainly during the deodorization step. The extent to which they are formed may depend on the oilseed or fruit being processed, the process used and the type of equipment installed (AOCS, 2016). Hence, the refined oil obtained from any oilseed source may vary in 3-MCPD ester content.

Based on reports of the analysis of foodstuffs in a number of countries, it appears that refined vegetable oil is a major contributor to the levels of 3-MCPD esters in food (EFSA, 2016). There appears to be little evidence that 3-MCPD esters are formed in food during processing or cooking, and there is a reasonable correlation between the levels of the 3-MCPD esters in the oils used and the amount they were used in.

Early studies of 3-MCPD esters in 20 retail food products found that all samples contained free 3-MCPD at approximately 9.6–82.7 μg/kg food and 3-MCPD esters at concentrations ranging from below the LOD to 6.1 mg/kg food (Svejkovka et al., 2004). The levels of bound 3-MCPD (monoesters and diesters of 3-MCPD with higher fatty acids) in the foodstuffs varied between the LOD (1.1 mg/kg of fat) and 36.8 mg/kg fat. Five foodstuffs of plant origin processed at high temperatures contained elevated levels of bound 3-MCPD (0.14–6.10 mg/kg). A high level of bound 3-MCPD (0.28 mg/kg) was also found in a sample of pickled fish.

High temperature of roasting of coffee surrogates and malts also results in high amounts of 3-MCPD esters, with levels of 145–1184 μg/kg found in coffee

surrogates and of 4.0–650 µg/kg in malts in the Czech Republic (Divinova, Dolezal & Velisek, 2007). The highest amounts were found in roasted barley and roasted malts, which could be a result of the high fat content and low water content of the roasted cereals and the high temperatures used (>200 °C) in roasting.

Dingel & Matissek (2015) found that 3-MCPD esters do not appear to be formed during deep frying, though this could be because of lower frying temperatures (160–188 °C; temperatures during deodorization may be up to 270 °C). In addition, the chlorine-containing compounds that are known 3-MCPD esters precursors may no longer have been present in the deodorized high-oleic sunflower oil used. Thus, the quality of the final product (and the presence of 3-MCPD esters in a food) may depend directly on the quality of the frying oil used.

2- and 3-MCPD esters were also found in more than 100 edible fats and oils and products containing fats/oils, such as cookies and cooking sprays, in Canada (Becalski et al., 2015a,b). Most of virgin/unprocessed/unrefined oils did not contain detectable levels of MCPD esters except for two samples of unrefined palm oils, which contained MCPD esters at 100–550 µg/kg. The reasons the unrefined oils contained MCPD equivalents was not clear, especially when compared with the Kuhlmann (2011) study, which found undetectable amounts of MCPD esters in the virgin or crude oils tested, including sesame oil. MacMahon et al. (2013) found levels of MCPD esters in toasted unrefined sesame oils comparable to those found by Becalski et al. (2015a,b) in toasted and unrefined sesame oils, indicating that the roasting process might not be the sole reason for the presence of MCPD esters in processed sesame oils.

MCPD ester levels were highly variable in processed oils/fats, reaching 17 mg/g, expressed as MCPD equivalents.

8. Food consumption and dietary exposure estimates

Previous JECFA assessments of 3-MCPD focused on exposure to free 3-MCPD from consumption of soy sauce, HVP and other processed foods. In the current evaluation, dietary exposure was estimated for 3-MCPD from all sources, including exposure from 3-MCPD esters. As reviewed in section 7, 3-MCPD esters are found in processed fats and oils and in foods containing fats and oils. Exposures to 3-MCPD esters from refined oil ingredients in infant formula are of special interest, because some infant formulas contain palm oil or palm olein, which may have higher concentrations of 3-MCPD esters relative to concentrations in other oils (MacMahon et al., 2013), and because formula is the sole or primary source of nutrition for many infants.

8.1 Concentrations in food used in the dietary exposure estimates

The Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database was queried for the period from 2012 to 2016 for records relating to 3-MCPD (esters, free form or total) in any food. The 2012 date was chosen due to concerns about the accuracy of 3-MCPD ester analyses conducted prior to 2012 and because analyses of samples collected prior to 2012 may not reflect 3-MCPD concentrations in products on the market today (EFSA, 2016).

The data extracted from the GEMS/Food contaminants database originated from 15 countries, representing eight of the 17 GEMS/Food cluster diets (Table 7). Data on 3-MCPD ester concentrations in foods were submitted by Brazil, Canada, China, Japan, Singapore and the USA. Each of these countries submitted data on 3-MCPD ester concentrations in oils; data submitted on other food categories varied by submitter. Data on 3-MCPD ester concentrations in infant formulas or follow-on formulas were submitted by Brazil, Canada, France, Germany, Greece, Italy, Japan, the Netherlands, Spain and the USA. Data on total 3-MCPD concentrations in selected foods (mainly teas, honey, seasonings and dietary supplements) were submitted by seven European countries (France, Germany, Greece, Ireland, Italy, the Netherlands, Spain and the United Kingdom).

Most (78%) of the 3-MCPD ester and total 3-MCPD concentration data were obtained from random sampling rather than from targeted sampling. However, because key data on fats and oils and on infant formulas from several countries (Canada, Japan, USA) were obtained using targeted sampling, these data were included in the exposure estimation.

Data on free 3-MCPD concentrations in selected foods (mainly sauces and seasonings) were submitted by the Philippines and Singapore. All of the submitted free 3-MCPD concentration data were obtained from random sampling.

3-MCPD concentration data provided in other units were converted to μg/kg food. Data provided by Japan on 3-MCPD ester concentrations in butter and margarine were reported per kg fat; these data were multiplied by a factor of 0.8 (i.e. assuming an 80% fat concentration in butter and margarine) to obtain concentrations per kg food. Data provided by Japan on 3-MCPD ester concentrations in infant formula were also reported per kg fat; these data were multiplied by a factor of 0.04 (i.e. assuming a maximum fat concentration of 4% in prepared formulas). 3-MCPD ester concentration data submitted for formula powders were converted to concentrations in prepared formulas assuming a dilution factor of 7.7 (EFSA, 2016). 3-MCPD ester concentration data submitted on formula concentrates were converted to concentrations in prepared formulas assuming a dilution factor of 2.

Table 7
3-MCPD concentration data from GEMS/Food contaminants database, 2012–2016

		3-MCPD data: foods included		
Country	**Cluster**	**Esters (no. of samples)**	**Free (no. of samples)**	**Total (no. of samples)**
Brazil	5	Fats/oils; follow-on formula; baked products; instant noodles; French fries; onion (cooked); garlic (cooked); snack foods; composite foods (250)	–	–
Canada	10	Fats/oils; infant formula; baked products (83)	–	–
China	9	Fats/oils; imitation coffees; bread; cereals/products nes; cheese; meat/products, raw; milk and dairy/products nes (478)	–	–
France	7	–	–	Chamomile, beef (unknown form), milk, cheese, chicken (unknown form), eggs (raw), yogurt, follow-on formula, honey, peppermint, pork (unknown form), tea, supplements (222)
Germany	8	–	–	Chamomile, beef (unknown form), milk, cheese, chicken (unknown form), eggs (raw), yogurt, follow-on formula, infant formula, herbs, honey, leafy greens, peppermint, pork (unknown form), tea, turkey (unknown form), vegetable dishes, supplements (1320)
Greece	6	–	–	Chamomile, beef (unknown form), milk, cheese, chicken (unknown form), eggs (raw), yogurt, infant formula, honey, peppermint, pork (unknown form), tea, supplements (96)
Ireland	15	–	–	Honey (23)
Italy	10	–	–	Chamomile, beef (unknown form), milk, cheese, chicken (unknown form), eggs, yogurt, infant formula, follow-on formula, peppermint, pork (unknown form), tea, supplements (141)
Japan	10	Fats/oils; infant formula; follow-on formula; formula for medical use (360)	–	–
Netherlands	11	–		Chamomile, beef (unknown form), milk, cheese, chicken (unknown form), chicken liver (unknown form), eggs, yogurt, infant formula, follow-on formula, peppermint, pork, pork liver, tea, supplements (287)
Philippines	9	–	Sauces (33)	–
Singapore	No cluster	Biscuits; oils (43)	Sauces; seasonings (409)	–

Table 7 (continued)

		3-MCPD data: foods included		
Country	**Cluster**	**Esters (no. of samples)**	**Free (no. of samples)**	**Total (no. of samples)**
Spain	10	–	–	Chamomile, beef (unknown form), beef liver (unknown form), cow milk, goat milk, cheese, chicken (unknown form), eggs (raw), yogurt, infant formula, follow-on formula, peppermint, pork (unknown form), pork liver (unknown form), tea, supplements (285)
United Kingdom	7	–	–	Chamomile, honey, ginseng, tea, supplements (225)
USA	10	Fats/oils; infant formula; toddler formula (269)	–	–

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; 3-MCPD: 3-(mono)chloro-1,2-propanediol; nes: not elsewhere specified; no.: number; USA: United States of America

For each food included in the GEMS/Food contaminants database, a lower-bound (LB) mean was calculated after setting concentrations to zero for samples for which the concentration was below the LOD. An upper-bound (UB) mean was calculated by setting nondetected sample concentrations to the LOD. Table 8 shows, by GEMS/Food cluster diet, the number of samples, per cent left censorship, LB mean and UB mean for 3-MCPD concentrations in foods based on sample dates of 2012–2016.

8.2 Food consumption data used in the dietary exposure estimates

The Committee used data from the FAO/WHO Chronic Individual Food Consumption Database – summary statistics (CIFOCOss) to estimate national exposures to 3-MCPD esters in countries for which recent published estimates were not available. CIFOCOss is a compendium of food consumption survey data collected from individuals over 2 or more days; the age groups for which data are included vary by country. Food consumption data expressed per kg bw were determined based on the actual body weights of survey respondents. The CIFOCOss food classification system includes some food groups from the Codex raw commodity classification system and others from the Codex General Standard for Food Additives. Further details are available at http://www.who.int/foodsafety/databases/en/.

The Committee used the GEMS/Food cluster diets to estimate international exposures to 3-MCPD esters. This database includes estimates of consumption for 17 GEMS/Food clusters. The diets were developed by grouping countries with similar eating patterns, and are based on Food and Agriculture Organization of the United Nations (FAO) food balance sheet data.

8.3 Assessments of dietary exposure

8.3.1 National estimates

Because toxicological evidence indicates that there are no concerns regarding acute exposures to 3-MCPD, the Committee evaluated chronic exposures only.

A comprehensive search for peer-reviewed original research on dietary exposure to 3-MCPD esters (expressed as 3-MCPD equivalents), free 3-MCPD or total 3-MCPD (i.e. esters plus free form) was conducted. Literature databases searched included Web of Science and PubMed. Search terms were "[3-monochloropropane-1,2-diol OR MCPD] AND [intake or exposure]". The search was limited to studies published in 2012 or later due to concerns about the accuracy of earlier 3-MCPD ester analyses and due to concerns that analyses of samples collected prior to 2012 may not reflect 3-MCPD concentrations in products on the market today (EFSA, 2016).

Table 8

Summary of data from the GEMS/Food contaminants database on concentrations of 3-MCPD (as 3-MCPD equivalents), by cluster diet

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
3-MCPD, esters or total																		
17 Prepared fruits (no dried & juice)																		
Banana, cooked[c]																		
No. of individual samples	1	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	50	–	–	–	–	50	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	50	–	–	–	–	50	–	–	–	–	–	–	–	–	–	–	–	–
22 Tree nuts (excluding groundnuts)																		
Hazelnut spread[c]																		
No. of individual samples	1	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	50	–	–	–	–	50	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	50	–	–	–	–	50	–	–	–	–	–	–	–	–	–	–	–	–
32 Roots and tubers processed																		
French fries; potato noisettes[c]																		
No. of individual samples	8	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	25	–	–	–	–	25	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	70	–	–	–	–	70	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	80	–	–	–	–	80	–	–	–	–	–	–	–	–	–	–	–	–
Potato or cassava sticks or chips[c]																		
No. of individual samples	26	–	–	–	–	26	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	291	–	–	–	–	291	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	291	–	–	–	–	291	–	–	–	–	–	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
41 Bulb vegetables																		
Garlic, chopped, cooked; onion, chopped, cooked[c]																		
No. of individual samples	8	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	526	–	–	–	–	526	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	526	–	–	–	–	526	–	–	–	–	–	–	–	–	–	–	–	–
44 Leafy vegetables (including Brassica leafy vegetables and seaweed)																		
Leaf lettuce; roman rocket; spinach (state unknown)																		
No. of individual samples	46	–	–	–	–	–	–	–	46	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	91	–	–	–	–	–	–	–	91	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	1	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	1	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
48 Other and mixed vegetables																		
Vegetable-based composites[c]																		
No. of individual samples	6	–	–	–	–	2	–	–	4	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	67	–	–	–	–	0	–	–	100	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	47	–	–	–	–	140	–	–	0	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	47	–	–	–	–	140	–	–	0	–	–	–	–	–	–	–	–	–
50 Herbs																		
Aniseed myrtle; chamomile; fennel seed; ginseng; peppermint (state unknown)																		
No. of individual samples	266	–	–	–	–	–	7	22	201	–	14	22	–	–	–	–	–	–
% samples < LOD or LOQ	55	–	–	–	–	–	71	59	54	–	71	41	–	–	–	–	–	–
LB mean (µg/kg)	83	–	–	–	–	–	34	34	93	–	44	85	–	–	–	–	–	–
UB mean (µg/kg)	90	–	–	–	–	–	35	36	101	–	45	86	–	–	–	–	–	–
61 Further processed cereals and by-products																		
Noodles/pasta, "raw"[c]																		
No. of individual samples	10	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	151	–	–	–	–	151	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	151	–	–	–	–	151	–	–	–	–	–	–	–	–	–	–	–	–
Other baked products[c]																		
No. of individual samples	21	–	–	–	–	5	–	–	–	–	6	–	–	–	–	–	–	–
% samples < LOD or LOQ	5	–	–	–	–	20	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	292	–	–	–	–	112	–	–	–	–	154	–	–	–	–	–	–	–
UB mean (µg/kg)	293	–	–	–	–	120	–	–	–	–	154	–	–	–	–	–	–	–
Bread, "raw"[c]																		
No. of individual samples	44	–	–	–	–	–	–	–	–	44	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–
LB mean (µg/kg)	233	–	–	–	–	–	–	–	–	233	–	–	–	–	–	–	–	–
UB mean (µg/kg)	233	–	–	–	–	–	–	–	–	233	–	–	–	–	–	–	–	–
Cereals and cereal-based products, nes[c]																		
No. of individual samples	22	–	–	–	–	–	–	–	–	22	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–
LB mean (µg/kg)	220	–	–	–	–	–	–	–	–	220	–	–	–	–	–	–	–	–
UB mean (µg/kg)	220	–	–	–	–	–	–	–	–	220	–	–	–	–	–	–	–	–
70 Sugars, honey, candies (excluding chocolate), sweeteners and molasses																		
Honey																		
No. of individual samples	670	–	–	–	–	–	–	107	540	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	95	–	–	–	–	–	–	97	98	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	0	0	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	1	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
80 Milk fats																		
Butter																		
No. of individual samples	37	–	–	–	–	8	–	–	–	–	29	–	–	–	–	–	–	–
% samples < LOD or LOQ	73	–	–	–	–	88	–	–	–	–	69	–	–	–	–	–	–	–
LB mean (µg/kg)	10	–	–	–	–	6	–	–	–	–	10	–	–	–	–	–	–	–
UB mean (µg/kg)	32	–	–	–	–	41	–	–	–	–	30	–	–	–	–	–	–	–
81 Mammalian fats (no milk fat)																		
Lard																		
No. of individual samples	24	–	–	–	–	–	–	–	–	–	24	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	266	–	–	–	–	–	–	–	–	–	266	–	–	–	–	–	–	–
UB mean (µg/kg)	266	–	–	–	–	–	–	–	–	–	266	–	–	–	–	–	–	–
84 Plant origin fat																		
Almond oil																		
No. of individual samples	3	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–
% samples < LOD or LOQ	33	–	–	–	–	–	–	–	–	–	33	–	–	–	–	–	–	–
LB mean (µg/kg)	1 051	–	–	–	–	–	–	–	–	–	1 051	–	–	–	–	–	–	–
UB mean (µg/kg)	1 054	–	–	–	–	–	–	–	–	–	1 054	–	–	–	–	–	–	–
ARA/DHA oil																		
No. of individual samples	3	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	378	–	–	–	–	–	–	–	–	–	378	–	–	–	–	–	–	–
UB mean (µg/kg)	378	–	–	–	–	–	–	–	–	–	378	–	–	–	–	–	–	–
Avocado oil																		
No. of individual samples	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	50	–	–	–	–	–	–	–	–	–	50	–	–	–	–	–	–	–
LB mean (µg/kg)	218	–	–	–	–	–	–	–	–	–	218	–	–	–	–	–	–	–
UB mean (µg/kg)	267	–	–	–	–	–	–	–	–	–	267	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Coconut oil																		
No. of individual samples	21	–	–	–	–	–	–	–	–	–	21	–	–	–	–	–	–	–
% samples < LOD or LOQ	24	–	–	–	–	–	–	–	–	–	24	–	–	–	–	–	–	–
LB mean (µg/kg)	266	–	–	–	–	–	–	–	–	–	266	–	–	–	–	–	–	–
UB mean (µg/kg)	272	–	–	–	–	–	–	–	–	–	272	–	–	–	–	–	–	–
Cottonseed oil																		
No. of individual samples	3	–	–	–	–	–	–	–	–	1	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	0	0	–	–	–	–	–	–	–
LB mean (µg/kg)	371	–	–	–	–	–	–	–	–	252	431	–	–	–	–	–	–	–
UB mean (µg/kg)	371	–	–	–	–	–	–	–	–	252	431	–	–	–	–	–	–	–
Flaxseed oil																		
No. of individual samples	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	50	–	–	–	–	–	–	–	–	–	50	–	–	–	–	–	–	–
LB mean (µg/kg)	44	–	–	–	–	–	–	–	–	–	44	–	–	–	–	–	–	–
UB mean (µg/kg)	144	–	–	–	–	–	–	–	–	–	144	–	–	–	–	–	–	–
Grapeseed oil																		
No. of individual samples	10	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	2 254	–	–	–	–	–	–	–	–	–	2 393	–	–	–	–	–	–	–
UB mean (µg/kg)	2 254	–	–	–	–	–	–	–	–	–	2 393	–	–	–	–	–	–	–
Groundnut oil																		
No. of individual samples	38	–	–	–	–	2	–	–	–	29	7	–	–	–	–	–	–	–
% samples < LOD or LOQ	16	–	–	–	–	0	–	–	–	10	43	–	–	–	–	–	–	–
LB mean (µg/kg)	1 353	–	–	–	–	215	–	–	–	1 694	266	–	–	–	–	–	–	–
UB mean (µg/kg)	1 357	–	–	–	–	215	–	–	–	1 696	283	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Hemp oil																		
No. of individual samples	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	50	–	–	–	–	–	–	–	–	–	50	–	–	–	–	–	–	–
LB mean (µg/kg)	20	–	–	–	–	–	–	–	–	–	20	–	–	–	–	–	–	–
UB mean (µg/kg)	24	–	–	–	–	–	–	–	–	–	24	–	–	–	–	–	–	–
Macadamia oil																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	100	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
UB mean (µg/kg)	8	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–
Maize germ oil																		
No. of individual samples	33	–	–	–	–	10	–	–	–	–	23	–	–	–	–	–	–	–
% samples < LOD or LOQ	12	–	–	–	–	20	–	–	–	–	9	–	–	–	–	–	–	–
LB mean (µg/kg)	263	–	–	–	–	281	–	–	–	–	254	–	–	–	–	–	–	–
UB mean (µg/kg)	278	–	–	–	–	291	–	–	–	–	272	–	–	–	–	–	–	–
Margarine																		
No. of individual samples	109	–	–	–	–	16	–	–	–	–	75	–	–	–	–	–	–	–
% samples < LOD or LOQ	5	–	–	–	–	0	–	–	–	–	1	–	–	–	–	–	–	–
LB mean (µg/kg)	629	–	–	–	–	267	–	–	–	–	607	–	–	–	–	–	–	–
UB mean (µg/kg)	631	–	–	–	–	267	–	–	–	–	607	–	–	–	–	–	–	–
Mayonnaise																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	100	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
UB mean (µg/kg)	99	–	–	–	–	–	–	–	–	–	99	–	–	–	–	–	–	–
MCT (medium chain triglycerides) oil																		
No. of individual samples	5	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–
% samples < LOD or LOQ	40	–	–	–	–	–	–	–	–	–	40	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
LB mean (µg/kg)	18	–	–	–	–	–	–	–	–	–	18	–	–	–	–	–	–	–
UB mean (µg/kg)	21	–	–	–	–	–	–	–	–	–	21	–	–	–	–	–	–	–
Oil, other or blends																		
No. of individual samples	47	–	–	–	–	13	–	–	–	–	29	–	–	–	–	–	–	–
% samples < LOD or LOQ	17	–	–	–	–	0	–	–	–	–	28	–	–	–	–	–	–	–
LB mean (µg/kg)	807	–	–	–	–	489	–	–	–	–	652	–	–	–	–	–	–	–
UB mean (µg/kg)	832	–	–	–	–	489	–	–	–	–	694	–	–	–	–	–	–	–
Olive oil																		
No. of individual samples	34	–	–	–	–	6	–	–	–	–	28	–	–	–	–	–	–	–
% samples < LOD or LOQ	53	–	–	–	–	33	–	–	–	–	57	–	–	–	–	–	–	–
LB mean (µg/kg)	412	–	–	–	–	1 362	–	–	–	–	209	–	–	–	–	–	–	–
UB mean (µg/kg)	450	–	–	–	–	1 378	–	–	–	–	251	–	–	–	–	–	–	–
Palm fruit oil																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	558	–	–	–	–	–	–	–	–	–	558	–	–	–	–	–	–	–
UB mean (µg/kg)	558	–	–	–	–	–	–	–	–	–	558	–	–	–	–	–	–	–
Palm kernel oil																		
No. of individual samples	3	–	–	–	–	1	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	135	–	–	–	–	170	–	–	–	–	117	–	–	–	–	–	–	–
UB mean (µg/kg)	135	–	–	–	–	170	–	–	–	–	117	–	–	–	–	–	–	–
Palm oil, refined or unspecified																		
No. of individual samples	41	–	–	–	–	16	–	–	–	2	23	–	–	–	–	–	–	–
% samples < LOD or LOQ	7	–	–	–	–	0	–	–	–	0	13	–	–	–	–	–	–	–
LB mean (µg/kg)	2 559	–	–	–	–	2 191	–	–	–	4 135	2 677	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
UB mean (µg/kg)	2 561	–	–	–	–	2 191	–	–	–	4 135	2 682	–	–	–	–	–	–	–
Palm oil, unrefined																		
No. of individual samples	5	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	20	–	–	–	–	20	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	240	–	–	–	–	240	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	250	–	–	–	–	250	–	–	–	–	–	–	–	–	–	–	–	–
Palm olein																		
No. of individual samples	8	–	–	–	–	1	–	–	–	–	7	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	3 770	–	–	–	–	1 300	–	–	–	–	4 122	–	–	–	–	–	–	–
UB mean (µg/kg)	3 770	–	–	–	–	1 300	–	–	–	–	4 122	–	–	–	–	–	–	–
Palm stearin																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	3 238	–	–	–	–	–	–	–	–	–	3 238	–	–	–	–	–	–	–
UB mean (µg/kg)	3 238	–	–	–	–	–	–	–	–	–	3 238	–	–	–	–	–	–	–
Pumpkinseed oil																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	100	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
UB mean (µg/kg)	8	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–
Rapeseed and mustard oil, refined or unspecified																		
No. of individual samples	89	–	–	–	–	13	–	–	–	52	18	–	–	–	–	–	–	–
% samples < LOD or LOQ	24	–	–	–	–	0	–	–	–	19	50	–	–	–	–	–	–	–
LB mean (µg/kg)	282	–	–	–	–	162	–	–	–	394	99	–	–	–	–	–	–	–
UB mean (µg/kg)	297	–	–	–	–	162	–	–	–	398	161	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Rapeseed and mustard oil, unrefined																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	100	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
UB mean (µg/kg)	99	–	–	–	–	–	–	–	–	–	99	–	–	–	–	–	–	–
Rice bran oil																		
No. of individual samples	26	–	–	–	–	–	–	–	–	–	25	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	587	–	–	–	–	–	–	–	–	–	531	–	–	–	–	–	–	–
UB mean (µg/kg)	587	–	–	–	–	–	–	–	–	–	531	–	–	–	–	–	–	–
Safflower seed oil																		
No. of individual samples	19	–	–	–	–	–	–	–	–	–	19	–	–	–	–	–	–	–
% samples < LOD or LOQ	11	–	–	–	–	–	–	–	–	–	11	–	–	–	–	–	–	–
LB mean (µg/kg)	690	–	–	–	–	–	–	–	–	–	690	–	–	–	–	–	–	–
UB mean (µg/kg)	711	–	–	–	–	–	–	–	–	–	711	–	–	–	–	–	–	–
Sesame seed oil, refined or unspecified																		
No. of individual samples	38	–	–	–	–	2	–	–	–	3	33	–	–	–	–	–	–	–
% samples < LOD or LOQ	26	–	–	–	–	0	–	–	–	0	30	–	–	–	–	–	–	–
LB mean (µg/kg)	170	–	–	–	–	530	–	–	–	522	116	–	–	–	–	–	–	–
UB mean (µg/kg)	197	–	–	–	–	530	–	–	–	522	147	–	–	–	–	–	–	–
Sesame seed oil, unrefined																		
No. of individual samples	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	939	–	–	–	–	–	–	–	–	–	939	–	–	–	–	–	–	–
UB mean (µg/kg)	939	–	–	–	–	–	–	–	–	–	939	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Shortening																		
No. of individual samples	48	–	–	–	–	9	–	–	–	–	39	–	–	–	–	–	–	–
% samples < LOD or LOQ	4	–	–	–	–	11	–	–	–	–	3	–	–	–	–	–	–	–
LB mean (µg/kg)	1 110	–	–	–	–	1 069	–	–	–	–	1 120	–	–	–	–	–	–	–
UB mean (µg/kg)	1 113	–	–	–	–	1 074	–	–	–	–	1 121	–	–	–	–	–	–	–
Soybean oil																		
No. of individual samples	72	–	–	–	–	12	–	–	–	37	22	–	–	–	–	–	–	–
% samples < LOD or LOQ	18	–	–	–	–	8	–	–	–	24	14	–	–	–	–	–	–	–
LB mean (µg/kg)	248	–	–	–	–	332	–	–	–	227	239	–	–	–	–	–	–	–
UB mean (µg/kg)	260	–	–	–	–	337	–	–	–	231	267	–	–	–	–	–	–	–
Sunflower seed oil, refined or unspecified																		
No. of individual samples	38	–	–	–	–	11	–	–	–	10	17	–	–	–	–	–	–	–
% samples < LOD or LOQ	24	–	–	–	–	0	–	–	–	10	47	–	–	–	–	–	–	–
LB mean (µg/kg)	453	–	–	–	–	189	–	–	–	1 143	217	–	–	–	–	–	–	–
UB mean (µg/kg)	479	–	–	–	–	189	–	–	–	1 145	275	–	–	–	–	–	–	–
Sunflower seed oil, unrefined																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	100	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
UB mean (µg/kg)	99	–	–	–	–	–	–	–	–	–	99	–	–	–	–	–	–	–
Walnut oil																		
No. of individual samples	2	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	1 750	–	–	–	–	–	–	–	–	–	1 750	–	–	–	–	–	–	–
UB mean (µg/kg)	1 750	–	–	–	–	–	–	–	–	–	1 750	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
85 Animal or vegetable fat, nes																		
Fats and oils, nes																		
No. of individual samples	66	–	–	–	–	–	–	–	–	66	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	6	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–
LB mean (µg/kg)	998	–	–	–	–	–	–	–	–	998	–	–	–	–	–	–	–	–
UB mean (µg/kg)	999	–	–	–	–	–	–	–	–	999	–	–	–	–	–	–	–	–
90 Milks (no other ingredients)																		
Cattle milk; goat milk; fermented milk																		
No. of individual samples	209	–	–	–	–	–	18	26	81	–	26	58	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	100	100	100	–	100	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
91 Dairy products (including whey, excluding milk fats)																		
Milk and dairy products, nes (including cheese)																		
No. of individual samples	182	–	–	–	–	4	3	3	14	148	3	11	–	–	–	–	–	–
% samples < LOD or LOQ	47	–	–	–	–	0	100	100	100	35	100	100	–	–	–	–	–	–
LB mean (µg/kg)	16	–	–	–	–	88	0	0	0	20	0	0	–	–	–	–	–	–
UB mean (µg/kg)	17	–	–	–	–	88	0	0	0	21	0	0	–	–	–	–	–	–
100 Mammalian (not marine) meat, unprocessed (including home-cooked)																		
Cattle meat; pig meat (state unknown)																		
No. of individual samples	159	–	–	–	–	–	16	20	74	–	20	29	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	100	100	100	–	100	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
101 Poultry (incl. pigeon) meat, unprocessed (incl. home-cooked)																		
Chicken meat; turkey meat (state unknown)																		
No. of individual samples	83	–	–	–	–	–	8	10	41	–	10	14	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	100	100	100	–	100	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
102 Mammalian offals, unprocessed (including home-cooked)																		
Cattle liver; pig liver (state unknown)																		
No. of individual samples	21	–	–	–	–	–	–	–	19	–	–	2	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	100	–	–	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	0	–	–	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–
103 Poultry offals, unprocessed (including home-cooked)																		
Chicken, edible offal; turkey, edible offal (state unknown)																		
No. of individual samples	10	–	–	–	–	–	–	–	1	–	–	9	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	100	–	–	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	0	–	–	0	–	–	–	–	–	–
UB mean (µg/kg)	1	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–
104 Meat and offals, nes (including reptiles and amphibians), unprocessed (incl. home-cooked)																		
Meat and meat products, nes, raw																		
No. of individual samples	29	–	–	–	–	–	–	–	–	29	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–
LB mean (µg/kg)	24	–	–	–	–	–	–	–	–	24	–	–	–	–	–	–	–	–
UB mean (µg/kg)	24	–	–	–	–	–	–	–	–	24	–	–	–	–	–	–	–	–
105 Meat and offals, processed (excluding marine)																		
Meat-based composite foods[c]																		
No. of individual samples	19	–	–	–	–	19	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
LB mean (µg/kg)	106	–	–	–	–	106	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	106	–	–	–	–	106	–	–	–	–	–	–	–	–	–	–	–	–
110 Eggs																		
Eggs and egg products, nes, raw																		
No. of individual samples	205	–	–	–	–	–	20	26	81	–	25	53	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	100	100	100	–	100	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	0	0	0	–	0	0	–	–	–	–	–	–
127 Processed aquatic animals																		
Seafood-based composite foods: codfish cakes[c]																		
No. of individual samples	2	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	60	–	–	–	–	60	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	60	–	–	–	–	60	–	–	–	–	–	–	–	–	–	–	–	–
131 Nonalcoholic beverages (excluding milk based beverages, stimulants and water)																		
Nonalcoholic beverage, nes																		
No. of individual samples	2	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	100	–	–	100	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	0	–	–	0	–	–	–	–	–	–
UB mean (µg/kg)	0	–	–	–	–	–	–	–	0	–	–	0	–	–	–	–	–	–
140 Coffee (or substitute) based beverages																		
Coffee imitation beverage																		
No. of individual samples	35	–	–	–	–	–	–	–	–	35	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	3	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–
LB mean (µg/kg)	7	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–
UB mean (µg/kg)	7	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
142 Tea and mate beverages																		
Tea, dried or solid (green tea; black tea; rooibos; herbs for tea)																		
No. of individual samples	393	–	–	–	–	–	2	117	254	–	6	14	–	–	–	–	–	–
% samples < LOD or LOQ	67	–	–	–	–	–	0	80	63	–	33	36	–	–	–	–	–	–
LB mean (µg/kg)	103	–	–	–	–	–	1 229	36	65	–	107	1 201	–	–	–	–	–	–
UB mean (µg/kg)	112	–	–	–	–	–	1 229	40	76	–	107	1 202	–	–	–	–	–	–
Tea, infusion																		
No. of individual samples	217	–	–	–	–	–	9	16	102	–	20	35	–	–	–	–	–	–
% samples < LOD or LOQ	33	–	–	–	–	–	56	50	32	–	60	37	–	–	–	–	–	–
LB mean (µg/kg)	4	–	–	–	–	–	4	1	2	–	1	7	–	–	–	–	–	–
UB mean (µg/kg)	4	–	–	–	–	–	4	1	2	–	1	7	–	–	–	–	–	–
170 Food for infants and small children																		
Infant formula powder[c]																		
No. of individual samples	115	–	–	–	–	–	2	–	3	–	108	2	–	–	–	–	–	–
% samples < LOD or LOQ	7	–	–	–	–	–	100	–	100	–	1	100	–	–	–	–	–	–
LB mean (µg/kg)	334	–	–	–	–	–	0	–	0	–	356	0	–	–	–	–	–	–
UB mean (µg/kg)	334	–	–	–	–	–	0	–	0	–	356	0	–	–	–	–	–	–
Infant formula concentrate[c]																		
No. of individual samples	4	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	0	–	–	–	–	–	–	–
LB mean (µg/kg)	479	–	–	–	–	–	–	–	–	–	479	–	–	–	–	–	–	–
UB mean (µg/kg)	479	–	–	–	–	–	–	–	–	–	479	–	–	–	–	–	–	–
Infant formula, prepared[c]																		
No. of individual samples	32	–	–	–	–	–	–	–	–	–	32	–	–	–	–	–	–	–
% samples < LOD or LOQ	9	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–
LB mean (µg/kg)	37	–	–	–	–	–	–	–	–	–	37	–	–	–	–	–	–	–
UB mean (µg/kg)	38	–	–	–	–	–	–	–	–	–	38	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Infant formula, prepared, including powders and concentrates converted to prepared[c]																		
No. of individual samples	151	–	–	–	–	–	2	–	3	–	144	2	–	–	–	–	–	–
% samples < LOD or LOQ	7	–	–	–	–	–	100	–	100	–	3	100	–	–	–	–	–	–
LB mean (µg/kg)	47	–	–	–	–	–	0	–	0	–	50	0	–	–	–	–	–	–
UB mean (µg/kg)	47	–	–	–	–	–	0	–	0	–	50	0	–	–	–	–	–	–
Follow-on formula powder[c]																		
No. of individual samples	85	–	–	–	–	40	–	3	7	–	30	5	–	–	–	–	–	–
% samples < LOD or LOQ	35	–	–	–	–	33	–	100	100	–	7	100	–	–	–	–	–	–
LB mean (µg/kg)	139	–	–	–	–	136	–	0	0	–	214	0	–	–	–	–	–	–
UB mean (µg/kg)	152	–	–	–	–	162	–	0	0	–	214	0	–	–	–	–	–	–
Follow-on formula, prepared, including powders and concentrates converted to prepared[c]																		
No. of individual samples	85	–	–	–	–	40	–	3	7	–	30	5	–	–	–	–	–	–
% samples < LOD or LOQ	35	–	–	–	–	33	–	100	100	–	7	100	–	–	–	–	–	–
LB mean (µg/kg)	18	–	–	–	–	18	–	0	0	–	28	0	–	–	–	–	–	–
UB mean (µg/kg)	20	–	–	–	–	21	–	0	0	–	28	0	–	–	–	–	–	–
Tea for infants and children (state unknown)																		
No. of individual samples	34	–	–	–	–	–	–	2	32	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	79	–	–	–	–	–	–	0	84	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	15	–	–	–	–	–	–	209	3	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	19	–	–	–	–	–	–	209	7	–	–	–	–	–	–	–	–	–
Free 3-MCPD (µg/kg food)																		
53 Spices & condiments																		
No. of individual samples	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
54 Sauces & vinegars																		
Black bean sauce																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hoisin sauce																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oyster sauce																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Soy sauce																		
No. of individual samples	379	–	–	–	–	–	–	–	–	33	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	83	–	–	–	–	–	–	–	–	21	–	–	–	–	–	–	–	–
LB mean (µg/kg)	38	–	–	–	–	–	–	–	–	435	–	–	–	–	–	–	–	–
UB mean (µg/kg)	39	–	–	–	–	–	–	–	–	436	–	–	–	–	–	–	–	–
Vinegar																		
No. of individual samples	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 8 (continued)

GEMS/Food level 2[a] / Statistic	GEMS/Food cluster diet																	
	World[b]	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
Sauces, other or unknown																		
No. of individual samples	41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Other foods																		
HVP powder																		
No. of individual samples	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
% samples < LOD or LOQ	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LB mean (µg/kg)	32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UB mean (µg/kg)	33	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

ARA: arachidonic acid; DHA: docosahexaenoic acid; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; HVP: hydrolysed vegetable protein; LB: lower bound (concentrations in food [nondetects set to zero]); LOD: limit of detection; LOQ: limit of quantification; nes: not elsewhere specified; No.: number; UB: upper bound (concentrations in food [nondetects set to the LOD]); 3-MCPD: 3-(mono)chloro-1,2-propanediol

[a] All GEMS/Food contaminants data for 3-MCPD for samples dated 2012 to present are included in this summary table and listed under the most relevant GEMS/Food (and CIFOCOss) category. In many cases, sample sizes are extremely small and may not be statistically reliable.

[b] In some cases, the number of samples contributing to the global total are greater than the sum of the samples for the individual clusters. This is because some countries have not been assigned to any cluster (Singapore), but have contributed monitoring data. All of these data were used in the calculation of global LB and UB means.

[c] 3-MCPD ester concentrations in these foods are likely influenced by the specific oil used in manufacture or cooking; concentrations may not be reflective of foods in individual countries, regionally or globally.

The literature search identified one study of exposure to 3-MCPD esters conducted in various regions of China (Li et al., 2015) and another conducted specifically in China, Hong Kong Special Administrative Region (SAR) (Chung et al., 2013). A Polish assessment of exposure to 3-MCPD esters (Starski et al., 2013) focused only on exposure from baked goods, and was not considered for further review because exposure estimates based on consumption of baked goods alone would underestimate total exposure. The only recent study on dietary exposure to free 3-MCPD was conducted in Brazil (Arisseto et al., 2013), and the only study that included estimates of dietary exposure to 3-MCPD from both esters and the free form in the total diet was a study of exposures in Europe (EFSA, 2016). In each of the studies of exposure to 3-MCPD esters (Li et al., 2015; Chung et al., 2013) and in the single study of exposure to total 3-MCPD (EFSA, 2016), it was assumed that 100% of 3-MCPD is released from 3-MCPD esters by hydrolysis in the digestive system. Exposure estimates found in the literature for each country or region are shown in Table 9 and summarized below.

The Committee also reviewed the 3-MCPD concentration data submitted to the GEMS/Food contaminants database to determine if exposures to 3-MCPD esters or to total 3-MCPD could potentially be estimated for countries other than those with recently published exposure estimates. Because oils are the primary source of 3-MCPD esters, and because concentrations in oils are known to vary geographically, the potential for estimating national 3-MCPD exposures was evaluated only for countries that submitted data on 3-MCPD ester concentrations in fats and oils. Of the countries that submitted data on 3-MCPD ester concentrations in oils, CIFOCOss data are available only for Brazil, Japan and the USA, and the potential for estimating national exposures was therefore evaluated only for those countries. Exposures were estimated using concentration data submitted to the GEMS/Food contaminants database, combined with data from CIFOCOss on total mean consumption (including consumers and nonconsumers) per kg bw per day to allow exposures to be summed over all relevant food groups. These exposures were estimated deterministically, by multiplying mean concentrations by mean food consumption levels.

(a) Brazil

Arisseto et al. (2013) estimated exposures to free 3-MCPD in foods using analytical data on 232 food samples collected in the city of Campinas, Brazil, between 2009 and 2011. Foods collected included soy sauces, cereal-based products, foods containing HVP and malt-derived ingredients, and smoked foods. For most of the products, two different lots were analysed. Samples were analysed for free 3-MCPD concentrations using GC-MS detection, after sample preparation based on Brereton et al. (2001) and Lim et al. (2005). Sauces and seasonings had

Table 9
Summary of national estimates of dietary exposure to 3-MCPD (as 3-MCPD equivalents) from the scientific literature

Mycotoxin / Country or region	Foods included	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer) in µg/kg bw per day	Major contributors[a]	Reference
3-MCPD esters						
China	Survey results: 9 types of refined oil, 6 types of crude oil, margarine and lard	China Health and Nutrition Survey, 2009, three 24-hour records of weighed and measured food	Children (7–10 years) Children (11–13 years) Adolescents (14–17 years) Adults (18–49 years) Adults (>50)	1.3 (1.8–3.8)[b] 0.9 (1.3–2.6) 0.8 (1.0–2.3) 0.7 (1.2–2.6) 0.7 (1.2–2.0)	Not reported	Li et al. (2015)
China, Hong Kong SAR	Survey results: oils and other foods thought to be potential sources of 3-MCPD esters as a result of frying, roasting or baking	Hong Kong Population-Based Food Consumption Survey 2005–2007, food frequency and two 24-hour recalls; data used not specified	Adults (18–69 years)	0.2 (0.5)	Noodles (24%); bakery wares (24%)	Chung et al. (2013)
Free 3-MCPD						
Brazil	Survey results: soy sauces, cereal-based products, foods containing HVP, foods containing malt-derived ingredients and smoked foods	*Analysis of Personal Food Consumption in Brazil* (IBGE, 2011), 1-day food record	Population (10+ years)	0.08 (0.44)[c]	Bread (63%), soups and broths (24%)	Arisseto et al. (2013)
Total 3-MCPD						
Europe	Data submitted by the food industry, German Federal Institute for Risk Assessment (BfR), Joint Research Centre of the European Commission (JRC) and other sources	National surveys based on at least two 24-hour recalls	Infants (<12 months)	0.8–0.9 (1.7–1.8)	Infant and follow-on formulas (>50%); vegetable fats and oils; cookies	EFSA (2016)
			Toddlers (≥12 to <36 months)	0.8–0.9 (1.6–1.9)[d]	Vegetable fats and oils; cookies; pastries and cakes; infant and follow-on formulas	
			Other children (36 months to <10 years)	0.7–0.8 (1.4–1.6)[d]	Pastries and cakes; margarine and similar; vegetable fats and oils; cookies	

Mycotoxin / Country or region	Foods included	Consumption data used	Population groups (age)	Estimated dietary exposure, mean (high consumer) in µg/kg bw per day	Major contributors[a]	Reference
Europe (continued)			Adolescents (10–18 years)	0.4–0.5 (0.8–0.9)[d]	Margarine and similar; fried or baked potato products; pastries and cakes	
			Adults (≥18 to <65 years)	0.3–0.3 (0.6–0.7)[d]	Margarine and similar; pastries and cakes; vegetable fats and oils	
			Elderly adults (≥65 to <75 years)	0.3–0.4 (0.6–0.7)[d]	Margarine and similar; pastries and cakes; vegetable fats and oils; bread and bread rolls	
			Very elderly adults (≥75 years)	0.3–0.3 (0.6–0.7)[d]	Margarine and similar; pastries and cakes; vegetable fats and oils; bread and bread rolls	
		Assumptions regarding infant formula consumption	Infants, formula fed	2.4 (3.2)		

bw: body weight; HVP: hydrolysed vegetable protein; LB: lower bound; 3-MCPD: 3-(mono)chloro-1,2-propanediol; SAR: Special Administrative Region; LOD: limit of detection; UB: upper bound

[a] Where no numerical indicator is shown, the proportion of exposure was not provided in the original document.

[b] Lower end of high consumer range: 97.5th percentile based on deterministic analyses; upper end: 95th percentile based on probabilistic analyses.

[c] High consumer exposure based on mean food consumption and 95th percentile concentration.

[d] Means are medians of lower bound (LB, nondetects set to zero) and upper bound (UB, nondetects set to the LOD) mean exposures across European surveys; high consumer exposures are medians of the LB–UB 95th percentile exposures across European surveys.

the highest average free 3-MCPD concentrations (about 221 µg/kg food), but concentrations in over 60% of the 45 sauce and seasoning samples analysed were below the LOD. Exposure to free 3-MCPD was estimated using the analytical data described above with results of *Analysis of Personal Food Consumption in Brazil* (IBGE, 2011), a 2008–2009 survey in which 34 003 individuals 10 years and older each provided a 1-day food record. Dietary exposure to free 3-MCPD was estimated at 0.08 µg/kg bw per day based on mean concentrations and mean food consumption levels. Upper level exposure was estimated at 0.44 µg/kg bw per day based on 95th percentile concentrations and mean food consumption levels.

Brazil submitted data to the GEMS/Food contaminants database on 3-MCPD ester concentrations in specific oils, butter, margarine, shortening, instant noodles and various oil-containing composite "snack foods". There were fewer than 10 samples for each type of snack food. Matches were found between the fats and oils included in the GEMS/Food contaminants database and fats and oils reported in the CIFOCOss for Brazil. However, the CIFOCOss consumption level in Brazil is zero for each oil at both the mean and upper percentile consumption levels, likely because most oil consumption is reported as part of food mixtures. Because an estimate of exposure to 3-MCPD from butter, margarine, instant noodles and snack foods alone would not provide a complete picture of 3-MCPD exposure, the Committee declined to estimate 3-MCPD exposures for Brazil.

(b) China

Li et al. (2015) analysed 3-MCPD ester concentrations in 143 samples of fats and oils collected from Chinese markets. Products collected included nine types of refined oils, six types of crude oils, and margarine and lard. The number of samples collected per oil ranged from three (of palm oil) to 18 (of rapeseed oil and soybean oil). Samples were analysed using the German Society for Fat Science method C-VI 18 (DGF, 2011) with GC-MS detection. Concentrations of 3-MCPD esters were highest in refined camellia oil, a cooking oil popular in southern China (Wang et al., 2006), with 3-MCPD concentrations ranging from 988 to 2586 µg/kg oil. 3-MCPD concentrations were also high in palm oil (1294–1646 µg/kg oil) and margarine (789–1602 µg/kg oil). Dietary exposures to 3-MCPD esters were estimated by combining the concentration data on fats and oils with data on oil consumption from the 2009 China Health and Nutrition Survey, a nationally representative survey of households and individuals conducted by the Carolina Population Center in the University of North Carolina (USA) and the Institute of Nutrition and Food Safety of CDC (China) (Popkin et al., 2010; Cui & Michael, 2012). Survey participants each provided 3 days of food records, with

weights or measurements for each food consumed. In a deterministic assessment of exposure, mean 3-MCPD ester concentrations were combined with mean, median and 97.5th percentile consumption of fats and oils. A probabilistic assessment was also conducted. In this assessment, the distribution of 3-MCPD ester concentrations was combined with the distribution of consumption using Monte Carlo simulation.

Li et al. (2015) reported exposures to 3-MCPD esters for subpopulations aged 7–10, 11–13, 14–17 and 18–49 years and ≥50 years, by sex. Estimated exposures to 3-MCPD esters from consumption of fats and oils were highest for children aged 7–10 years. Mean exposures for this age group were 1.3 μg/kg bw per day for both boys and girls based on the deterministic assessment, and 1.2 μg/kg bw per day based on the probabilistic assessment. Li et al. (2015) estimated that adults aged 18–49 years and children aged 7–10 years have 95th percentile 3-MCPD ester exposures of 2.6 and 3.8 μg/kg bw per day, respectively, based on a probabilistic analysis that combined distributions of food consumption data with distributions of 3-MCPD ester concentrations. It should be noted that the deterministic and probabilistic analyses in this study were each based on very few analytical samples.

An earlier study of exposure to 3-MCPD esters was conducted in China, Hong Kong SAR, by Chung et al. (2013), who estimated exposures to 3-MCPD esters from consumption of 290 samples of foods. Foods collected for analysis included oils and other foods thought to be potential sources of 3-MCPD esters as a result of frying, roasting or baking. Oils collected included canola, corn, grapeseed, olive and peanut (3–4 samples each). Palm oil was not collected for analysis. Samples were analysed using an indirect method (enzymatic hydrolysis and GC-MS detection) developed by Chung & Chan (2012). Dietary exposures to 3-MCPD esters were estimated by combining the results of these analyses with data on food consumption by adults (18–69 years) from the Hong Kong Population-Based Food Consumption Survey 2005–2007 (FEHD, 2010). Dietary exposures to 3-MCPD were estimated to be 0.2 μg/kg bw per day for average consumers (including 0.013 μg/kg bw per day from oil) and 0.5 μg/kg bw per day for upper-level (95th percentile) consumers. The authors noted that the 3-MCPD ester exposure from oil could potentially have been underestimated because the survey respondents may have had difficulty quantifying their total oil consumption. In a separate analysis of exposure based on the concentrations of 3-MCPD esters in oil and on oil disappearance in China, Hong Kong SAR, Chung et al. (2013) estimated exposure to 3-MCPD esters from all oil sources to be 0.15 μg/kg bw per day. However, the authors also noted that total 3-MCPD ester exposures may have been overestimated due to selection bias introduced by targeting foods thought to be potential sources of 3-MCPD esters.

(c) Europe (total 3-MCPD)

EFSA (2016) estimated dietary exposures to total 3-MCPD (esters and free form) using concentration data submitted by the food industry, the German Federal Institute for Risk Assessment (BfR), the Joint Research Centre of the European Commission (JRC) and other sources. Foods considered in the assessment included fats, oils, grain products, meat/poultry/fish products, savoury sauces and other food sources of 3-MCPD. Where applicable, AOCS-approved methods were used for all analyses. Data on 3-MCPD concentrations were combined with FoodEx-coded food consumption data at the individual level from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) (EFSA, 2011a,b; Huybrechts et al., 2011; Merten et al., 2011) to estimate chronic total exposures to 3-MCPD. The Comprehensive Database includes data from 41 surveys in 23 different European countries, representing a total sample of 78 990 individuals.

Dietary exposures to total 3-MCPD were estimated for each survey and for each age group. These age groups included infants (<12 months), toddlers (≥12 to <36 months), other children (≥36 months to <10 years), adolescents (≥10 years to <18 years), adults (≥18 years to <65 years), elderly adults (≥65 years to <75 years) and very elderly adults (≥75 years). Dietary exposures were also estimated for infants fed formula only, based on an assumed intake of 170 g/kg bw per day.

The minimum, median and maximum of mean total dietary 3-MCPD exposures across surveys were reported for each age group. The median of mean total 3-MCPD exposures across surveys ranged from 0.7 to 0.9 µg/kg bw per day for infants, toddlers and other children. The median of high-level (95th percentile) exposures across surveys for these age groups ranged from 1.4 to 1.9 µg/kg bw per day. The median of mean total exposures for adolescents and adults ranged from 0.3 to 0.5 µg/kg bw per day, and the median of high-level (95th percentile) exposures ranged from 0.6 to 0.9 µg/kg bw per day.

Major food contributors to 3-MCPD exposure varied by age group. For infants, who were noted as being a heterogeneous group including both formula and breast milk consumers, infant formula provided over 50% of exposure; vegetable fats and oils and cookies also were major contributors. For toddlers, vegetable fats and oils, cookies, pastries and cakes, and infant and follow-on formulas were the major contributors to 3-MCPD exposure. For other children, foods contributing most to exposure were pastries and cakes, margarine and similar foodstuffs, vegetable fats and oils, and cookies. For adolescents, margarine and similar foodstuffs, fried or baked potato products, and pastries and cakes were important contributors to the 3-MCPD exposure. For adults, margarine and similar foodstuffs, pastries and cakes, and vegetable fats and oils had the greatest

contributions to 3-MCPD exposures across surveys. Major food contributors to 3-MCPD exposure for the two age groups of older adults (65–75 years; ≥75 years) were margarine and similar foodstuffs, pastries and cakes, vegetable fats and oils, and bread and bread rolls.

For formula-fed infants, dietary exposure to 3-MCPD was estimated at 2.4 µg/kg bw per day based on the mean 3-MCPD formula concentration and at 3.2 µg/kg bw per day based on the 95th percentile formula concentration.

The major strength of this study is that it includes estimates of total 3-MCPD exposure based on concentrations in baked, roasted and fried foods. These estimates may therefore be more accurate than exposures that would be predicted based on the oil contents of those foods alone. However, the study is somewhat limited by the assumption that 3-MCPD concentrations are the same across all European countries and that data may be summarized over multiple surveys, each conducted using different methodology (e.g. different numbers of days of intake for which data were collected, different age groups represented).

(d) Japan

Data on concentrations of 3-MCPD esters submitted by Japan were limited to data on specific fats and oils. Consumption values for Japan are included in the CIFOCOss for the following categories: "butter; mammalian fats (except milk fats) and skin, nes [not elsewhere specified]"; "vegetable oils, nes"; and "vegetable fats (excluding oil), nes". Data on concentrations of 3-MCPD esters in lard were used for the category "mammalian fats (except milk fats)". Data on concentrations of 3-MCPD esters in margarine and shortening were used for the category "vegetable fats (excluding oil), nes". An overall 3-MCPD concentration to use for "vegetable oils (nes)" was determined by weighting concentrations in individual oils by Food and Agriculture Organization Corporate Statistical Database (FAOSTAT; http://www.fao.org/faostat/en/#home) 2016 data on per capita availability of oils in Japan. Data used in estimating exposures to 3-MCPD esters for Japan are shown in Table 10.

Exposures in Japan, based on mean consumption levels of butter, lard, margarine, shortening and vegetable oils, were estimated at 0.1–0.2 µg/kg bw per day for children and 0.1 µg/kg bw per day for the general population (see Table 12 below). Upper-level exposures (90th percentile) were estimated at 0.2–0.4 µg/kg bw per day for children and 0.2 µg/kg bw per day for the general population. Exposures from vegetable oils represented over 70% of total exposure, with most of the remainder contributed by vegetable fats (i.e. margarine and shortening).

Table 10
GEMS/Food contaminants data used for estimating exposure to 3-MCPD esters (as 3-MCPD equivalents) in Japan

CIFOCOss code	Description	No. of samples	LOD (µg/kg fat)	% <LOD	LB–UB mean (µg/kg food)[a]
02.2.1	Butter	40	30–40	68	10–32
MF 0100	Mammalian fats (except milk fats) and skin, nes[b]	23	30–40	0	260–260
02.1.2	Vegetable fats (excluding oil), nes[c]	98	40–50	0	853–853
OR 0172	Vegetable oils, nes[d]	72	40–200	34.7	212–365

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – summary statistics; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; 3-MCPD: 3-(mono)chloro-1,2-propanediol; LOD: limit of detection; nes: not elsewhere specified; No.: number; UB: upper bound
[a] Nondetects of LB concentrations in food set to zero; nondetects of UB concentrations in food set to the LOD.
[b] Lard.
[c] Margarine and shortening.
[d] Concentration data submitted by Japan for individual oils were weighted based on FAOSTAT (2016) data on oil availability for Japan for 2011.

(e) USA

Data submitted by the USA were limited to data on concentrations of 3-MCPD esters in specific vegetable oils. Data used in estimating exposures to 3-MCPD esters for the USA are shown in Table 11.

For the USA, mean exposure to 3-MCPD esters from consumption of oils was estimated at 0.3 µg/kg bw per day for children less than 6 years of age. The 90th percentile exposure was estimated at 0.6 µg/kg bw per day for this age group (Table 12). Mean and 90th percentile adult exposures were estimated at 0.2 and 0.4 µg/kg bw per day, respectively. Major contributors to exposure were soybean oil (70%) and peanut oil (10%). Because the USA estimates of oil consumption in the CIFOCOss represent consumption from all sources, including margarine, shortening and food mixtures, the 3-MCPD ester exposure estimates based on these data represent total exposures from consumption of oil.

(f) Multiple countries – infant formula

Data submitted to the GEMS/Food contaminants database on 3-MCPD ester concentrations in infant formulas were used in estimating dietary exposure to 3-MCPD esters for infants exclusively fed infant formula during the first 6 months of life. The methodology used to estimate exposure to carrageenan in infant formula at the seventy-ninth JECFA meeting (Annex 1, reference *220*) was used to estimate exposure to dietary 3-MCPD esters for formula-fed infants. Median infant formula consumption estimates were derived from estimated energy requirements (EERs) for fully formula-fed infants (Table 13). Standard body weights and EERs for male and female infants aged 1, 3 and 6 months were

Table 11
GEMS/Food contaminants data used for estimating dietary exposure to 3-MCPD esters (as 3-MCPD equivalents) in the USA

CIFOCOss code	Description	No. of samples	LOD (µg/kg fat)	% <LOD	LB–UB mean (µg/kg food)[a]
OR 0495	Rapeseed oil (including canola)	8	8	12.5	158–159
OR 0665	Coconut oil	16	8	25	294–296
OR 0691	Cottonseed oil	2	8	0	431–431
OC 0693	Linseed oil	1	8	0	88–88
OR 0645	Maize oil	10	8	0	293–293
OR 0305	Olive oil	10	8	40	282–285
OR 0696	Palm oil	16	8	12.5	2 789–2 790
OR 0697	Peanut oil and butter	5	8	40	296–299
OR 0699	Safflower seed oil	15	8	0	841–841
OR 0700	Sesame seed oil	3	8	0	285–285
OR 0541	Soybean oil	19	8	0	277–277
OR 0702	Sunflower seed oil	8	8	12.5	412–413

CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – summary statistics; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; 3-MCPD: 3-(mono)chloro-1,2-propanediol; UB: upper bound
[a] Nondetects of LB concentrations in food set to zero; nondetects of UB concentrations in food set to the LOD.

Table 12
Estimated national dietary exposures to 3-MCPD esters (as 3-MCPD equivalents) based on GEMS/Food contaminants data

			Estimated exposures to 3-MCPD esters (µg/kg bw per day)	
Country	Exposure sources	Population	LB–UB mean[a]	LB–UB P90[a,b]
Japan	Fats and oils	Children	0.1–0.2	0.2–0.4
		General population	0.1–0.1	0.2–0.2
USA	Oils	Children <6 years	0.3–0.3	0.6–0.6
		General population	0.2–0.2	0.4–0.4

bw: body weight; GEMS/Food Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; 3-MCPD: 3-(mono)chloro-1,2-propanediol; LOD: limit of detection; P90: 90th percentile; UB: upper bound; USA: United States of America
[a] Nondetects of LB concentrations in food set to zero; nondetects of UB concentrations in food set to the LOD.
[b] Estimated at twice the mean exposures.

Table 13
Estimated infant formula consumption amounts for infants up to 6 months of age

Sex	Age (months)	Median body weight (kg)[a]	EERs (kcal/day)[a]	Formula consumption (mL/day)[b]
Male	0–1	4.6	560	836
	2–3	6.3	629	939
	5–6	7.9	662	988

Table 13 (continued)

Sex	Age (months)	Median body weight (kg)[a]	EERs (kcal/day)[a]	Formula consumption (mL/day)[b]
Female	0–1	4.4	509	760
	2–3	5.8	585	873
	5–6	7.3	626	934

EER: estimated energy requirement
[a] Median body weights and EERs reported according to the Joint FAO/WHO/UNU expert report on human energy requirements (FAO/WHO/UNU, 2004).
[b] Volume of ingested formula based on a standard energy density of 67 kcal per 100 mL to meet an infant's energy requirements in full.

taken from daily human energy requirements defined by FAO/WHO/UNU (2004). The report of the seventy-ninth meeting of JECFA noted that the EERs of formula-fed infants are greater than those of breastfed infants and that this disparity decreases with increasing age.

Dietary exposures to 3-MCPD esters from consumption of infant formula were also estimated using high (95th percentile) daily energy intakes reported by Fomon (1993) for formula-fed infants to capture possible brand loyalty to products having high concentrations of 3-MCPD esters. Formula-fed males and females age 1 month have EERs of 122 and 117 kcal/kg bw per day, respectively (FAO/WHO/UNU, 2004). Fomon (1993) reported maximum 95th percentile energy intakes for male and female infants at 14–27 days of age of 148.7 and 146.0 kcal/kg bw per day, respectively. For all dietary exposure estimates, a common formula energy density of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily.

Exposures to 3-MCPD esters from consumption of follow-on formula were estimated for infants 6 months of age, assuming exclusive consumption of formula, and for infants 12 months of age, assuming that the caloric intake from follow-on formula is 13.7% of the total caloric intake (Boggio et al., 1999).

Data on concentrations of 3-MCPD esters (or total 3-MCPD) for prepared infant formulas are shown in Table 14. Data on 3-MCPD esters (or total 3-MCPD) concentrations in infant formulas and/or follow-on formulas were submitted by Brazil, Canada, France, Germany, Greece, Italy, Japan, the Netherlands, Spain and the USA.

Data submitted by France, Germany, Greece, Italy, the Netherlands and Spain indicate that 3-MCPD was not detected in any sample of infant formula (n = 8) or follow-on formula powders (n = 17). The LOD and LOQ specified for the formulas were 0.1 and 0.33 µg/kg powder, respectively. The estimated UB mean in diluted formula is less than 1 µg/kg, regardless of whether the UB is based on the LOD or LOQ. Based on these limited data, therefore, it appears

Table 14

Estimates of dietary exposure to 3-MCPD esters (as 3-MCPD equivalents) for prepared formula–fed infants (concentrations are expressed as ready to consume)

Age (months)	Sex	3-MCPD ester concentration in formula[a] Mean (P95) (µg/kg formula)	No. of samples	Exposure to 3-MCPD esters (µg/kg bw per day)			
				Based on mean/median kcal needs[b]		Based on P95 consumption[c]	
				Mean	P95	Mean	P95
Infant formula							
Canada							
0–1	M + F	37	32	7	–	9	–
2–3	M + F			6	–	–	–
5–6	M + F			5	–	–	–
European countries[d]							
0–6	M + F	<1	8	<1	–	<1	–
Japan							
0–1	M + F	36	23	7	–	8	–
2–3	M + F			6	–	–	–
5–6	M + F			5	–	–	–
USA							
0–1	M	51 (109)	89	10	21	12	25
0–1	F			9	19	12	25
2–3	M + F			8	17	–	–
5–6	M + F			7	14	–	–
Follow-on formula							
Brazil							
6	M	19	40	2	–	–	–
6	F			3	–	–	–
12	M + F			0	–	–	–
European countries[e]							
6–12	M + F	<1	17	<1	–	–	–
Japan							
6	M	34	30	4	–	–	–
6	F			5	–	–	–
12	M + F			1	–	–	–

bw: body weight; F: female; LOD: limit of detection; M: male; 3-MCPD: 3-(mono)chloro-1,2-propanediol; No.: number; P95: 95th percentile

[a] Mean and P95 values represent both lower-bound (nondetects set to zero) and upper-bound (nondetects set to the LOD) values. P95 concentrations were determined only where the number of samples exceeded 60.

[b] Based on median body weights and estimated energy requirements reported according to the Joint FAO/WHO/UNU expert report on human energy requirements (FAO/WHO/UNU, 2004). Volume of ingested formula based on a standard energy density of 67 kcal/100 mL to meet an infant's energy requirements in full. Gram weights of formulas estimated based on an assumed density of 1.05 g/mL.

[c] Based on 95th percentile energy intake in formula-fed infants reported by Fomon (1993). For infants 12 months of age, the volume of formula is equivalent to 13.7% of energy requirements (Boggio et al., 1999).

[d] Germany, Greece, Italy, Netherlands, Spain.

[e] France, Germany, Italy, Netherlands, Spain.

that there is essentially no 3-MCPD exposure from infant formula or follow-on formula consumed in these countries.

Estimates of dietary exposures to 3-MCPD esters from infant formula sampled in Canada, Japan and the USA, based on estimated median formula intakes, are shown in Table 14. Mean 3-MCPD ester concentrations were similar for prepared infant formulas from Canada (37 µg/kg formula) and Japan (36 µg/kg formula). Estimated exposures to 3-MCPD esters from consumption of infant formula in these countries ranged from 7 µg/kg bw per day at 0–1 month of age to 5 µg/kg bw per day at 5–6 months.

Concentrations of 3-MCPD esters in USA infant formulas were 51 µg/kg formula at the mean and 109 µg/kg formula at the 95th percentile. USA infant exposures to 3-MCPD esters from consumption of formula ranged from 10 µg/kg bw per day for males at 0–1 month of age to 7 µg/kg bw per day at 5–6 months. Exposures to 3-MCPD esters based on the 95th percentile concentration were approximately double the mean exposures, ranging from 21 µg/kg bw per day at 0–1 month of age to 14 µg/kg bw per day at 5–6 months. It should be noted that the relatively high estimates of exposure to 3-MCPD esters for infants in the USA fed prepared formula are likely due to the inclusion of palm oil and/or palm olein as ingredients in many formulas. No information was available on major oil ingredients in infant formula samples not from the USA.

Estimates of dietary exposure to 3-MCPD esters from infant formula based on mean 95th percentile formula intakes by infants in Canada, Japan and the USA during the first month of life are 9, 8 and 12 µg/kg bw per day, respectively. Estimated exposures based on 95th percentile formula intakes and 95th percentile 3-MCPD ester concentrations in the USA are 25 µg/kg bw per day (Table 14).

Estimates of dietary exposure to 3-MCPD esters from follow-on formula at 6 months of age are 2–3 µg/kg bw per day for infants in Brazil and 4–5 µg/kg bw per day for infants in Japan.

8.3.2 International estimates

International exposures to 3-MCPD esters were estimated using GEMS/Food cluster diet data on per capita consumption amounts per adult person in g/day, divided by a body weight of 60 kg. Rough estimates of upper-level (90th percentile) exposures were obtained by doubling the estimated mean exposures.

(a) 3-MCPD esters

The Committee reviewed the available GEMS/Food contaminants data on 3-MCPD esters and determined that very few data other than those for fats and oils could be appropriately used in estimating international dietary exposures to

3-MCPD esters. Therefore, the Committee estimated cluster diet exposures to 3-MCPD using GEMS/Food contaminants data on fats and oils alone. It should be noted that the methods underlying some of these data were not provided and, where known, may not have been validated in international collaborative studies.

The Committee determined 3-MCPD ester concentrations for GEMS/Food cluster diets level 2 category 84 (vegetable fat) using available GEMS/Food contaminants data to calculate mean concentrations in individual oils. Factors for weighting data on individual oils in each cluster diet were derived by weighting FAOSTAT (FAO, 2016) data on per capita availability of specific oils in each country by the relative population of each country included in the cluster (World Bank, 2016). Concentrations of 3-MCPD esters in oil used in these calculations were those for the specific cluster diet, where available; the global concentration was used where no cluster-specific data were available for that oil. 3-MCPD ester concentrations estimated using GEMS/Food contaminants data on oils, weighted using the factors shown in Table 15, are shown in Table 16.

Table 17 shows estimated mean dietary exposures to 3-MCPD esters from fat and oil sources. Mean exposures to 3-MCPD esters range from 0.15 µg/kg bw per day for cluster diet G14 (LB) to 1.66 µg/kg bw per day for cluster diet G11 (UB).

(b) Free 3-MCPD

Previous mean estimates of international exposure to free 3-MCPD ranged from 0.28 to 3.41 µg/kg bw per day (Annex 1, references *184*). These estimates were based largely on data from sources in Europe. Since then, regulatory bodies have greatly reduced concentrations in soy sauce and HVP. The 2016 EFSA assessment showed that recent mean total exposures were far below the 2007 levels, and that soy sauce and other sources of free 3-MCPD are minor contributors to mean total 3-MCPD exposures. In another recent study, Arisseto et al. (2013) estimated mean exposure to free 3-MCPD exposure from all sources to be 0.08 µg/kg bw per day for the population aged 10 years and older in Brazil.

The Committee reviewed current GEMS/Food contaminants data on free 3-MCPD concentrations in foods to determine how to best use these data for estimating international exposures, given that international standards for soy sauce and HVP, the major sources of free 3-MCPD exposure, vary from country to country. Mean free 3-MCPD concentrations in soy and other savoury sauces based on GEMS/Food contaminants data from both Singapore and the Philippines were 38–39 µg/kg sauce (LB–UB), but 435–436 µg/kg sauce (LB–UB) based only on data from the Philippines (see Table 8). The concentrations from soy sauce in the Philippines were used as conservative estimates of 3-MCPD concentrations in soy sauce worldwide in estimating 3-MCPD exposures.

Table 15

Factors developed for weighting 3-MCPD ester concentrations in vegetable oils to derive an overall concentration for vegetable fat, by GEMS/Food cluster

	Per cent of total oil consumption[a] per GEMS/Food cluster																
Vegetable oil	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Coconut oil	0.5	0.1	2.6	1.6	1.9	1.1	6.2	1.0	5.5	1.2	7.7	13.7	1.1	71.3	1.4	0.0	35.7
Cottonseed oil	13.5	2.4	3.2	1.5	7.8	7.9	2.3	0.4	4.4	1.9	0.0	–	4.8	0.0	0.0	2.9	–
Groundnut oil	1.3	0.1	12.6	0.4	8.3	0.8	1.0	0.3	7.6	0.8	9.4	–	20.4	1.5	0.2	2.3	0.6
Maize germ oil	1.6	1.2	1.1	16.0	1.4	4.3	2.6	1.3	1.4	5.9	5.2	4.3	1.7	0.5	1.9	0.0	–
Olive oil	4.4	0.9	0.4	4.9	0.6	9.7	7.2	24.2	0.3	6.5	6.2	4.6	0.1	0.3	8.9	0.2	0.9
Palm oil	41.3	0.3	64.7	31.0	17.4	7.7	7.0	10.4	30.0	2.1	28.0	–	44.7	1.8	12.7	51.1	39.8
Palm kernel oil	0.4	0.0	3.8	1.5	0.9	2.7	0.8	0.7	4.6	0.6	0.0	–	14.6	3.1	0.1	0.6	14.2
Rapeseed and mustard oil	6.1	3.9	0.1	5.7	14.2	3.5	32.9	18.8	14.5	14.0	7.5	–	0.7	0.7	22.1	0.1	–
Rice bran oil	0.1	–	–	–	3.0	–	–	0.0	1.0	0.6	–	–	–	11.1	–	–	–
Sesame seed oil	0.6	0.0	1.2	2.2	0.7	1.8	0.4	0.3	2.0	0.2	0.0	0.2	1.7	1.0	0.1	8.9	0.0
Soybean oil	17.7	3.4	8.9	21.8	34.5	30.3	19.3	21.1	27.1	52.7	28.6	26.5	4.7	7.5	11.1	8.9	8.3
Sunflower seed oil	12.5	87.9	1.5	13.4	9.4	30.1	20.4	21.5	1.7	13.5	7.6	50.8	5.6	1.3	41.7	25.1	0.6

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; 3-MCPD: 3-(mono)chloro-1,2-propanediol

[a] Based on oil availability (g per capita) (FAOSTAT, 2016) in cluster countries, weighted by population of each country.

Table 16

Concentrations of 3-MCPD esters (as 3-MCPD equivalents) in vegetable fat, derived using weighting factors, by GEMS/Food cluster

	Concentration of 3-MCPD esters (µg/kg oil) per GEMS/Food cluster																
Statistic	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Lower-bound mean	1 685	710	2 334	1 427	895	897	887	975	2 770	428	1 443	692	1 953	805	1 010	1 943	1 612
Upper-bound mean	1 722	749	2 366	1 468	896	941	930	1 022	2 774	519	1 484	734	1 984	838	1 051	1 979	1 644

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; 3-MCPD: 3-(mono)chloro-1,2-propanediol

[a] Derived using GEMS/Food data on 3-MCPD esters, weighted based on relative oil availability (g per capita) (FAOSTAT, 2016) in cluster countries, and on population of each country.

Table 17

Estimated exposures to 3-MCPD esters (as 3-MCPD equivalents) from consumption of fats and oils, by GEMS/Food cluster

GEMS/Food level 2[a] / Statistic	Exposure to 3-MCPD esters[a] (µg/kg bw per day) per GEMS/Food cluster																
	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16	G17
80 Milk fats[b]																	
LB mean	0.001	0.001	0.000	0.001	0.000	0.001	0.003	0.004	0.000	0.001	0.004	0.000	0.000	0.000	0.002	0.000	0.001
UB mean	0.003	0.003	0.000	0.003	0.003	0.003	0.011	0.014	0.000	0.004	0.011	0.001	0.000	0.000	0.008	0.000	0.002
81 Mammalian fats (no milk fat)[c]																	
LB mean	0.009	0.020	0.003	0.004	0.007	0.002	0.018	0.051	0.014	0.025	0.056	0.025	0.004	0.004	0.063	0.004	0.011
UB mean	0.009	0.020	0.003	0.004	0.007	0.002	0.018	0.051	0.014	0.025	0.056	0.025	0.004	0.004	0.063	0.004	0.011
84 Plant origin fat, weighted by availability of specific oils in populations within clusters[d]																	
LB mean	0.884	0.353	0.806	1.066	0.402	0.500	0.753	0.873	0.898	0.426	1.339	0.166	0.728	0.144	0.728	0.479	0.608
UB mean	0.903	0.373	0.817	1.096	0.402	0.525	0.790	0.916	0.899	0.516	1.378	0.176	0.739	0.150	0.757	0.488	0.620
85 Animal or vegetable fat, nes (assume concentrations same as 84 Plant origin fat, above)																	
LB mean	0.003	0.016	0.006	0.017	0.002	0.002	0.057	0.017	0.010	0.008	0.210	0.112	0.005	0.001	0.026	0.004	0.099
UB mean	0.003	0.017	0.006	0.018	0.002	0.002	0.060	0.018	0.010	0.009	0.216	0.119	0.006	0.001	0.027	0.004	0.101
Total exposures from fats and oils																	
LB mean	0.90	0.39	0.81	1.09	0.41	0.50	0.83	0.95	0.92	0.46	1.61	0.30	0.74	0.15	0.82	0.49	0.72
UB mean	0.92	0.41	0.83	1.12	0.41	0.53	0.88	1.00	0.92	0.55	1.66	0.32	0.75	0.16	0.86	0.50	0.73

bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; nes: not elsewhere specified; UB: upper bound

[a] Assumes 60 kg body weight for individuals in all clusters.

[b] Derived using GEMS/Food data for butter.

[c] Derived using GEMS/Food data for lard.

[d] Derived using GEMS/Food data on 3-MCPD esters, weighted based on relative oil availability (g per capita) (FAOSTAT, 2016) in cluster countries, and on population of each country.

Data submitted to the GEMS/Food contaminants database on concentrations of free 3-MCPD in foods other than soy sauce were limited mainly to data on seasonings and HVP. It is difficult to estimate exposure based on these data as GEMS/Food cluster diets do not include consumption estimates for seasonings or HVP. In the evaluation of free 3-MCPD at the fifty-seventh JECFA meeting (Annex 1, reference *154*), international exposures from consumption of HVP were estimated based on an assumption that one eighth of the diet, approximately 180 g, consists of savoury foods that might contain free 3-MCPD, and on an assumption that those foods contain a mean residual concentration of free 3-MCPD of 12 µg/kg food. These exposures were estimated at 2 µg/person per day, which is equivalent to 0.03 µg/kg bw per day based on an average body weight of 60 kg. The UB mean concentration provided by EFSA (2016) on free 3-MCPD concentrations in composite foods was 12 µg/kg, indicating that the previous JECFA assumptions may still be relevant. However, in the current assessment the Committee used the Arisseto et al. (2013) estimate of 0.08 µg/kg bw per day free 3-MCPD exposure in Brazil as a more conservative estimate of cluster-based exposures to free 3-MCPD from foods other than soy sauce.

(c) Total 3-MCPD

Table 18 shows estimated total exposures to 3-MCPD. Mean exposures to 3-MCPD range from 0.2 µg/kg bw per day for cluster diet G14 to 1.7 µg/kg bw per day for cluster diet G11. Estimated 90th percentile exposures range from 0.4 µg/kg bw per day for cluster diet G14 to 3.4 µg/kg bw per day for cluster diet G11. Vegetable fats and oils were the major contributors to 3-MCPD exposure in all clusters.

8.4 Limitations of assessment of dietary exposure to 3-MCPD

The national and international assessments of dietary exposure to 3-MCPD conducted by the Committee are based on extremely limited usable data on concentrations of both 3-MCPD esters and free 3-MCPD. Methods of analysis were not provided for most of the data submitted to the GEMS/Food contaminants database; although data on 3-MCPD esters sampled prior to 2012 were not used due to concerns about methods of analysis used before 2012, it is possible that data on 3-MCPD esters sampled after 2012 were also obtained using inappropriate methods. Given that the method of analysis for these contaminants is important in determining the reliability of the concentration data, it would be useful for data submitters to provide this information in the future.

Estimates of exposure to 3-MCPD esters reflect exposures from consumption of fats and oils only, and do not reflect any positive or negative

Table 18

Estimated mean and 90th percentile exposures to total 3-MCPD (esters and free, as 3-MCPD equivalents), by GEMS/Food cluster

	Exposure to total 3-MCPD[a] (µg/kg bw per day) per GEMS/Food cluster																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Fats and oils[b]																	
LB	0.90	0.39	0.81	1.09	0.41	0.50	0.83	0.95	0.92	0.46	1.61	0.30	0.74	0.15	0.82	0.49	0.72
UB	0.92	0.41	0.83	1.12	0.41	0.53	0.88	1.00	0.92	0.55	1.66	0.32	0.75	0.16	0.86	0.50	0.73
Soy sauce[c]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Other sources of free 3-MCPD[d]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Total mean exposure																	
LB	1.0	0.5	0.9	1.2	0.5	0.6	0.9	1.0	1.0	0.6	1.7	0.4	0.8	0.2	0.9	0.6	0.8
UB	1.0	0.5	0.9	1.2	0.5	0.6	1.0	1.1	1.0	0.7	1.7	0.4	0.8	0.2	0.9	0.6	0.8
Estimated 90th percentile exposure[e]																	
LB	2.0	1.0	1.8	2.4	1.0	1.2	1.8	2.0	2.0	1.2	3.4	0.8	1.6	0.4	1.8	1.2	1.6
UB	2.0	1.0	1.8	2.4	1.0	1.2	2.0	2.2	2.0	1.4	3.4	0.8	1.6	0.4	1.8	1.2	1.6

bw: body weight; LB: lower bound (concentrations in food [nondetects set to zero]); GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; 3-MCPD: 3-(mono)chloro-1,2-propanediol; UB: upper bound (concentrations in food [nondetects set to the LOD])

[a] Assumes 60 kg body weight for individuals in all clusters.

[b] Derived using GEMS/Food data on 3-MCPD esters, weighted based on relative oil availability (g per capita) (FAOSTAT, 2016) in cluster countries, and on population of each country.

[c] Based on a free 3-MCPD concentration of 436 µg/kg in soy sauce.

[d] Based on results of Arisseto et al. (2013).

[e] Estimated at twice the mean.

changes in 3-MCPD ester concentrations that may occur during cooking processes. National exposures could be estimated only for Japan and the USA; although data on 3-MCPD ester concentrations in fats and oils were available for Brazil and Canada, national estimates for these countries could not be made due to limitations in CIFOCOss data. Countries that have relevant consumption data (for individuals with 2 or more days of data from national dietary surveys) are encouraged to submit their data to CIFOCOss to enable a broader range of estimates to be calculated in the future.

Ranges of concentrations of 3-MCPD esters in specific oils are wide, and are known to vary geographically depending on mitigation strategies used in oil production. However, data on concentrations of 3-MCPD in oils were available for only three of the 17 GEMS/Food clusters, and global average concentrations were therefore used for all other clusters in estimating international exposures. Countries are encouraged to submit additional data on 3-MCPD concentrations to the GEMS/Food contaminants database to reduce the uncertainty in international estimates of dietary exposure.

Data available on free 3-MCPD concentrations in foods were limited to data on soy sauce and other savoury sauces, seasonings and HVP submitted by the Philippines and Singapore. The Committee used assumptions developed for a previous JECFA assessment, along with data from one recent study conducted in Brazil, to estimate exposures to free 3-MCPD from other sources. Data on free 3-MCPD concentrations in foods from other countries would be useful to ensure a smaller degree of uncertainty in the estimates.

9. Dose–response analysis and estimation of toxic/carcinogenic risk

9.1 Identification of key data for risk assessment

9.1.1 Pivotal data from biochemical and toxicological studies

Based on experimental evidence in vitro and in rats, 3-MCPD esters are assumed to be substantially hydrolysed to free 3-MCPD in the gastrointestinal tract. Orally administered 3-MCPD appears to be more toxic in rats than in mice. The 3-MCPD esters 3-MCPD monopalmitate, 3-MCPD dipalmitate and 3-MCPD dioleate were of similar or lower toxicity than 3-MCPD following oral administration to rats. Therefore, the dose–response analysis focused on 3-MCPD rather than on 3-MCPD esters. The two oral long-term studies on toxicity and carcinogenicity in rats (Sunahara, Perrin & Marchesini, 1993; Cho et al., 2008a) were considered

the pivotal studies for the present evaluation, and kidney and possibly the male reproductive organs the critical target organs.

9.2 General modelling considerations

9.2.1 Selection of data

Two oral long-term studies of toxicity and carcinogenicity with 3-MCPD administered in drinking-water to rats were considered suitable for dose–response analysis (Sunahara, Perrin & Marchesini, 1993; Cho et al., 2008a).

9.2.2 Measure of exposure

In both studies, 3-MCPD was administered in drinking-water for 2 years to male and female rats. Sunahara, Perrin & Marchesini (1993) conducted their study with Fischer 344 rats, while Cho et al. (2008a) used SD rats. (See section 2.4.3 for additional details and tabular results from both studies.)

9.2.3 Measure of response

In both studies, statistically significant effects were noted mainly in the kidney of both sexes and in males in testis.

9.2.4 Selection of mathematical model

Dose–response modelling was conducted with the United States Environmental Protection Agency (USEPA) Benchmark Dose Software (BMDS version 2.6.1) using the Weibull, multistage, gamma, quantal-linear, logistics, log-logistic, probit and log-probit models. As there was some question of the use of the unrestricted models, i.e. the upper-bound curve that was used to compute the lower 95% confidence limit on the benchmark dose (BMDL) for each of these models was highly supralinear, the Committee followed the *Principles for Modelling Dose–Response for the Risk Assessment of Chemicals* (FAO/WHO, 2009) and compared the restricted models to the model-average estimate (Wheeler & Bailer, 2007). This estimate was computed using the default version of the software (Wheeler & Bailer, 2008) and including all models except the quantal-quadratic model, and using the Bayesian information criterion to compute the model-average weights.

9.2.5 Benchmark dose estimates

Benchmark doses were calculated for the most sensitive end-point, namely renal tubular hyperplasia in rats, with a similar dose–response relationship in both pivotal studies: Sunahara, Perrin & Marchesini (1993) and Cho et al. (2008a).

Table 19 shows the benchmark dose for a 10% inhibition (BMD_{10}) and lower 95% confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) estimates from the target end-point using the most sensitive model (restricted log-logistic). Here the Cho et al. (2008a) study produces the lowest $BMDL_{10}$ of 0.87 mg/kg bw per day. As the log-logistic model was the only restricted model that fit the data adequately (i.e. $P > 0.1$) for the Cho et al. (2008a) data (Fig. 3), the value was compared with model-averaging as well as the unrestricted model across both studies and sexes. These model-averaging results are also shown in Table 19. The model-average estimates are consistent with the log-logistic estimates for both the BMD_{10} and $BMDL_{10}$ estimates across studies, which gives additional support for the use of the restricted log-logistic model.

Additional details on mathematical models for renal tubular hyperplasia in male and female SD and F344 rats are shown in Table 20 and Table 21. Here the values of the unrestricted supralinear models are also reported for comparison.

A report submitted to the Committee by the Grocery Manufacturers Association (Dourson & Parker, 2016) reviewed previous BMD calculations (Hwang et al., 2009; Abraham, Mielke & Lampen, 2012; Rietjens et al., 2012; EFSA, 2016) and provided a new one. Dourson & Parker (2016) and Hwang et al. (2009) considered the same datasets (renal tubular hyperplasia in male rats from the Cho et al. (2008a) study) and the same model (restricted log-logistic) as most suitable, and derived the same BMD_{10} and $BMDL_{10}$ as the Committee at the present meeting. However, the rationale for rejection of the use of the unrestricted models, which were used by EFSA (2016) and others, differed. JECFA rejected the highly supralinear models based on its general considerations for dose–response modelling (FAO/WHO, 2009). Dourson & Parker (2016) applied criteria outlined in USEPA's *Benchmark Dose Technical Guidance* (USEPA, 2012) and rejected the unrestricted models due to high (>2) BMD/BMDL ratios.

10. Comments

10.1 Biochemical aspects

10.1.1 3-MCPD esters

In vitro, various 3-MCPD esters were shown to be substrates for porcine pancreatic lipases and, particularly sn-1-monoesters, were hydrolysed rapidly and almost completely as determined by the release of 3-MCPD (>95% within 1 minute at 37 °C). Although a diester (3-MCPD palmitate-oleate) was also efficiently hydrolysed in the same test system, the hydrolysis occurred at a slower

Table 19
Results of benchmark dose modelling

Study / species, strain, study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)
Cho et al. (2008a)				
SD rat, 2-year study of toxicity and carcinogenicity (drinking-water)	0, 1.97, 8.27, 29.5	Renal tubular hyperplasia		
		Male	1.21[a]	0.87[a]
			1.29[b]	0.89[b]
		Female	27.6[a]	18.2[a]
			28.0[b]	20.4[b]
Sunahara, Perrin & Marchesini (1993)				
F344 rat, 2-year study of toxicity and carcinogenicity (drinking-water)	0.11, 1.1, 5.2, 28.3	Renal tubular hyperplasia		
		Male	1.64[a]	1.08[a]
			2.47[b]	1.74[b]
		Female	1.89[a]	1.30[a]
			1.96[b]	1.60[b]

BMD_{10}: benchmark dose for a 10% inhibition; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
[a] Restricted log-logistic.
[b] Model average.

Fig. 3
BMD_{10} estimation with the restricted log-logistic model for renal tubular hyperplasia in male SD rats

BMD_{10}: benchmark dose for a 10% response; BMDL: lower 95% confidence limit on the benchmark dose; BMR: benchmark response
Source: Cho et al. (2008a)

Table 20
Dose–response modelling of renal tubular hyperplasia in male and female SD rats[a]

	Males					Females				
Model	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	*P* value	AIC	Acceptable	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	*P* value	AIC	Acceptable
Restricted models										
Logistic	5.62	4.60	0.00	208.99	No	29.1	24.1	0.52	75.309	Yes
Log-logistic	1.21	0.87	0.61	194.76	Yes	27.6	18.2	0.32	77.075	Yes
Probit	5.36	4.47	0.00	208.40	No	27.8	22.5	0.47	75.419	Yes
Log-probit	3.98	2.96	0.01	204.75	No	25.9	18.3	0.32	77.050	Yes
Weibull	2.14	1.66	0.07	198.99	No	28.0	18.6	0.32	77.078	Yes
Multistage 2°	2.14	1.66	0.07	198.99	No	26.3	19.5	0.54	75.225	Yes
Quantal linear	2.14	1.66	0.07	198.99	No	23.5	14.4	0.17	78.261	Yes
Gamma	2.14	1.66	0.07	198.99	No	27.2	18.4	0.32	77.067	Yes
Unrestricted models										
Unrestricted gamma	0.53	0.07	0.92	195.83	Comparison	27.2	18.4	0.32	77.067	Comparison
Unrestricted log-logistic	0.83	0.22	0.57	196.15	Comparison	27.6	18.2	0.32	77.075	Comparison
Unrestricted log-probit	0.92	0.28	0.54	196.20	Comparison	25.9	17.0	0.32	77.050	Comparison
Unrestricted Weibull	0.63	0.13	0.81	195.88	Comparison	28.0	18.6	0.32	77.078	Comparison

AIC: Akaike information criterion; BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
Source: Cho et al. (2008a)

Table 21
Dose–response modelling of renal tubular hyperplasia in male and female F344 rats

	Males					Females				
Model	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	*P*- value	AIC	Acceptable	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	*P* -value	AIC	Acceptable
Restricted models										
Logistic	6.43	5.24	0.05	193.09	No	8.49	6.91	0.00	200.0	No
Log-logistic	1.64	1.08	0.97	189.16	Yes	1.89	1.30	0.22	185.3	Yes
Probit	6.00	4.96	0.06	192.65	No	7.94	6.54	0.00	199.2	No
Log-probit	4.55	3.36	0.13	191.19	Yes	5.29	3.94	0.00	195.6	No
Weibull	2.59	1.95	0.59	188.22	Yes	3.24	2.46	0.02	190.0	No
Multistage 2°	2.59	1.95	0.59	188.22	Yes	3.24	2.46	0.02	190.0	No
Quantal linear	2.59	1.95	0.59	188.22	Yes	3.24	2.46	0.02	190.0	No
Gamma	2.59	1.95	0.59	188.22	Yes	3.24	2.46	0.02	190.0	No
Unrestricted models										
Unrestricted gamma	1.22	0.22	0.79	189.23	Comparison	0.83	0.29	0.18	185.9	Comparison
Unrestricted log-logistic	1.64	0.42	0.97	189.16	Comparison	1.23	0.41	0.14	186.7	Comparison
Unrestricted log-probit	1.80	0.58	0.85	189.20	Comparison	1.56	0.47	0.16	186.4	Comparison
Unrestricted Weibull	1.32	0.28	0.84	189.21	Comparison	0.84	0.33	0.23	185.4	Comparison

AIC: Akaike information criterion; BMD_{10}: benchmark dose for a 10% response; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
Source: Sunahara, Perrin & Marchesini (1993)

rate (>95% within 90 minutes at 37 °C) (Seefelder et al., 2008). In another in vitro study, approximately 40% of 3-MCPD dioleate was hydrolysed mainly to 3-MCPD-sn-2-monooleate, releasing only small amounts of free 3-MCPD, after a 3-hour incubation with porcine pancreatic lipase at 37 °C (Kaze et al., 2016).

Following oral administration of equimolar 3-MCPD and 3-MCPD dipalmitate to rats, significant amounts of 3-MCPD in the blood were detected (86% based on a comparison of AUC values for free 3-MCPD), demonstrating efficient hydrolysis. The C_{max} of 3-MCPD was approximately 5 times lower following administration of 3-MCPD dipalmitate compared with 3-MCPD. In support of the efficiency of the hydrolysis, no 3-MCPD dipalmitate was detected in blood, kidney, liver or fat following oral administration. Excretion of free 3-MCPD in urine (within 72 hours) and faeces (within 48 hours) after dosing was also investigated and was found to be similar for equimolar doses of 3-MCPD and its dipalmitate, with 2.0% and 2.4% of the dose as 3-MCPD in urine and 0.5% and 1.4% in faeces, respectively (Abraham et al., 2013). After oral administration of 3-MCPD dipalmitate to rats, measured urinary metabolites included, in decreasing order, DHPMA and free 3-MCPD; no (<LOD) β-chlorolactic acid was detected (Barocelli et al., 2011). Based on the available data, substantial hydrolysis of 3-MCPD esters (monoesters and diesters) to 3-MCPD in the gastrointestinal tract has been demonstrated. For the purpose of the current assessment, complete hydrolysis of the 3-MCPD esters is assumed.

10.1.2 3-MCPD

3-MCPD appears to be detoxified by glutathione conjugation, yielding *S*-(2,3-dihydroxypropyl)cysteine and DHPMA. It can also be oxidized to β-chlorolactic acid and further to oxalic acid (Annex 1, reference *155*). In the study by Barocelli et al. (2011), DHPMA was detected in urine of rats at a higher percentage than free 3-MCPD, and only traces (<1% of the dose) of β-chlorolactic acid were excreted, suggesting a more important role of the glutathione pathway than previously considered.

10.2 Toxicological studies

10.2.1 3-MCPD esters

In rats and mice, some 3-MCPD diesters and 3-MCPD sn-1-monoesters are acutely less toxic (oral LD_{50} values ranging from 1780 to >5000 mg/kg bw, corresponding to 332 to >941 mg/kg bw 3-MCPD equivalents) (Liu et al., 2012, 2017; Li et al., 2013) than 3-MCPD (oral LD_{50} values of 118–291 mg/kg bw) (Ericsson & Baker, 1970; Qian et al., 2007). The Committee noted that the differences in acute

toxicity were likely due to the lower maximal plasma concentration of 3-MCPD following administration of the 3-MCPD ester.

Short-term oral exposure to 3-MCPD dipalmitate, 3-MCPD dioleate or 3-MCPD monopalmitate revealed the kidney to be the main target organ in rats, with effects generally occurring at doses above 2 mg/kg bw per day expressed as 3-MCPD. Renal effects included increased relative kidney weight; at higher doses, histopathological changes included tubular epithelial hyperplasia, glomerular lesions and accumulation of hyaline casts. Effects on male reproductive organs (increased testis weight, histopathological findings in testes and epididymis) and liver weight increase were generally seen at doses equal to and greater than 30 mg/kg bw per day expressed as 3-MCPD (Barocelli et al., 2011; Li et al., 2013; Onami et al., 2014a). The target organs and relative potencies were in general similar for the 3-MCPD esters 3-MCPD dipalmitate, 3-MCPD monopalmitate and 3-MCPD dioleate in comparison with 3-MCPD, which supports the assumption of substantial hydrolysis of 3-MCPD esters in the gastrointestinal tract to 3-MCPD.

No in vitro genotoxicity studies were available. In an in vivo genotoxicity study, 4 weeks of oral exposure (5 days/week gavage dosing) to the 3-MCPD esters 3-MCPD dipalmitate, 3-MCPD monopalmitate and 3-MCPD dioleate and also free 3-MCPD at equimolar doses (40 mg/kg bw per day expressed as 3-MCPD) in transgenic F344 *gpt* delta rats produced no positive results in the micronucleus assay with bone marrow, the *Pig-a* mutation assay with red blood cells and the *gpt* assay in kidney and testis (Onami et al., 2014b).

There were no oral long-term toxicity or developmental toxicity studies identified for any 3-MCPD esters. 3-MCPD dipalmitate administered to male rats at oral doses of 100–200 mg/kg bw per day on 5 consecutive days caused infertility, which was partly reversible. The infertility was described as comparable with that occurring with equimolar doses of 3-MCPD (Rooney & Jackson, 1980).

10.2.2 3-MCPD

In previous 3-MCPD evaluations by the Committee, the 2-year carcinogenicity study in Fischer 344 rats by Sunahara, Perrin & Marchesini (1993) (Annex 1, reference *155*) was considered the critical study, with kidney identified as the main target organ. A LOEL of 1.1 mg/kg bw per day was identified for renal tubular hyperplasia as the most sensitive end-point. Although the Committee at that meeting noted some increased incidences of benign renal, mammary and testicular tumours, they were considered to be secondary to observed increases in chronic progressive nephropathy and/or endocrine imbalance due to hormonally mediated Leydig cell tumours. In addition, no genotoxic potential has been demonstrated in vivo for 3-MCPD.

New oral studies with 3-MCPD that have become available since the previous evaluation include short-term toxicity studies in mice and rats, long-term toxicity studies in mice and rats and a 26-week carcinogenicity study in transgenic CB6F1 *ras*H2-Tg mice.

In a short-term oral toxicity study conducted with 3-MCPD in mice, a NOAEL of 18.05 mg/kg bw per day was identified, based on testicular effects (Cho et al., 2008b).

In a short-term oral toxicity study conducted with 3-MCPD in rats, the kidney and the testes were identified as critical target organs. Nephrotoxicity was particularly severe in females, resulting in death due to acute renal failure at 29.5 mg/kg bw per day in 35% of females. Effects observed in both sexes included various histopathological findings in the kidneys (e.g. tubular epithelial hyperplasia, glomerular lesions and accumulation of hyaline casts), which appeared to be mainly restricted to the middle and high doses (7.4 and 29.5 mg/kg bw per day). Testicular effects, including degeneration of seminiferous tubules and decreases in spermatid density, were mainly observed at the highest dose (29.5 mg/kg bw per day) (Barocelli et al., 2011). The Committee noted that there were deficiencies in the reporting of this study.

In a 2-year oral (drinking-water) carcinogenicity study in mice, no increases in neoplastic or non-neoplastic lesions were observed up to the highest dose tested, 31.0 mg/kg bw per day (Jeong et al., 2010). There was no significant increase in tumour incidence in a 26-week gavage carcinogenicity study in transgenic CB6F1 *ras*H2-Tg mice, with a LOAEL of 10 mg/kg bw per day, based on increased relative kidney weight (Lee et al., 2016).

In a 2-year oral (drinking-water) carcinogenicity study in SD rats, there were dose-related increased incidences of renal nephropathy and tubular hyperplasia, with the hyperplasia more frequently observed in males, being significantly different from controls at all doses (1.97, 8.27 and 29.5 mg/kg bw per day) in males. Atrophy and arteritis/periarteritis in testes were also significantly increased compared with controls at all doses, although not in a dose-related manner. Increased incidences of renal cell tumours (adenoma or carcinoma) in both sexes and of Leydig cell tumours in males were observed, being significantly different from controls at the highest dose (Cho et al., 2008a).

10.3 Observations in humans

No clinical or epidemiological studies were identified.

10.4 Analytical methods

The initial work in the area of 3-MCPD was focused on the determination of the free 3-MCPD or unesterified 3-MCPD. In analysing food for 3-MCPD, there appeared to be 3-MCPD-containing food categories that were unlikely to contain soy sauce or HVP. Subsequent reports indicated that perhaps oils and fats could be a common precursor for this contaminant. Analysis of oils and fats led to confirmation of the presence of variable concentrations of 3-MCPD fatty acid esters in this category. Early method development for oils and fats was hampered by the lack of analytical standards for the large family of possible 3-MCPD monoesters and diesters. This situation has been rectified fairly recently, allowing for method development directed at the measurement of the 3-MCPD esters to commence.

There are two main approaches to 3-MCPD ester analysis, which include (1) the measurement of 3-MCPD esters after individual esters have been converted to MCPD through the cleavage of fatty acid moieties using either acidic or alkaline conditions or using enzymatic cleavage and (2) the measurement of intact MCPD esters. These methods are described as indirect methods and direct methods, respectively (Crews et al., 2013). 3-MCPD esters are frequently distinguished in the literature from 3-MCPD through reference to bound MCPD (intact 3-MCPD esters) and free 3-MCPD, respectively (Crews et al., 2013).

The extraction of MCPD esters from homogeneous food samples is performed using solvent to extract the lipid from samples and generally includes the addition of isotopically labelled 3-MCPD analogues to allow for recovery correction during sample preparation. Labelled standards are generally deuterated analogues of free MCPD or MCPD esters, although ^{13}C analogues have been used.

Indirect methods are based on derivatization of the free MCPD using PBA and heptafluorobutyrylimidazole, following cleavage of fatty acid methyl esters under acidic or alkaline conditions or via enzymatic cleavage, prior to indirect analysis (Crews et al., 2013). Analysis is generally performed using GC-MS, although early research into the determination of MCPD was also performed using gas chromatography with flame ionization detection and electron capture detection (Wenzl, Lachenmeier & Gokmen, 2007).

In contrast, direct measurement allows for analysis of the MCPD esters without the need for derivatization. Two steps of solid-phase extraction are generally used for the cleanup of extracts prior to direct analysis (Dubois et al., 2012). Analysis is performed with LC-MS (MacMahon, Ridge & Begley, 2014). Until recently, the lack of analytical standards for individual MCPD esters has had an impact on the ability to perform analysis of the esters directly.

3-MCPD LODs reported using indirect methods have ranged widely, with older data collected in the early 2000s having much higher limits (e.g. 5000 µg/kg) than more recently developed data (<10 µg/kg). Direct methods require the determination of an LOD for each of the esters measured, and the LODs can span more than 1 order of magnitude (10–160 µg/kg). A collaborative study was also performed to compare results obtained for MCPD esters in oils using the German Society for Fat Science (DGF) and the SGS Germany GmbH methods, showing that both methods would accurately determine 3-MCPD concentrations in fats and oils, with the assumption that glycidol was the only 3-MCPD-forming substance in the oil (Fiebig, 2011).

A number of reference methods are available for the measurement of MCPD, including the AOAC first action for an official method for the analysis of free 3-MCPD in a variety of foods (AOAC, 2005). More recently, the AOCS developed three official methods pertaining to the analysis of MCPD esters exclusively in oils and fats. All three AOCS methods result in the determination of the 2- and 3-MCPD-equivalent concentrations, with a concurrent determination of glycidol-equivalent concentrations (AOCS, 2013a,b,c).

10.5 Sampling protocols

Although the Codex Alimentarius Commission has not established specific sampling protocols for MCPD or its esters, general guidelines on sampling have been developed (FAO/WHO, 2004). Best practices have been established for numerous contaminants and include the collection of samples by qualified individuals using containers that are clean and non-reactive and that protect samples from contamination or damage during transport and storage. Sampling of commercial food products must ensure that samples collected are representative of the lot. Therefore, collection of multiple samples (incremental samples) from within the lot is recommended and may be used to form an aggregate sample from which laboratory samples may be analysed. Prior to the subsampling for laboratory analysis, homogenization of the aggregate sample should be performed, consistent with good laboratory practices. Sample collection must be focused on food commodities that are relevant to MCPD and MCPD esters (e.g. fats, oils, foods containing these products).

10.6 Effects of processing

3-MCPD esters are generated during the refining of crude oils and fats. The formation of 3-MCPD esters in oils has been associated with deodorization using high temperatures (Smidrkal et al., 2016). Unrefined oils contain a variety

of compounds that contribute to the formation of 3-MCPD esters, including acylglycerols, phospholipids, free fatty acids and chlorinated compounds. The details of the implementation of refining practices performed prior to deodorization have a critical impact on 3-MCPD ester formation, as does the temperature at which the deodorization is performed. The preliminary steps in the refining process include degumming or washing, neutralization, bleaching and deodorization (Pudel et al., 2011). The condition of fruit, etc., used for making oils will also have an impact on the levels of 3-MCPD esters, with bruised components contributing to higher levels of these contaminants (Gibon, De Greyt & Kellens, 2007).

Degumming of oil removes phospholipids and is generally performed at relatively low temperatures (80–120 °C). Neutralization of oils involves interaction of the oil with sodium carbonate or bicarbonate to lower the acid value (increase the pH), prior to deodorization. The bleaching process involves exposure of oils to bleaching clays to remove phospholipids from the oil. The final stage of oil refining is known as deodorization, where, in addition to acid treatment, oils are heated at higher temperatures (generally >200 °C). The refining process has been investigated to determine the impact on formation of glycidyl esters and MCPD esters, although the majority of research has focused on the MCPD esters owing to their earlier discovery.

It has been reported that palm oil has the highest concentrations of 3-MCPD esters compared with other oil types (e.g. soybean, rapeseed, sunflower) (Kuhlmann, 2011; Weisshaar, 2011). Palm oil is known not to contain high levels of phospholipids; therefore, the degumming step is often not performed with this oil, or dry degumming (treatment with citric or phosphoric acid) is performed. As a result of the elevated 3-MCPD concentrations in palm oil, palm oil has been the focus of much investigation related to 3-MCPD ester formation and identification of mitigation strategies (Destaillats et al., 2012; Matthaus & Pudel, 2014). Other groups have examined seed oils (virgin and refined) as part of their investigation into where 3-MCPD formation is most important.

Unlike the glycidyl esters, formation of 3-MCPD esters is not directly correlated with increased temperature, particularly above 240 °C. 3-MCPD ester formation does occur at temperatures corresponding to the deodorization of oils (generally >200 °C).

10.7 Prevention and control

Strategies to prevent and control the formation of 3-MCPD esters in oils and fats have been mainly focused on raw material pretreatment, the refining conditions and purification adsorbents. Recent reports indicate that efforts to control the

level of 3-MCPD esters in edible oils should begin with the selection and washing of the raw material. In the pretreatment of oilseeds, organic chlorine–containing compounds are considered to be the main donors in the formation of 3-MCPD esters (Matthaus et al., 2011). Washing raw material before refining with water or ethanol could remove those critical reactants and reduce 3-MCPD ester-forming capability (Li et al., 2016c). Another important strategy for the prevention and control of the formation of 3-MCPD esters in oils is the optimization of refining conditions, including water degumming, bleaching additives, steam distillation and thermal treatment. Control of temperature of the steam distillation and neutralization before the deodorization step have the greatest impact on the prevention and control of 3-MCPD ester formation. Dual deodorization using a short first step at a high temperature (250–270 °C) combined with a second longer step at a lower temperature (200 °C) also shows significant reduction of 3-MCPD ester formation. The application of adsorbents and additives, including calcinated zeolite, synthetic magnesium silicate and antioxidants, following deodorization further reduces the formation of 3-MCPD esters.

It can be concluded that strategies to prevent and control the formation of 3-MCPD esters in final oil products include:

- selection of raw material with low precursor content;
- removal of precursors using chemical treatment at mid-range temperatures;
- deodorization performed with a neutral pH at temperatures <240 °C;
- adoption of dual deodorization protocols;
- utilization of adsorbents to remove 3-MCPD esters in post-treatment.

The Committee noted the commitment of FEDIOL to continue to reduce the levels of 3-MCPD esters in refined vegetable oils and encouraged the organization to continue to reduce the levels of these contaminants.

10.8 Levels and patterns of contamination in food commodities

Vegetable oils and fats are regional in their production and are consumed in relatively higher proportions in the production area than in importing countries (FAOSTAT database: http://www.fao.org/faostat/en/#home). Therefore, it can be seen that palm oil and its products are the major fats consumed in South-east Asia, but less dominant in Europe and North America, whereas soybean oil is dominant in North and South America, and rapeseed and sunflower seed oils are more common in Europe. The pattern of consumption of individual oils in any

finished food is further complicated, as a mixture of oils is often used to give a food a particular texture or structure.

It is apparent that 3-MCPD esters are formed in the processing of vegetable oils mainly during the deodorization step. The extent to which they are formed may depend on the oilseed or fruit being processed and the process being used. Hence, the refined oil obtained from any oil source may vary in 3-MCPD ester content. From reports of the analysis of foodstuffs in a number of countries, it appears that refined vegetable oil is a major contributor to the levels of 3-MCPD esters found in food (EFSA, 2016). It should be noted that methods for the analysis of 3-MCPD esters in foods, other than for fats and oils, have not been subjected to full collaborative study, and it is not clear if the same samples were analysed by any of the laboratories involved in the provision of the majority of the results received from the USA, Canada and the European Union in response to the call for data. Although these methods themselves might exhibit reasonable precision for different food types, their accuracy has not been evaluated under rigorous conditions. Recently, a collaborative study has been organized for the analysis of contaminants in high-fat foods (margarines and mayonnaise), but no international work on other food types has been initiated.

Early studies of 3-MCPD esters in retail food products indicated that free 3-MCPD occurred at approximately 9.6–82.7 µg/kg food (Svejkovka et al., 2004), whereas the levels of 3-MCPD esters (monoesters and diesters) varied between the LOD (1.1 mg/kg fat) and 36.8 mg/kg fat. Foodstuffs of plant origin processed at high temperatures also contained elevated levels of 3-MCPD esters (0.14–6.10 mg/kg), as did coffee surrogates and malts following high-temperature roasting (0.145–1.184 mg/kg and 0.004–0.65 mg/kg, respectively) (Divinova, Dolezal & Velisek, 2007). In contrast, most virgin/unprocessed/unrefined oils do not contain detectable levels of MCPD esters (Kuhlmann, 2011; Becalski et al., 2015a,b). Levels of MCPD esters in toasted unrefined sesame oils found by MacMahon, Begley & Diachenko (2013b) were comparable with the levels of MCPD esters found in toasted and unrefined sesame oils by Becalski et al. (2015a,b), indicating that the roasting process might not be the sole reason for the presence of MCPD esters in processed sesame oils. There appears to be little evidence that further 3-MCPD esters are formed in food during processing or cooking.

Dingel & Matissek (2015) indicated that 3-MCPD esters are not formed during the deep frying process, perhaps because of the lower frying temperatures used (160–188 °C) or because chlorine-containing compounds were no longer present in the deodorized high-oleic sunflower oil used.

10.9 Food consumption and dietary exposure assessment

The Committee searched the scientific literature using PubMed and Web of Science to identify estimates of exposure to 3-MCPD, which includes 3-MCPD esters (expressed as 3-MCPD equivalents), free 3-MCPD and total 3-MCPD (i.e. 3-MCPD esters and free 3-MCPD), published from 2012 to 2016. The year 2012 was chosen as the start date owing to concerns about the accuracy of 3-MCPD ester analyses conducted prior to that time and to concerns that analyses of samples collected prior to 2012 may not reflect concentrations of 3-MCPD in products currently on the market. The Committee also estimated exposures to 3-MCPD at the national and international levels based on data submitted to the GEMS/Food contaminants database. It should be noted that the analytical methods underlying some of the 3-MCPD concentration data were not provided and, where known, may not have been among those validated in international collaborative studies. A summary of estimates of 3-MCPD dietary exposure is shown in Table 22.

Published estimates of mean dietary exposure to 3-MCPD esters ranged from 0.2 μg/kg bw per day for adults to 1.3 μg/kg bw per day for children aged 7–10 years (Chung et al., 2013; Li et al., 2015). Li et al. (2015) estimated that adults 18–49 years of age and children 7–10 years of age have 95th percentile dietary exposures to 3-MCPD esters of 2.6 and 3.8 μg/kg bw per day, respectively, based on a probabilistic analysis that combined distributions of food consumption data with distributions of 3-MCPD ester concentrations.

Mean and high (95th percentile) dietary exposures to free 3-MCPD were 0.08 and 0.44 μg/kg bw per day, respectively, in the population 10 years and older in Brazil (Arisseto et al., 2013).

Published estimates of dietary exposure to total 3-MCPD are limited to those reported by EFSA (2016) for Europe. The median of the mean dietary exposures to total 3-MCPD across European surveys ranged from 0.3 μg/kg bw per day for adults (≥18 to <65 years) and elderly adults (≥65 years to <75 years) to 0.9 μg/kg bw per day for infants (<12 months); the median of the high (95th percentile) dietary exposures for these age groups ranged from 0.6 μg/kg bw per day for elderly adults to 1.7 μg/kg bw per day for infants and toddlers (≥12 to <36 months). For infants exclusively fed infant formula, dietary exposure to 3-MCPD esters was estimated to be 2.4 μg/kg bw per day, based on the mean formula concentration. Exposure was also calculated using the 95th percentile 3-MCPD concentration in formula to capture brand loyalty to products having high concentrations of 3-MCPD esters; this exposure was estimated to be 3.2 μg/kg bw per day.

Table 22
Summary of estimates of dietary exposures to 3-MCPD (esters and total)

		Range of estimated dietary exposures (µg/kg bw per day)[a]			
		Mean consumption		High-percentile consumption[b]	
Source of estimate	Population	Mean concentration	P95 concentration[c]	Mean concentration	P95 concentration[c]
National					
Committee and literature	Adults	0.2–0.7		0.5–2.6[d]	
	Children/adolescents	0.4–1.3		0.8–3.8[d]	
	Infants[e]	<0.1–10	15–21	<0.1–12	25
National					
Committee	Adults[f]	0.2–1.7		0.4–3.4	

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound; 3-MCPD: 3-(mono)chloro-1,2-propanediol
[a] Includes LB and UB estimates.
[b] 90th or 95th percentile, depending on the assessment.
[c] The 95th percentile concentration was estimated only where the number of samples was greater than 60.
[d] Upper end of range based on probabilistic assessment that combined distributions of food consumption data with distributions of 3-MCPD ester concentrations.
[e] Includes all estimates for infants from infant and follow-on formula and from mixed diets for 0–12 months of age. Based on data on 3-MCPD ester concentrations in infant formula from the GEMS/Food contaminants database and the literature.
[f] Per capita estimates based on a body weight of 60 kg.

10.9.1 National estimates

The Committee estimated national dietary exposures to 3-MCPD esters for Japan and the USA based on concentration data on fats and oils submitted to the GEMS/Food contaminants database and on consumption data from the CIFOCOss database. Estimates of dietary exposure to 3-MCPD esters ranged from 0.1 µg/kg bw per day (mean consumption by the general population, Japan) to 0.6 µg/kg bw per day (90th percentile consumption by children, USA).

The Committee also estimated dietary exposure to 3-MCPD esters for infants exclusively consuming infant formula using GEMS/Food contaminants data and data on infant energy requirements. Mean dietary exposures to 3-MCPD esters from formula were estimated for infants in Canada and Japan as 5–7 µg/kg bw per day, depending on age, and in the USA as 7–10 µg/kg bw per day, based on estimated median formula consumption.

Dietary exposure to 3-MCPD esters for young infants was also estimated based on 95th percentile 3-MCPD concentrations to capture brand loyalty to products having high concentrations of 3-MCPD esters. This estimate was possible only for the USA as a result of sample size considerations. These dietary exposures to 3-MCPD for infants in the USA were estimated to be 21 and 25 µg/kg bw per day for median and 95th percentile formula consumption, respectively. The high dietary exposures to 3-MCPD esters estimated for formula-fed infants in the USA are likely due to the inclusion of palm oil and/or palm olein as

ingredients in many infant formulas in the USA. No information was available on major oil ingredients in non-USA infant formula samples.

10.9.2 International estimates

The Committee estimated international per capita dietary exposures to 3-MCPD (esters, free and total) for adults based on concentration data on fats, oils and soy sauce from the GEMS/Food contaminants database and on consumption data from the GEMS/Food cluster diets. 3-MCPD concentration data submitted by Brazil, Canada, China, Japan, Singapore and the USA were used in the analyses; global averages were used in analyses for clusters with no concentration data. Dietary exposures to free 3-MCPD from consumption of HVP were estimated based on assumptions used in the previous JECFA assessment.

International estimates of mean dietary exposure to total 3-MCPD ranged from 0.2 µg/kg bw per day for cluster G14 (Comoros, Fiji, Kiribati, Papua New Guinea, Solomon Islands, Sri Lanka, Vanuatu) to 1.7 µg/kg bw per day for cluster G11 (Belgium, the Netherlands). Dietary exposures at the 90th percentile were estimated to be 0.4–3.4 µg/kg bw per day (Table 22). These exposures largely reflect contributions from fats and oils; free 3-MCPD did not contribute significantly to dietary exposure to total 3-MCPD.

10.10 Dose–response analysis

The main target organs for 3-MCPD and its esters in rats and for 3-MCPD in mice were the kidneys and the male reproductive organs. 3-MCPD was carcinogenic in two rat strains, but not in mice. Oral long-term studies on toxicity and carcinogenicity in the F344 rat by Sunahara, Perrin & Marchesini (1993; previously evaluated by JECFA) and in the SD rat by Cho et al. (2008a; new study) were considered the pivotal studies for risk assessment (Table 23), and renal tubular hyperplasia was considered the most sensitive end-point. In accordance with JECFA guidance on dose–response modelling, all models in the USEPA's BMDS suite (version 2.6.1) were fitted to the data from Sunahara, Perrin & Marchesini (1993) and Cho et al. (2008a) using the software's default constraints for restricted models. For the restricted models and the Cho et al. (2008a) data, the only model having acceptable fit, i.e. *P*-values greater than 0.1, was the log-logistic model. As a comparison, the $BMDL_{10}$s were computed from unrestricted models; all of the unrestricted models estimated the $BMDL_{10}$ at unrealistically low doses. For further comparison, the model-averaging software of Wheeler & Bailer (2008), which is available in source code as supplemental material, was used to compute the model-average estimate to compare the estimates based upon the log-logistic model. For this comparison, all models, except the quantal-

Table 23
Results of benchmark dose modelling in two studies in rats

Species / study type (route of administration) Reference	Doses (mg/kg bw per day)	Critical end-point	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)
SD rat, 2-year study of toxicity and carcinogenicity (drinking-water) Cho et al. (2008a)	0, 1.97, 8.27, 29.5	Renal tubular hyperplasia		
		Male	1.21[a]	0.87[a]
			1.29[b]	0.89[b]
		Female	23.5[c]	14.4[c]
			28.0[b]	20.4[b]
F344 rat, 2-year study of toxicity and carcinogenicity (drinking-water) Sunahara, Perrin & Marchesini (1993)	0.11, 1.1, 5.2, 28.3	Renal tubular hyperplasia		
		Male	1.64[a]	1.08[a]
			2.47[b]	1.74[b]
		Female	1.89[a]	1.30[a]
			1.96[b]	1.60[b]

BMD_{10}: benchmark dose for a 10% inhibition; $BMDL_{10}$: lower 95% confidence limit on the benchmark dose for a 10% response; bw: body weight
[a] Restricted log-logistic model.
[b] Model average.
[c] Restricted quantal-linear model.

quadratic, were included in the analysis, and the Bayesian information criterion was used to compute the model-average weights. The model-average $BMDL_{10}$ estimates were close to the values provided by the log-logistic model, which gave support for the use of the log-logistic model. For the two studies using the BMDS suite, the BMD_{10} estimates for male rats ranged between 1.21 and 4.55 mg/kg bw per day, with 95% $BMDL_{10}$s ranging between 0.87 and 3.36 mg/kg bw per day. Female rats had a larger range between the two studies. Here, BMD_{10} estimates ranged between 1.89 and 29.1 mg/kg bw per day, with 95% $BMDL_{10}$s ranging between 1.30 and 24.1 mg/kg bw per day. For the assessment, the lowest $BMDL_{10}$ was 0.87 mg/kg bw per day for renal tubular hyperplasia in male rats from the Cho et al. (2008a) study using the restricted log-logistic model.

11. Evaluation

Experimental evidence indicates that 3-MCPD esters are substantially hydrolysed to 3-MCPD in the gastrointestinal tract and elicit toxicity as free 3-MCPD. The Committee therefore based its evaluation on the conservative assumption of complete hydrolysis of 3-MCPD esters to 3-MCPD. Whereas the experimental data supporting substantial hydrolysis are derived from studies with post-weaning animals, the Committee concluded that the capacity of the neonate to hydrolyse fatty acids in the gut is efficient, and therefore the same assumption of substantial hydrolysis could be extended to this age group.

The main target organs for 3-MCPD and its esters in rats and for 3-MCPD in mice are the kidneys and the male reproductive organs. 3-MCPD was carcinogenic in two rat strains, but not in mice. No genotoxic potential has been demonstrated in vivo for 3-MCPD. Two long-term carcinogenicity studies with 3-MCPD in rats were identified as pivotal studies, and renal tubular hyperplasia was identified as the most sensitive end-point (Sunahara, Perrin & Marchesini, 1993; Cho et al., 2008a). The lowest $BMDL_{10}$ for renal tubular hyperplasia was calculated to be 0.87 mg/kg bw per day for male rats (Cho et al., 2008a). After application of a 200-fold uncertainty factor, the Committee established a group PMTDI of 4 μg/kg bw for 3-MCPD and 3-MCPD esters, singly or in combination, expressed as 3-MCPD equivalents (rounded to one significant figure). The overall uncertainty factor of 200 incorporates a factor of 2 related to the inadequacies in the studies of reproductive toxicity.

The previous PMTDI of 2 μg/kg bw for 3-MCPD, established at the fifty-seventh meeting and retained at the sixty-seventh meeting, was withdrawn.

The Committee noted that there are no published collaboratively studied methods for the determination of 3-MCPD esters in complex foods in contrast to the situation with fats and oils; therefore, caution should be applied when interpreting analytical data from complex foods.

The Committee further noted that there was uncertainty in comparing the reported levels in the same foods from different regions because of the lack of interlaboratory comparisons and the absence of data arising from proficiency testing schemes.

The Committee noted that estimated dietary exposures to 3-MCPD for the general population, even for high consumers (up to 3.8 μg/kg bw per day), did not exceed the new PMTDI. Estimates of mean dietary exposure to 3-MCPD for formula-fed infants, however, could exceed the PMTDI by up to 2.5-fold for certain countries (e.g. 10 μg/kg bw per day in the first month of life).

While the current evaluation was specific to the request for an evaluation of 3-MCPD esters, the Committee was aware that 2-MCPD esters can be detected in some of the same foods as 3-MCPD esters. There are, however, currently limited food occurrence data available for 2-MCPD and 2-MCPD esters in the GEMS/Food contaminants database, and the toxicological database is currently insufficient to allow a hazard characterization.

11.1 Recommendations

The Committee recommends that appropriate efforts to reduce concentrations of 3-MCPD esters and 3-MCPD in infant formula continue to be implemented.

The Committee recommends that additional international collaborative studies should be undertaken on methods of analysis for 3-MCPD esters in relevant fat- or oil-containing foods in order to remove the uncertainty surrounding the accuracy of the data submitted to the GEMS/Food contaminants database for use in future evaluations.

To address the uncertainty associated with reproductive effects, experimental studies would be required to elucidate the potential reproductive toxicity of 3-MCPD esters, including exposure of newborns.

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Sterigmatocystin

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1. Explanation 776
2. Biological data 777
2.1 Biochemical aspects 777
2.1.1 Absorption, distribution and excretion 777
2.1.2 Biotransformation 779
(a) In vivo 779
(b) In vitro 779
2.1.3 Effects on enzymes and other biochemical parameters 782
2.1.4 Physiologically based pharmacokinetic modelling 783
2.1.5 Transfer of sterigmatocystin into human food 783
2.2 Toxicological studies 784
2.2.1 Acute toxicity 784
(a) Median lethal dose (LD_{50}) studies 784
(b) Oral toxicity 784
(c) Non-oral routes of administration 785
(d) Acute toxicity in domestic animals 786
2.2.2 Short-term studies of toxicity 786
(a) Mice 786
(b) Rats 786
(c) Guinea-pigs 787
(d) Monkeys 787
(e) Domestic animals 788
2.2.3 Long-term studies of toxicity and carcinogenicity 788
(a) Oral administration 788
(b) Non-oral administration 798
2.2.4 Genotoxicity 799
(a) In vitro 799
(b) In vivo 802

(c) DNA adducts 802
2.2.5 Reproductive and developmental toxicity 804
2.2.6 Special studies 804
(a) Toxicity in vitro 804
(b) Neurotoxicity 807
(c) Immunotoxicity 807
(d) Other types of study 809
2.2.7 Sterigmatocystin and aflatoxins or other mycotoxins 810
2.3 Observations in domestic animals/veterinary toxicology 814
2.4 Observations in humans 814
3. Analytical methods 815
3.1 Chemistry 815
3.1.1 Sterigmatocystin as precursor of aflatoxins and biosynthetic pathway 816
3.2 Description of analytical methods 819
3.2.1 Introduction 819
3.2.2 Screening tests 820
3.2.3 Quantitative methods 821
3.2.4 Quantitative methods for modified sterigmatocystin 824
4. Sampling protocols 824
5. Effects of processing 826
5.1 Sorting and cleaning 826
5.2 Dry and wet milling 826
5.3 Thermal processing 826
5.4 Alkaline treatment 827
5.5 Fermentation 827
5.6 Modified, hidden and bound sterigmatocystin 827
6. Prevention and control 827
6.1 Preharvest control 829
6.1.1 Tillage and soil types 829
6.1.2 Planting resistant cultivars 829
6.1.3 Prevention of sterigmatocystin production in fungi 829
6.1.4 Sowing date 829
6.1.5 Biocontrol of sterigmatocystin-producing fungi 829
6.1.6 Fungicides and inhibitors of sterigmatocystin synthesis 829
6.1.7 Harvesting 829
6.2 Postharvest control 829
6.2.1 Storage of food products and commodities 829
6.3 Decontamination 831
6.3.1 Irradiation 831
6.3.2 Chemical detoxification 831
6.3.3 Microbial degradation 831
6.3.4 Enzymatic degradation 831
6.3.5 Prevention of absorption of sterigmatocystin in gastrointestinal tract by adsorbents 831
6.4 HACCP and integrated mycotoxin management system 832
7. Levels and patterns of contamination in food commodities 832
7.1 Surveillance data 832
7.1.1 Contamination in WHO regions 832

(a) African Region 832
(b) Region of the Americas 833
(c) European Region 833
(d) Eastern Mediterranean Region 833
(e) Western Pacific Region 833
7.1.2 Contamination in countries as reported in published papers 833
(a) African Region 833
(b) Region of the Americas 839
(c) South-East Asia Region 839
(d) European Region 839
(e) Eastern Mediterranean Region 840
(f) Western Pacific Region 840
7.1.3 Contamination in feed 841
7.1.4 Co-occurrence of sterigmatocystin with other mycotoxins 841
8. Food consumption and dietary exposure estimates 843
8.1 Surveillance data 843
8.2 National estimates 844
8.3 International estimates 845
9. Dose–response analysis and estimation of toxic/carcinogenic risk 846
9.1 General modelling considerations 846
9.1.1 Selection of data 846
9.1.2 Measure of exposure 847
9.1.3 Measure of response 847
9.1.4 Selection of mathematical models 847
9.2 BMD analysis 847
10. Comments 849
10.1 Biochemical aspects 849
10.2 Toxicological studies 850
10.2.1 Sterigmatocystin and aflatoxin B_1 853
10.3 Observations in domestic animals/veterinary toxicology 854
10.4 Observations in humans 855
10.5 Analytical methods 855
10.6 Sampling protocols 856
10.7 Effects of processing 856
10.8 Prevention and control 857
10.9 Levels and patterns of contamination 857
10.10 Food consumption and dietary exposure assessment 858
10.10.1 International estimates 858
10.11 Dose–response analysis 859
11. Evaluation 859
11.1 Recommendations 860
12. References 861

1. Explanation

Sterigmatocystin is a toxic fungal secondary metabolite (mycotoxin) that is mainly produced by more than a dozen species of *Aspergillus* as well as by a number of phylogenetically and phenotypically different fungal genera (Rank et al., 2011; Jurjević et al., 2013; Hubka et al., 2016). It is a polyketide-derived mycotoxin with Chemical Abstracts Service (CAS) No. 10048-13-2 and International Union of Pure and Applied Chemistry (IUPAC) name (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3′,2′:4,5]furo[2,3-c]xanthen-7-one.

Human and animal exposure can occur if there is contamination of food or feed by a sterigmatocystin-producing fungus. Sterigmatocystin contamination mainly takes place during storage and has been reported in foods such as cheese and beer (Veršilovskis, Bartkevičs & Miķelsone, 2008), peanuts (Varga et al., 2013), crisp bread, rye wholemeal, white bread and muesli (Mol et al., 2016), rice (Rofiat et al., 2015) and chilli (Yogendrarajah et al., 2014a) and in feed (Warth et al., 2012; Biancardi & Dall'Asta, 2015).

Structurally, sterigmatocystin is closely related to aflatoxins (Fig. 1). Sterigmatocystin is an intermediate in the biosynthetic pathway for aflatoxins (Fig. 2; Keller, Kantz & Adams, 1994; Yu, Bhatnagar & Ehrlich, 2002). *Aspergillus versicolor* and *A. nidulans* do not contain the enzymes necessary for the conversion of sterigmatocystin into aflatoxin (Sweeney & Dobson, 1999). Only a few examples of co-occurrence with other mycotoxins, principally aflatoxin B_1 (AFB_1), have been reported in the literature (Abramson et al., 1999; Yogendrarajah et al., 2014a,b).

Sterigmatocystin has not previously been evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee reviewed sterigmatocystin at the present meeting at the request of the Codex Committee on Contaminants in Foods.

The literature search for biological data was built on the review conducted by the European Food Safety Authority (EFSA, 2013), updated by papers published after 2011. The search was performed in PubMed and Scopus. The literature search on the occurrence of and dietary exposure to sterigmatocystin was run using three databases (Scopus, PubMed and Ovid) and a cut-off date of 2000.

Fig. 1
Sterigmatocystin

Fig. 2
Biosynthesis of aflatoxins from sterigmatocystin

AFL: aflatoxin; OMSTC: *O*-methylsterigmatocystin;
STC: sterigmatocystin
Source: Carbone et al. (2007)

2. Biological data

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Daily doses of sterigmatocystin (8 mg/kg body weight [bw] equally divided over 13 days) were administered in feed to immature (50–150 g) and mature (200–300 g) Sprague Dawley rats of both sexes (n = 5/sex per group), housed in metabolic cages for urine and faeces collection. On day 14, an 8 mg/kg bw dose of ^{14}C-labelled sterigmatocystin dissolved in wheat germ oil was given to the rats by gavage. Sterigmatocystin was extracted from *A. versicolor* (purity not known). Five rats of each sex and age group were killed at 3, 6, 12, 24, 48 and 96 hours. In each sex and age group of rats, the sterigmatocystin plasma levels versus time curves contained multiple peaks interpreted as evidence for enterohepatic recirculation.

Apart from the gastrointestinal tract, the liver exhibited the largest area under the cumulative tissue level versus time curves. Other tissues that displayed high tissue levels were kidneys and adrenals. Statistically significant differences in tissue levels of radioactivity due to age and sex were observed. Mature males showed the highest tissue levels and immature females the lowest. The highest concentration of radioactivity in serum appeared 3 hours after administration in immature and mature females, between 3 and 6 hours after administration in mature males and around 12 hours after administration in immature males. In plasma, the gradual decline in the log-linear phase resulted in long half-lives, ranging from 61.5 hours (in immature females) to 130 hours (in mature males), suggesting a potential for accumulation. The elimination of ^{14}C-labelled sterigmatocystin averaged 10% of the total administered dose in urine and between 64% and 92% in the faeces, according to the age and sex group. The faecal excretion after 96 hours was higher in males (90% in average) compared with females (65%). The excretion in urine appeared complete by 96 hours, with at least 90% excreted after 48 hours. There were no significant differences in the urinary elimination of males and females (10% and 9%, respectively). Overall, the rate of absorption could be as high as 77–100% in adult rats based on the measurement of 8–10% of administered radioactivity excreted in urine and another 67–92% of the radioactivity excreted in faeces (Walkow et al., 1985).

Wang et al. (1991) treated orally 30 male Wistar rats with ^{3}H-labelled sterigmatocystin (1.85×10^{7} Bq; radioactive purity 95%). The animals were placed randomly in pairs in metabolic cages. The highest concentration of radioactivity in serum appeared 3 hours after administration and the half-life of distribution was 0.5 hour. The radioactivity was concentrated mainly in liver, stomach, kidney, duodenum and lung and to a lesser extent in fat, muscle, testis, rectum and bone. The half-life of excretion was 43.9 hours. By 48, 96 and 144 hours, 56.4%, 62.4% and 64.4% had been excreted in faeces and 20.1%, 21.0% and 23.8% in urine. The authors suggested that the biliary excretion may be the major route of excretion of sterigmatocystin (Wang et al., 1991).

One male vervet monkey received an oral dose of 18 mg/kg bw of ^{14}C-labelled sterigmatocystin (extracted from *A. versicolor*; 99.5% pure) and was killed 40 hours later. The bile was collected and mixed with the bile of two other male monkeys (weighing 3.60 and 4.00 kg), each equipped with a bile duct canula and given a daily oral dose of 10 mg/kg bw sterigmatocystin for 10 days. The combined bile (8.7 mL) was diluted with distilled water and equilibrated with an equal volume of chloroform. Radioactivity was monitored on both phases. The major portion of the dose (~70%) was excreted unchanged via the faeces (Steyn & Thiel, 1976). During the 40 hours before termination, the urine of the treated vervet monkey was collected. Four other male vervet monkeys received orally unlabelled sterigmatocystin at a dose of 14 mg/kg bw. Their urine was also

collected for 40 hours. To obtain sufficient urinary metabolites of sterigmatocystin, the same vervet monkeys were used repeatedly at intervals longer than 1 week. In this way, a total of 720 mg sterigmatocystin was administered to the four monkeys. A glucuronic acid conjugate was identified in the urine and in the bile. Total absorption could be as high as 85% based on excretion in the faeces of about 70% of the administered radioactivity and in the urine of about 15% of the administered radioactivity (Thiel & Steyn, 1973; Steyn & Thiel, 1976).

After intraperitoneal injection into Fischer rats (100–150 g; n = 4 per group; 1 rat for each dose served as the control) of 1, 4, 8 or 16 mg/kg bw of sterigmatocystin extracted from *A. versicolor* (purity unknown) and dissolved in dimethyl sulfoxide (DMSO), 3.1% of the administered dose was quantified in the urine within 24 hours after administration, using indirect enzyme-linked immunosorbent assay (ELISA) that could detect both sterigmatocystin glucuronide and DNA adducts (Olson & Chu, 1993a).

2.1.2 Biotransformation

(a) In vivo

Three in vivo studies provide some information on phase II metabolites of sterigmatocystin, identifying a glucuronide conjugate as the major or the only metabolite in bile and urine.

In vervet monkeys orally administered ^{14}C-labelled sterigmatocystin, the glucuronide conjugate was the major metabolite identified in the urine and the bile. No evidence was found to suggest that any other conjugates (e.g. sulfates) of sterigmatocystin were present in the bile. This result was confirmed by a treatment of the metabolite with β-glucuronidase. Unlike AFB_1, which undergoes *O*-demethylation before conjugation with glucuronic acid, sterigmatocystin already contains a free phenolic hydroxyl group (Thiel & Steyn, 1973; Steyn & Thiel, 1976).

The glucuronide was the only metabolite identified (by high-performance liquid chromatography [HPLC]) in the urine of Fischer rats (100–150 g) receiving sterigmatocystin (extracted from *A. versicolor*; purity unknown) by intraperitoneal injection (4, 8 or 16 mg/kg bw; three rats per group). This result was confirmed by treatment of the metabolite with β-glucuronidase. Sulfatase hydrolysis resulted in no change (Olson & Chu, 1993b).

(b) In vitro

According to Essigmann et al. (1979, 1980), the formation of reactive epoxide could explain DNA adducts observed both by incubation of sterigmatocystin (origin and purity not specified) with DNA under cell-free conditions in the presence of rat liver microsomes and by perfusion of isolated rat liver. From

the chemical structure and stereochemistry of this adduct, the authors deduced that an *exo*-sterigmatocystin-1,2-oxide, that is, an epoxide of sterigmatocystin with the epoxide ring in *trans* position to the bulky rest of the molecule, was the metabolite that reacted with DNA. The quantitative yield of adduct indicated that this metabolite was a major product of the in vitro metabolism of sterigmatocystin (Essigmann et al., 1979, 1980).

Sterigmatocystin-1,2-oxide can be chemically synthesized, as shown by a team of researchers. After incubation of sterigmatocystin with human liver microsomes, the glutathione conjugate of sterigmatocystin-1,2-oxide was detected (Baertschi et al., 1989; Raney et al., 1992).

Cabaret et al. (2010, 2011) studied the in vitro metabolism of sterigmatocystin incubated with recombinant cytochrome P450 enzymes (CYP1A1, 1A2, 2A6, 2A13 and 3A4) or in primary culture of porcine tracheal epithelial cells (PTEC). Sterigmatocystin was uniformly enriched with ^{13}C to confirm the relationship between the parent toxin and the metabolites. Human recombinant P450 enzymes were designed to identify the metabolites resulting from P450 action. Only CYP1A1 and 3A4 formed metabolites. CYP1A1 led to the formation of two oxidized metabolites identified by the authors as monohydroxysterigmatocystin (M1) and dihydroxysterigmatocystin (M2). In the presence of cytosolic glutathione and glutathione *S*-transferase, a glutathione adduct (M3) was observed. The authors suggested the formation of a transient reactive epoxide of sterigmatocystin, as suggested by Essigmann et al. (1979). In PTEC, only one metabolite was observed, identified as a glucurono-conjugate (M4). Cabaret et al. (2010, 2011) concluded that sterigmatocystin is mainly detoxified in respiratory cells through glucuronidation and is unable to produce significant amounts of reactive epoxide metabolites. In PTEC treated with β-naphthoflavone (a CYP inducer) prior to sterigmatocystin incubation, two other products were detected, a sulfo-conjugate (M5) and a glucurono-conjugate (M6) of monohydroxysterigmatocystin. The authors of the study commented that these in vitro results obtained with porcine epithelial cells would need to be confirmed in human epithelial cells (Cabaret et al., 2010, 2011).

Krol (2011) expressed some concerns about the identification of the three metabolites by Cabaret et al. (2010, 2011) as their structures were only identified through electrospray ionization mass spectrometry (ESI-MS) data. According to Krol (2011), a number of more plausible structures could account for the observed results. He proposed, as an example, that monohydroxysterigmatocystin (M1) could be a CYP1A1-mediated product of the hydroxylation of the parent compound to either a catechol or a *para*-hydroquinone. Further aromatic hydroxylation on either ring would result in a metabolite consistent with dihydroxysterigmatocystin (M2). Oxidation of M1 to a quinone (either an *ortho*-quinone or a *para*-quinone) followed by glutathione conjugation would yield a

glutathione adduct on the aromatic ring (M3). Krol (2011) concluded that in order to determine the exact structure of the metabolites, a rigorous structural analysis would need to be performed.

Pfeiffer, Fleck & Metzler (2014) provided data in favour of the Krol (2011) proposal. They identified the catechol 9-hydroxysterigmatocystin as the major metabolite formed by human and rat hepatic microsomes, via hydroxylation of the aromatic ring. No sterigmatocystin-1,2-oxide and only small amounts of sterigmatocystin-1,2-dihydrodiol were detected in microsomal incubations, suggesting that epoxidation is a minor pathway compared to catechol formation. Catechol formation was also much more pronounced than furofuran epoxidation in the microsomal metabolism of 11-methoxysterigmatocystin (MSTC). In support of this preference for catechol formation, only trace amounts of the thiol adduct of the 1,2-oxides but large amounts of the thiol adducts of the 9-hydroxy-8,9-quinones were obtained when *N*-acetyl-L-cysteine was added to the microsomal incubations of sterigmatocystin and MSTC. In addition to hydroxylation at C-9, smaller amounts of 12c-hydroxylated, 9,12c-dihydroxylated and 9,11-dihydroxylated metabolites were formed. This study suggests that hydroxylation of the aromatic ring, yielding a catechol, represents a major and novel pathway in the oxidative metabolism of sterigmatocystin and MSTC, which may contribute to the toxic and genotoxic effects of these mycotoxins. Hydroxylation of sterigmatocystin at position 12c is analogous to the hydroxylation of AFB_1 at position 9a. The catechols 9-hydroxysterigmatocystin and 9-hydroxy-MSTC, upon further oxidation to *ortho*-quinones, form adducts with thiols such as *N*-acetyl-L-cysteine (Pfeiffer, Fleck & Metzler, 2014).

Evidence for the ability of various CYP isoforms to produce reactive metabolites of sterigmatocystin comes from studies using genotoxicity assays. For example, several CYPs have been tested for their activation potential to form DNA-damaging compounds in two *Salmonella typhimurium* strains (SOS response). Results showed an activation of sterigmatocystin by human CYP3A4, 1A1 and 1A2 but not by rat CYP1A1 and 1A2, nor by human CYP1B1 (Shimada et al., 1992, 1996a). The activation was more efficient by human CYP3A4 than by CYP3A5 (Yamazaki et al., 1995) and by adult liver microsomes than by fetal liver microsomes or adult lung microsomes (Shimada et al., 1996b). In genetically engineered immortal V79 Chinese hamster cell lines expressing rat liver CYP1A1, 1A2 and 2B1, sterigmatocystin was activated and induced micronuclei, whereas no micronuclei were observed in normal cells in the absence of S9 (Ellard & Parry, 1993). In the yeast *Saccharomyces cerevisiae* expressing rat CYP2B1 exposed to sterigmatocystin, Black et al. (1992) observed a dose-dependent increase in the mutation frequency, whereas in the control strain (not expressing CYP2B1) no increase was observed.

Chinese hamster lung cells expressing human fetus–specific CYP3A7 or adult CYP3A4 and exposed to sterigmatocystin showed 10 times higher cytotoxicity than control cells not expressing CYP3A7 or CYP3A4 (Hashimoto et al., 1995).

Further work of Cabaret et al. (2014) was on 5-methoxysterigmatocystin, using as before human recombinant CYP450s and PTEC primary cultures. CYP1A1 metabolized 5-methoxysterigmatocystin into hydroxy-*nor*-methoxysterigmatocystin, *nor*-methoxysterigmatocystin and dihydroxy-methoxysterigmatocystin. CYP1A2 led to monohydroxymethoxysterigmatocystin. The presence of glutathione did not lead to any detectable metabolite. In PTEC, 5-methoxysterigmatocystin metabolism resulted in a glucurono-conjugate of 5-methoxysterigmatocystin, a sulfo-conjugate and a glucurono-conjugate of monohydroxymethoxysterigmatocystin. The authors concluded that 5-methoxysterigmatocystin is mainly detoxified in respiratory cells through conjugation. However, while the formation of a reactive epoxide intermediate was suggested for sterigmatocystin in recombinant CYP450s, this was not the case for 5-methoxysterigmatocystin.

2.1.3 Effects on enzymes and other biochemical parameters

The effects of sterigmatocystin on liver cytochrome P450-dependent monooxygenases, on the production of reactive oxygen species and on lipid peroxidation were studied in male Wistar rats exposed for 30 days to a diet contaminated with *A. versicolor*. Fresh bread was inoculated with the fungus and allowed to grow for 21 days at 29 °C. The bread was then treated with chloroform to kill the organism, dried, powdered and mixed with commercial animal feed. The estimated dose of sterigmatocystin was 0.2 mg/kg bw per day. The feed was given to a group of six rats weighing 70–75 g at the beginning of the study. An additional group of six rats received a normal diet.

In the liver, the treatment significantly decreased the levels of reduced glutathione, ascorbic acid, α-tocopherol and thiol status. Catalase activity was decreased whereas superoxide dismutase and glutathione peroxidase activities were increased. Higher formation of hydroxyl radical and hydrogen peroxide was observed. In addition, the levels of cytochrome P450, cytochrome b_5, cytochrome b_5 reductase and cytochrome *c* reductase were increased. The markers of lipid peroxidation (levels of thiobarbituric acid reactive substances, conjugated dienes and lipid hydroperoxides) were elevated. The levels of serum marker enzymes of liver damage (γ-glutamyltransferase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and ornithine carbamoyltransferase) were elevated. Overall results suggest that generation

of free radicals induced depletion of antioxidants. This led to enhanced lipid peroxidation (Sivakumar et al., 2001).

2.1.4 Physiologically based pharmacokinetic modelling

No data were available.

2.1.5 Transfer of sterigmatocystin into human food

A. versicolor, the mould species known to produce sterigmatocystin, is normally found in cheese, particularly hard cheese, which may become infected with the mould during production and storage. Van Egmond et al. (1978) investigated potential carry-over of sterigmatocystin in two cows fed with approximately 5–10 mg sterigmatocystin per day for 2 weeks and found none in milk (limit of detection [LOD] of 1 µg/kg milk). Using thin-layer chromatography (TLC) and based on the LOD, the authors calculated that less than 0.4% of sterigmatocystin was transferred into milk.

Lafont, Siriwardana & Lafont (1979) conducted a study in France on the occurrence of sterigmatocystin in 235 cheese samples and detected the toxin at 45, 125 and 330 µg/kg only in the outer 2 cm layer of three hard cheeses.

In the Netherlands, sterigmatocystin was detected in the outer layer (1–2 cm) of mouldy cheese at a concentration up to 9000 µg/kg (Northolt et al., 1980; Northolt & van Egmond, 1982). Van Egmond, Northolt & Paulsch (1982) studied the distribution and stability of sterigmatocystin over surface rind layers of Gouda cheeses at different maturation stages and temperatures and found that the levels of sterigmatocystin decrease rapidly from the outer to the inner layers. Other studies showed that distribution of sterigmatocystin in cheese inoculated with *A. versicolor* and *A. nidulans* was within 8 mm of the surface (Engel & Teuber, 1980).

Bartos & Matyas (1982) examined 66 hard cheese samples from local retail shops in Czechoslovakia and found that two samples of Edam cheese contained sterigmatocystin at 7.5 µg/kg and 17.5 µg/kg and one sample of Moravian Block cheese contained sterigmatocystin at 7.5 µg/kg. Abd Alla et al. (1996) and Metwally et al. (1997) used TLC to analyse 100 cheese samples collected from local markets in Egypt and found that 35% contained sterigmatocystin at between 10 and 63 µg/kg. The reports do not indicate that milk was contaminated with the sterigmatocystin. Other studies in Germany (Nowotny et al., 1983), South Africa (Luck et al., 1976), Spain (Lopez-Diaz et al., 1996) and the United Kingdom (MAFF, 1998) found no sterigmatocystin in cheese samples.

Veršilovskis, Van Peteghem & De Saeger (2009) used liquid chromatography with tandem mass spectrometry (LC-MS/MS) with an LOD of 0.03 µg/kg and a limit of quantification (LOQ) of 0.1 µg/kg to analyse eight Latvian and 13 Belgian cheese samples. Two Belgian cheeses were found to have

sterigmatocystin at a concentration of 0.52 and 1.23 µg/kg. Sterigmatocystin also was detected in four of the Latvian samples at above the LOD but below the LOQ.

With little published information on occurrence of sterigmatocystin in milk and/or animal products such as meat and eggs as a result of feed contamination, the EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that there were insufficient data to assess the rate of carry-over of sterigmatocystin into milk (EFSA, 2013).

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Median lethal dose (LD_{50}) studies

Median lethal dose (LD_{50}) values of sterigmatocystin are shown in Table 1.

Large differences in the acute oral toxicity of sterigmatocystin in different strains of rats have been described in the literature, but the details were not available to the Committee (cited in Ohtsubo, Saito & Kimura, 1978).

(b) Oral toxicity

The main histological findings in Wistar-derived male and female rats after a single administration of sterigmatocystin were necrosis of the liver and kidneys. The site of necrosis in the liver differed according to the route of administration, that is, necrosis was centrilobular following oral dosing versus periportal after intraperitoneal injection. Moreover, when the wheat germ oil vehicle was used, an extensive zone of fatty change surrounded the areas of necrosis.

Rats receiving sterigmatocystin (extracted from maize contaminated by *Bipolaris* sp.; 95% pure; dissolved in dimethylformamide or wheat germ oil) at 100–150 mg/kg bw orally showed slight degenerative changes in the liver and, in some cases, single-cell necrosis. At higher doses (208 or 300 mg/kg bw) extensive centrilobular necrosis was present with haemorrhage in the necrotic area. In some livers, the major portion of all lobules was necrotic. In the kidney, lesions increased in severity with increase in the dose of toxin, irrespective of the solvent. At doses between 10 and 100 mg/kg bw, sterigmatocystin caused cortical haemorrhage, hyaline casts in the collecting tubules and pyknotic nuclei in the tubular cells at the corticomedullary junctions. There was also hyaline degeneration or necrosis of tubular epithelial cells. At higher doses (100–144 mg/kg bw), lesions were accompanied by degeneration and necrosis of glomeruli with hyaline thickening of the basement membrane. Massive haemorrhage and severe necrosis of the renal tubules and glomeruli occurred at high doses (144 and 300

Table 1
Summary of oral or intraperitoneal acute toxicity for sterigmatocystin

Animal	Sex	Solvent	Route	LD_{50} (mg/kg bw)	95% CI	Reference
Rat	M	Dimethylformamide	p.o.	166	113–224	Purchase & van der Watt (1969)[a]
Rat	F	Wheat germ oil	p.o.	120	92–155	Purchase & van der Watt (1969)[a]
Rat	M	Dimethylformamide	i.p.	60	46–77	Purchase & van der Watt (1969)[a]
Rat	M	Wheat germ oil	i.p.	65	37–109	Purchase & van der Watt (1969)[a]
Vervet monkey	M	DMSO	i.p.	32	15–70	van der Watt & Purchase (1970a)

bw: body weight; CI: confidence interval; DMSO: dimethyl sulfoxide; i.p.: intraperitoneal; LD_{50}: median lethal dose; p.o.: oral
[a] Purchase & van der Watt (1969) extracted sterigmatocystin from maize contaminated by *Bipolaris* sp. (95% pure) and dissolved in dimethylformamide or wheat germ oil. Each dose was tested in a group of four Wistar-derived male or female rats (100–150 g bw). The authors highlighted that the LD_{50} values may not be as accurate as the confidence limits suggest due to the poor solubility of sterigmatocystin in the solvents used; the LD_{50} values may reflect the combined effects of toxin and solvent.
[b] The sterigmatocystin used by van der Watt & Purchase (1970a) was produced in the laboratory (80% pure) and dissolved in DMSO. Each dose of sterigmatocystin was tested in two monkeys by i.p. injection and two control monkeys received DMSO only.

mg/kg bw), but marked degenerative change with necrosis was also evident at lower doses (10–100 mg/kg bw) (Purchase & van der Watt, 1969).

(c) Non-oral routes of administration

Rats administered sterigmatocystin extracted from maize contaminated by *Bipolaris* sp. (95% pure) and dissolved in dimethylformamide or wheat germ oil via intraperitoneal injection (30–200 mg/kg bw) were seen to develop lesions in liver and kidney similar to those observed in rats treated orally (Purchase & van der Watt, 1969).

Vervet monkeys were given an intraperitoneal injection of sterigmatocystin (80% pure; dissolved in DMSO) at doses of 0, 15, 32, 70 or 150 mg/kg bw (two animals/dose compared to two controls receiving DMSO only). Necropsy of all animals (those that died before study end and those killed at the end of the 10-day study period) confirmed the liver and the kidney as the target organs. Dose-related severity of the lesions was observed. At 15 mg/kg bw, the liver was enlarged with small foci of necrosis. Icterus was observed at 70 mg/kg bw and above with large areas of haemorrhagic necrosis. Early bile duct proliferation was observed at 32 mg/kg bw and was more pronounced at 70 mg/kg bw. In the kidney, lesions included degenerating glomeruli with oedema, fatty changes and necrosis of the tubular epithelium (van der Watt & Purchase, 1970a).

Fujii et al. (1976) tested the toxicity of sterigmatocystin by subcutaneous administration in newborn (BALB/c × DBA/2)F1 mice. The animals received within 24 hours of birth a single subcutaneous injection of a 1% gelatine suspension of sterigmatocystin (extracted from *A. versicolor*; 99.9% pure) at 0.5, 1, 5, 10, 50 or 100 mg/kg bw. There were between four and 11 animals per dose

(39 in total from nine dams). At doses higher than 10 mg/kg bw all the mice died within 5 days of treatment. There were no deaths at doses of 0.5 and 1 mg/kg bw.

Acute toxicity of sterigmatocystin by intratracheal instillation (exposure from indoor environments) was investigated in 21- to 28-day-old male mice by Miller et al. (2010). Inflammation of the lung and mucus production were observed 12 hours after intratracheal instillation with a single dose of 4×10^{-5} mol (138.4 µg) of commercial sterigmatocystin per kg of fresh lung, a dose that the study authors considered comparable to possible human exposure (Miller et al., 2010).

(d) Acute toxicity in domestic animals

According to EFSA (2013), acute oral and intraperitoneal toxicity studies in poultry confirmed the liver and kidney as the main target organs for toxicity (Salam & Shanmugasundaram, 1983; Sreemannarayana, Frohlich & Marquardt, 1986, 1987, 1988; Sreemannarayana et al., 1986; Sayed, 1993; cited in EFSA, 2013).

2.2.2 Short-term studies of toxicity

(a) Mice

No data were available.

(b) Rats

The sequence of histopathological changes in the liver of rats after 2–16 weeks of exposure to sterigmatocystin was investigated by van der Watt & Purchase (1970b). Male weanling Wistar rats from the laboratory colony were exposed to sterigmatocystin (extracted from maize contaminated by *Bipolaris* sp.; 95% pure) at 100 mg/kg feed (equivalent to 5–10 mg/kg bw per day using the default factor of 0.1 for young rats and 0.05 for adult rats [WHO, 2016]). At the beginning of the experiment, the rats weighed 50 g. A control group received a standard diet. Each group consisted of 16 rats. Two rats of each group were killed every 2 weeks. The first results after only 2 weeks of exposure showed a diffuse single-cell necrosis and few necrotic foci without inflammation. The Kupffer cells were prominent, and the hepatocytes exhibited marked variations in the size of both nuclei and cells. After 4 weeks of exposure, in addition to the liver changes, there was a distinct disruption of the normal hepatic lobular arrangement. After 6 weeks, the livers showed periportal necrosis with diffuse single-cell necrosis of the remaining parenchymal cells. A few periportal necrotic foci were accompanied by focal infiltrations of round cells. At 8 weeks, necrosis was more diffuse, in both the central and periportal areas. At this stage, disruption of the lobular arrangement was complete and accompanied by hepatocellular necrosis, marked nuclear pleiomorphism, proliferation of bile duct epithelial cells and round-cell infiltration commencing in the portal tracts and extending between the

hepatocytes. Small hyperplastic areas could be observed throughout the disrupted parenchymal tissue. At 12 weeks of exposure, macroscopic examination showed hyperplastic foci containing fat and glycogen-laden hepatocytes with numerous double nuclei and mitotic figures. Finally, at 16 weeks the large hyperplastic nodules were surrounded by degenerating and necrotic hepatocytes. The extent of the bile duct epithelial proliferation did not progress beyond that seen after 8 weeks of exposure (van der Watt & Purchase, 1970b).

In a study of male Wistar rats exposed for 30 days to a diet contaminated with *A. versicolor* at an estimated dose of sterigmatocystin at 0.2 mg/kg bw per day (see section 2.1.3 for details), histological examination showed degenerated parenchymal hepatocytes, necrosis and Kupffer cell proliferation (Sivakumar et al., 2001).

(c) Guinea-pigs

Effects of sterigmatocystin extracted from *A. versicolor* (purity unknown) were studied in guinea-pigs after 2 weeks of daily exposure at 4.2 mg/animal (Richard et al., 1978). The toxin was given orally in capsules. At the beginning of the experiment, the animals weighed 475–520 g. A control group received no treatment. Each group consisted of 10 animals. However, two animals treated with sterigmatocystin alone died before the end of the experiment. The most severe changes observed in guinea-pigs given sterigmatocystin were diffuse fatty degeneration of hepatocytes and focal necrosis without any particular lobular distribution. A proliferation of Kupffer cells and an infiltration by neutrophils were apparent in the necrotic foci. Mild to moderate bile duct hyperplasia was present in five guinea-pigs. In two guinea-pigs, this was accompanied by changes typical of fatty degeneration and single-cell necrosis. These changes were not seen in the five other treated guinea-pigs. No effects on total serum protein were observed but sterigmatocystin significantly reduced complement activity (α_2-globulin) in guinea-pig serum. No effects were observed on kidneys. Weight loss was significant.

(d) Monkeys

In a study in vervet monkeys given sterigmatocystin (extracted from maize contaminated by *Bipolaris* sp.; 95% pure) intragastrically at a dose of 20 mg/kg bw every 14 days for 12 months (number of animals not reported), liver biopsies were performed at 9-week intervals for histological examination (no further details available). After 4–6 months, chronic hepatitis was observed. Continued treatment resulted in aggressive hepatitis with single-cell necrosis of hepatocytes, progressive growth of the fibrous septa and disruption of the lobular architecture. After 12 months of exposure, large hyperplastic nodules, containing hepatocytes with pleomorphic nuclei, were observed (Purchase & van der Watt, 1970a).

(e) Domestic animals

The following studies were reviewed by EFSA (2013).

Exposure of male Warren chicks to sterigmatocystin at concentrations of 1.6 or 0.8 g/kg feed resulted in 60% mortality within 8 days of feeding. The surviving chicks were fed a control diet for 7 weeks. After this period, grey spots on the liver surface, an increase in the kidney mass, elevated serum alkaline phosphatase and decreased triglycerides were observed. Another group of chickens received a diet with an increasing concentration of sterigmatocystin from 20 to 50 mg/kg feed over 7 weeks. After this period, severe liver cirrhosis and fatty degeneration were observed. In addition, cellular necrosis and intercellular inflammation were diagnosed, and body-weight gains and feed conversion rates were reduced in comparison with the control group. Serum biochemical parameters were altered. Aspartate aminotransferase, γ-glutamyltransferase and creatine kinase activities were increased. Total protein, triglycerides and cholesterol were reduced. However, alanine aminotransferase, lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, urea, creatinine and uric acid were not affected. Haematological findings included symptoms of anaemia (Sayed, 1993).

In sheep, no adverse health effects were observed in females exposed to a diet with increasing concentrations of sterigmatocystin from 2 to 16 mg/kg feed over 100 weeks and observed until 6 months after the last exposure (Bohm & Sayed, 1994).

In pigs, exposure to sterigmatocystin at 30 μg/kg feed (duration not specified) led to decreased feed intake, depression, incidental diarrhoea and alteration in blood biochemical parameters such as transaminase values (alanine aminotransferase and aspartate aminotransferase), γ-glutamyltransferase and alkaline phosphatase as well as increased concentrations of bilirubin and urea. Postmortem analysis showed severe alterations of the liver tissue with multiple, often large, necrotic spots (Kovalenko et al., 2011).

In dairy cattle, a dietary exposure of approximately 5–10 mg of sterigmatocystin per animal per day for 2 weeks (only two dairy cows in the exposed group and one control) induced no adverse health effects (van Egmond et al., 1978).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Oral administration

Mice

Kempff, Pitout & van der Watt (1973) studied the activities of nucleic acid and alkaline deoxyribonucleases in the liver of mice exposed to sterigmatocystin for 371 days (53 weeks). The experimental animals were random-bred Onderstepoort Albino male mice from the researchers' colony. Mice were exposed daily to

sterigmatocystin (purity unknown) in the diet at 0.2 mg per mouse (no details provided on the dose estimate). Groups of animals were killed at predetermined intervals (not specified); each group consisted of four treated animals and four controls. Results from the eight groups of mice killed during the 371-day period indicated that long-term exposure to sterigmatocystin had virtually no effect on the specific activities of mouse liver nucleic acid and alkaline deoxyribonucleases. No histological changes were observed in the livers (Kempff, Pitout & van der Watt, 1973).

Zwicker, Carlton & Tuite (1974) investigated the long-term toxic effects and carcinogenicity in ICR white Swiss mice of commercial sterigmatocystin considered as pure (S-p), of sterigmatocystin administered in the form of a rice culture of *A. versicolor* (S-rc) and of a rice culture of *Penicillium viridicatum* (P-rc).

Weanling mice (3 weeks old) were divided into six groups and fed sterigmatocystin at 5 mg/kg feed (equivalent to 0.75 mg/kg bw per day using the default factor of 0.15 [WHO, 2016]) and/or a 7.5% content of a rice culture of *P. viridicatum* (P-rc) for 2-week periods alternating with 2-week periods on the control diet, for a total of 54–58 weeks. This feeding schedule was adopted because previous studies had shown that mice would not survive continuous feeding of a 7.5% *P. viridicatum* diet. A control group was fed only the base diet. The total number of mice per treatment was not the same in all the groups; neither were there equal numbers of males and females due to errors in sexing the mice at weaning. The study design is shown in Table 2.

The incidence of pulmonary tumours in mice in S-p and S-rc feed groups is shown in Table 3. There was no difference in mortality and incidence of pulmonary neoplasms between male and female mice. The only other neoplasms observed were malignant lymphoma in one control male, one control female and one female fed S-rc. In most of the mice, there were multiple pulmonary tumours classified as adenomas or adenocarcinomas arising from the bronchial and alveolar epithelium (Zwicker, Carlton & Tuite, 1974).

Enomoto et al. (1982) exposed female BDF1 mice to sterigmatocystin at a dietary concentration of 0, 30 or 120 mg/kg feed (equivalent to 0, 4.5 and 18 mg/kg bw per day using the default factor of 0.15 [WHO, 2016]) for 55 or 40 + 11 weeks. However, the authors noted that the feed intake in the high-dose group was reduced and the body weight was about 20% less than the controls after 40 weeks. The sterigmatocystin was isolated from *A. versicolor* (purity mentioned as checked but not specified). The mice were 5 weeks old at the beginning of the experiment and were divided into three groups. The control group (n = 50) was fed the base diet; the low-dose group (n = 55) was fed a diet containing sterigmatocystin at 30 mg/kg feed for 55 weeks; and the high-dose group (n = 55) was fed a diet containing sterigmatocystin at 120 mg/kg feed for 40 weeks, then a

Table 2
Experimental design of the study conducted by Zwicker, Carlton & Tuite (1974)

Group	No. of mice		Sterigmatocystin (mg/kg diet)		
	Males	Females	Culture (S-rc)[a]	Commercial (S-p)[b]	P-rc[c] (% in diet)
1 (control)	19	21	0	0	0
2	42	47	0	0	7.5
3	18	10	0	5	0
4	16	16	0	5	7.5
5	34	35	5	0	0
6	33	46	5	0	7.5

no.: number
[a] Sterigmatocystin administered in the form of a rice culture of *A. versicolor*.
[b] Commercial sterigmatocystin considered to be pure.
[c] Rice culture of *Penicillium viridicatum*.

Table 3
Incidence[a] of pulmonary tumours in male and female ICR white Swiss mice exposed to sterigmatocystin in the diet

Group	Incidence of pulmonary tumours[a]						Total incidence
	Adenomatosis		Adenoma		Adenocarcinoma		
	Males	Females	Males	Females	Males	Females	
1 (control)	3/16	1/21	2/16	2/21	0/16	0/21	4/37 (11%)
3 (S-p)[b]	10/15	6/10	12/15	9/10	1/15	8/10	21/25 (84%)
5 (S-rc)[c]	16/22	18/33	14/22	19/33	2/22	1/33	33/55 (60%)

[a] Incidence expressed as number of mice with pulmonary tumour / number of mice examined and, in parentheses, the number of mice with the finding as a percentage of the number of mice examined.
[b] Commercial sterigmatocystin, considered to be pure, at 5 mg/kg feed (equivalent to 0.75 mg/kg bw per day).
[c] Sterigmatocystin at 5 mg/kg feed (equivalent to 0.75 mg/kg bw per day) administered in the form of a rice culture of *A. versicolor*.
Source: Zwicker, Carlton & Tuite (1974)

control diet for 4 weeks (to compensate for the poor intake of the contaminated feed) and then again the treatment diet with 120 mg/kg feed for another 11 weeks. Five mice of each group were killed at week 43, 10 animals at week 68 and the remaining survivors at week 73.

The incidence of hepatic tumours and angiosarcoma of brown fat after week 43 is shown in Table 4. No tumours were found in any of the mice killed at week 43. No vascular changes were observed in control mice except for peliosis-like lesions in the spleens of two mice. Two tumours were observed in the control group: one leiomyosarcoma of the uterus and one leiomyoma of the uterus. Other tumours observed in the high-dose group included lung adenoma (12), angiosarcoma of the ovary (three), sarcoma of the uterus (one), leiomyoma of the uterus (one) and squamous cell carcinoma of the auditory gland duct (one). In

Table 4
Incidence of tumours in female BDF1 mice exposed to sterigmatocystin in the diet

Dietary concentration of sterigmatocystin	Time of kill (week)	No. killed	Incidence (*n*)[a]				
			Hepatocellular adenoma	Hepatocellular carcinoma	Hepatic haemangio-endothelioma	Hepatic angiosarcoma	Angiosarcoma of brown fat
0 mg/kg feed	43	5	0	0	0	0	0
	58[b]	1	0	0	0	0	0
	68	10	0	0	0	0	0
	73	34	0	0	0	0	0
	Total	–	0	0	0	0	0
30 mg/kg feed (equivalent to 4.5 mg/kg bw per day)	43	5	0	0	0	0	0
	68	10	1	0	8	5	1
	43–68[c]	21[d]	0	0	0	19	1
	73	17	0	1	6	10	4
	Total[e]	–	1/53	1/53	14/53	34/53	6/53
120 mg/kg feed (equivalent to 18 mg/kg bw per day)	11[b]	1	0	0	0	0	0
	43	5	0	0	0	0	0
	68	10	0	0	NR	0	2
	61–68[c]	23[f]	0	0	2	0	17
	73	12	2	0	2	0	8
	Total[e]	–	2/51	0/51	4/51	0/51	27/51

bw: body weight; no.: number; NR: not reported
[a] Incidence expressed as number of mice with the tumour.
[b] Died at time indicated.
[c] Died or killed when moribund at time indicated.
[d] Two of the 23 mice killed were not examined because of cannibalism or autolysis.
[e] Results expressed as number of mice with the tumour / number of mice examined.
[f] Four of the 27 mice killed were not examined because of cannibalism or autolysis.
Source: Enomoto et al. (1982)

the low-dose group, other tumours included lung adenoma (five), angiosarcoma of the lung (one), angiosarcoma of the ovary (one), papilloma of the oral cavity (one), leiomyosarcoma of the uterus (one) and leukaemia (one) (Enomoto et al., 1982).

The Committee noted that the results of this study show that sterigmatocystin has two targets for carcinogenicity: the hepatocytes (hepatocellular adenoma and carcinoma) and the blood vessels (hepatic haemangioendothelioma, hepatic angiosarcoma, angiosarcoma of brown fat).

Rats

In the Purchase & van der Watt (1970b) study, rats were exposed to sterigmatocystin in the diet or by gavage for 52 weeks. The sterigmatocystin (purity ~88%) was extracted from *Bipolaris* spp. in maize meal. The toxin was dissolved in chloroform

and added to a portion (~5%) of the diet. The chloroform was removed in a forced draught oven at 50 °C. This remaining portion of the diet was then mixed with stock diet to give the required sterigmatocystin content. Similarly, for the gavage portion of the study, sterigmatocystin was dissolved in chloroform and added to a weighed amount of sunflower seed oil. The chloroform was removed under vacuum until the weight of the flask was within 0.1% of the expected weight. The final traces of chloroform were removed by bubbling nitrogen through the oil standing in a boiling water bath.

Wistar-derived weanling rats were divided into eight groups each consisting of five males and five females. The animals were caged separately. Groups 1, 2 and 3 received ad libitum a diet containing sterigmatocystin at 10, 20 or 100 mg/kg feed (equivalent to 0.5, 1 and 5 mg/kg bw per day using the default factor of 0.05 [WHO, 2016]) for the first 6 months. The authors calculated a daily intake of 0.15, 0.3 and 1.5 mg/rat on the assumption of a food intake of 15 g/day. These dietary concentrations were increased to 15, 30 or 150 mg/kg for the second 6 months (corresponding to 0.75, 1.5 and 7.5 mg/kg bw per day). The rats were then placed on a stock ration until termination at 123 weeks.

Groups 4, 5 and 6 received by gastric intubation doses of 0.15, 0.3 or 1.5 mg/rat of sterigmatocystin dissolved in 0.5 mL sunflower seed oil 5 days a week for 52 weeks.

Groups 7 and 8 were control groups; group 7 received 0.5 mL sunflower seed oil on 5 days/week for 52 weeks and group 8 received no treatment.

In the high-dose group, 7/10 rats died between weeks 5 and 18. At week 42, 50/60 rats exposed to sterigmatocystin in the diet or by gavage survived, and 39/50 rats developed hepatocellular carcinoma. The incidence of hepatic tumours is shown in Table 5. Eight tumours of other types were seen in various organs (liver, uterus, ovary, spleen and omentum) and acanthotic changes occurred in the stomachs of 85% of the treated rats. No tumours were observed in the two control groups (Purchase & van der Watt, 1970b).

Kempff, Pitout & van der Watt (1973) studied the activity of nucleic acid and alkaline deoxyribonucleases in the liver of rats exposed to sterigmatocystin for 335 days (48 weeks). The experimental animals were random-bred male Wistar-derived Albino rats from their colony and had a mean weight of 100 g at the beginning of the experiment (~4 weeks old). The rats received sterigmatocystin (purity unknown) by gavage (20 mg/kg bw) once a week, except between days 140 and 170. Controls received the same volume of DMSO only. Two treated and two control animals were killed by decapitation at 3-week intervals. The specific activity of nucleic acid deoxyribonuclease started to increase after 80 days of exposure, reached a peak at day 230 when hyperplastic nodules were first detected macroscopically and then decreased close to normal (control) values when neoplasia commenced (from day 300). At day 335, nodular hyperplasia

Table 5
Incidence of hepatic tumours in male and female Wistar-derived rats exposed to sterigmatocystin in the diet or by gavage

Dose of sterigmatocystin	No. of rats at study start	No. of rats at week 42	Incidence (*n*)[a]	
			Hyperplastic nodular liver[b]	Hepatocellular carcinoma
Control (no treatment)	5 M + 5 F	9	0	0
Control (sunflower seed oil by gavage)	5 M + 5 F	10	0	0
0.15 mg/rat by gavage	5 M + 5 F	10	2	4
0.3 mg/rat by gavage	5 M + 5 F	9	2	5
1.5 mg/rat by gavage	5 M + 5 F	9	0	9
10 or 15 mg/kg feed[c]	5 M + 5 F	9	1	8
20 or 30 mg/kg feed[c]	5 M + 5 F	10	0	10
100 or 150 mg/kg feed[c]	5 M + 5 F	3	0	3

F: female; M: male; no.: number
[a] Incidence expressed as number of rats with the tumour.
[b] Hepatocellular adenoma.
[c] Dose was increased from 10, 20 or 100 mg/kg feed ad libitum for the first 6 months to 15, 30 or 150 mg/kg for the second 6 months.
Source: Purchase & van der Watt (1970b)

and foci of adenomata were observed in the liver sections of these animals. The authors did not provide any details on the numbers of animals with tumours at each sampling time (Kempff, Pitout & van der Watt, 1973).

The hepatocarcinogenicity of rice contaminated with *A. versicolor* was investigated by Ohtsubo, Saito & Kimura (1978) in male Donryu rats (6 weeks old). A strain of *A. versicolor* isolated from soybean was inoculated into minimally polished (2%) rice and cultured for 14 days at 25 °C. The rice culture was air-dried at 40–50 °C, powdered and mixed with a basal rat diet before pelleting. Two batches of rice culture, weighing 2.5 and 7 kg, contained sterigmatocystin at 300 and 175 mg/kg feed, respectively. To obtain a concentration of sterigmatocystin in the diet of 5 or 10 mg/kg feed (equivalent to 0.25 and 0.5 mg/kg bw per day using the default factor of 0.05 [WHO, 2016]), mouldy rice was added at a rate of 1.7% or 3.3% for batch 1 and a rate of 2.9% or 5.7% for batch 2. Chemical analysis of the mouldy rice for sterigmatocystin was carried out by gas–liquid chromatography (5-methoxysterigmatocystin not analysed). The authors did not provide any information on other mycotoxins in the mouldy rice. Each group (0, 5 or 10 mg/kg; n = 20/per group) was fed the diets for up to 709 days (101 weeks).

Before day 460 (week 66), about one third of the rats in the two test groups and three of the control animals died sporadically as a result of a chronic bronchopulmonary infection. Between day 465 (when the first animal with a tumour died) and day 709 (when survivors were killed), 13 rats in each test group died. Twelve of the 20 controls survived until the end of the experiment.

The incidence of hepatocellular carcinomas is shown in Table 6. There was no significant difference in tumour incidence at the two dose levels used. Tumours were, in almost all cases, multiple and associated with hyperplastic nodules without cirrhosis. There was no peritoneal dissemination in any rat, even when the tumours were relatively large and characterized by necrosis and haemorrhage. Pulmonary metastases were seen in three rats in each treatment group. The authors suggested that the Donryu strain is more resistant to sterigmatocystin than other strains (such as Wistar) and that the effects observed in this study at 5 mg/kg diet would probably occur with levels of less than 1 mg/kg in more susceptible strains (Ohtsubo, Saito & Kimura, 1978).

Terao, Aikawa & Kera (1978) investigated carcinogenesis in male Wistar rats (4 weeks old; treatment group, n = 15; control group, n = 30) exposed to sterigmatocystin (isolated from *A. versicolor* and purified; purity not specified) in the diet at a concentration of 0 and 10 mg/kg feed (equivalent to 0 and 0.5 mg/kg bw per day using the default factor of 0.05 [WHO, 2016]) for 54 weeks, followed by an observation period of 15 weeks (total period 69 weeks).

After the first 5 weeks, one animal from each group was killed and necropsied. Hepatocellular carcinomas, which were first observed after 54 weeks, were seen at study end in 8/15 (53%) exposed rats (Table 7). No metastases were found. The controls did not develop any tumours. In rats treated for between 40 and 50 weeks, multiple grey-white nodules up to 1 mm in diameter were visible on the surface of the liver. Histological examination revealed that these nodules were clear-cell foci or neoplastic nodules. In the ensuing 12 weeks, these nodules developed into hepatic carcinomas. Circumscribed, firm, grey-white neoplastic lesions (diameter 0.5–2.0 cm) were occasionally present in every liver lobe.

The authors also studied a possible combined effect of nitrosodimethylamine and sterigmatocystin on carcinogenesis in male rats fed diets containing sterigmatocystin (10 or 1 mg/kg feed) and nitrosodimethylamine (1 or 10 mg/kg feed). The number of hepatic carcinomas was increased to 15/20 in the group given sterigmatocystin at 10 mg/kg feed plus nitrosodimethylamine at 1 mg/kg feed (Terao, Aikawa & Kera, 1978).

In a review paper, Terao (1983) reported the results of another carcinogenicity study in male Wistar rats, without providing the details of the protocol. Daily doses in the diet were 15, 75 or 150 µg/day for more than 92 weeks. The number of rats with hepatocellular carcinoma was 8/22 in the low-dose group, 24/25 in the mid-dose group and 12/12 in the high-dose group (Table 8).

Table 6

Incidence[a] of hepatic tumours in male Donryu rats exposed to rice contaminated with *A. versicolor* in the diet

Dietary concentration of sterigmatocystin (mg/kg feed)	No. of rats at study start	Effective no. of rats[b]	Incidence (*n*)[a]				
				Hepatocellular carcinomas			
			Hepatic tumours	Well differentiated	With tubular pattern	Poorly differentiated	Accompanied by haemangiosarcoma
0	20	17	0	0	0	0	0
5	20	13	11	9	6	5	1
10	20	13	12	12	5	3	2

no.: number
[a] Incidence expressed as number of mice observed with tumours.
[b] Number of rats surviving at time of appearance of the first tumour.
Source: Ohtsubo, Saito & Kimura (1978)

Table 7

Incidence[a] of hepatic carcinomas in male Wistar rats exposed to sterigmatocystin in the diet

Dietary concentration of sterigmatocystin (mg/kg feed)	No. of rats at study start (*n*)	Effective no. of rats (*n*)[b]	Incidence of hepatocellular carcinoma (*n*)[a]	Mean time to necropsy (weeks)
0	30	25	0	69
10	15	14	8	60

no.: number
[a] Incidence expressed as number of mice observed with hepatic carcinomas.
[b] Number of rats surviving at time of appearance of the first tumour (at 54 weeks).
Source: Terao, Aikawa & Kera (1978)

Table 8

Incidence[a] of hepatic carcinomas in male Wistar rats exposed to sterigmatocystin in the diet

Dietary dose of sterigmatocystin (µg/rat per day)	Incidence of hepatic carcinoma[a]	Time hepatic carcinoma found
15	8/22 (36%)	86 ± 6 weeks
75	24/25 (96%)	68 ± 5 weeks
150	12/12 (100%)	58 ± 4 weeks

[a] Incidence expressed as number of rats with the finding / number of rats examined and, in parentheses, the number of rats with the finding as a percentage of the number of rats examined.
Source: Terao (1983)

Terao (1983) also reported that *O*-acetylsterigmatocystin induced hepatocarcinoma in 11/15 male Wistar rats fed a diet with *O*-acetylsterigmatocystin at 10 mg/kg for 54 weeks.

Maekawa et al. (1979) studied hepatic changes in male ACI/N rats exposed to sterigmatocystin (isolated from *A. versicolor* and purified; purity not specified) in the diet for more than 2 years at concentrations of 0, 0.1, 1 or 10 mg/kg feed (equivalent to 0, 0.005, 0.05 and 0.5 mg/kg bw per day using the default factor of 0.05 [WHO, 2016]). At study start, the rats were 11 weeks old; they were observed until 118 weeks of age for the high-dose group (n = 36), 122 weeks for the mid-dose group (n = 36), 121 weeks for the low-dose group (n = 36) and 121 weeks for the control group (n = 12).

Large variation in individual survival times was observed. Mean survival time varied from 84 weeks (mid-dose group) to 105 weeks in the control group. Multiple tumours were observed (see Table 9 for incidence and Table 10 for histological changes). There is an apparent dose-related effect for total liver tumours. However, although the authors stated that there were five liver tumours in the high-dose group (see Table 9), the breakdown of the histological types accounts only for four tumours (see Table 10). There were no significant differences in the incidences of other tumours such as testis, adrenal gland, etc., in exposed groups compared with controls. There was no dose–effect relationship on mean survival time. Central necrosis and hyperplastic foci both exhibited a clear dose-dependent relationship. Hyperplastic foci consisted of normal or larger vacuolated or eosinophilic cells, usually not demarcated from surrounding cells and without obvious disruption of the liver architecture. Maekawa et al. (1979) suggested that ACI/N rats may be more resistant to hepatocarcinogenic effects of sterigmatocystin than strains such as the Donryu rats used by Ohtsubo, Saito & Kimura (1978).

Monkeys

Thorgeirsson et al. (1994) reported the interim results of a long-term study, started in 1975, in 30 adult monkeys (species not specified) treated orally once a week with sterigmatocystin at 1.0 or 2.0 mg/kg bw (corresponding to 0.14 and 0.29 mg/kg bw per day, according to EFSA, 2013) dissolved in DMSO. The number of animals per dose was not specified and a specific control group for this experiment was not mentioned (although there was a control group with at least five monkeys treated with DMSO). As of 1993 (estimated time of exposure 18 years), 10 treated monkeys (33%; five in each dose group) developed one or more malignant tumours. (The types of tumours observed are shown in Table 11.) Some monkeys died without tumours but with diagnoses of myocarditis, sepsis, toxic hepatitis, cirrhosis, bronchopneumonia, acute renal tubular necrosis

Table 9

Tumour incidence[a] and mean survival time of male ACI/N rats exposed to sterigmatocystin in the diet

Dietary concentration of sterigmatocystin (mg/kg feed)	No. of rats at study start	Effective no. of rats[b]	Incidence (*n*)[a]					Mean survival time (weeks)
			All tumours	Liver	Testis	Adrenal gland	Others	
0	12	11	7 (64%)	0	4	1	4[c]	105 (71–121)
0.1	36	27	13 (48%)	0	5	1	8[d]	93 (75–121)
1	36	29	6 (21%)	1	2	1	3[e]	84 (58–122)
10	36	26	10 (39%)	5	4	2	5[f]	87 (75–118)

no.: number
[a] Incidence expressed as number of rats with the finding and, in parentheses, number of rats with the finding as a percentage of the number of rats examined.
[b] Number of surviving rats at study end.
[c] 1 pancreas, 1 lung, 1 salivary gland, 1 intestine.
[d] 3 pituitary gland, 2 urinary bladder, 2 skin, 1 pancreas, 1 subcutaneous tissue, 1 tongue, 1 colon.
[e] 1 skin, 1 bone marrow, 1 mesenterium.
[f] 2 colon, 1 kidney, 1 forestomach, 1 intestine, 1 subcutaneous tissue, 1 pituitary gland.
Source: Maekawa et al. (1979)

Table 10

Histological changes in liver of male ACI/N rats exposed to sterigmatocystin in the diet

Dietary concentration (mg/kg feed)	Effective no. of rats[b]	Incidence (*n*)[a]				
		Central necrosis	Hyperplastic focus or area	Hyperplastic nodule	Hepatocellular carcinoma	Haemangiosarcoma
0	11	2	1	0	0	0
0.1	27	7	4	0	0	0
1	29	14	9	0	0	1
10	26	16	21	3	1	3

[a] Results expressed as number of rats with the finding.
[b] Number of surviving rats at study end.
Source: Maekawa et al. (1979)

Table 11

Incidence of tumours in adult monkeys[a] exposed orally to sterigmatocystin

Dose per week (mg/kg bw per week)	No. of animals with hepatic tumours	Types of tumours
1.0	5	7 hepatocellular carcinomas
2.0	5	2 cholangiocarcinomas
		1 cholangiosarcoma
		1 renal cell carcinoma

bw: body weight; no.: number
[a] Species not specified; $n = 30$, although the number of animals per dose was not specified and a specific control group for this experiment was not mentioned.
Source: Thorgeirsson et al. (1994)

and necrotizing colitis. In surviving monkeys, laparoscopy showed extensive liver damage (Thorgeirsson et al., 1994).

(b) Non-oral administration

Carcinogenicity of sterigmatocystin has also been observed after intraperitoneal, subcutaneous and dermal administration in rats and mice (Dickens, Jones & Waynforth, 1966; Purchase & van der Watt, 1973; Fujii et al., 1976; Terao, 1978). Liver tumours were reported in all these studies.

Sterigmatocystin (origin and purity unknown) was injected subcutaneously as a fine suspension in arachis oil into six male rats (strain unknown; weighing initially 100 g) twice weekly for 24 weeks at a dose of 0.5 mg/rat. A control group of six rats was injected with the oil alone. The animals were observed for 65 weeks. The first tumour was observed at week 47. At the end of the 65 weeks of observation, three of the treated rats had local sarcoma. In addition, liver tumours were observed in two treated rats (one hepatocellular tumour and one cholangioma) (Dickens, Jones & Waynforth, 1966).

Purchase & van der Watt (1973) studied the carcinogenicity of sterigmatocystin by dermal administration. The shaved skin of Wistar-derived rats (80 g at the start of the study; five groups of 10 animals) was treated with 1 mg of sterigmatocystin in DMSO or in acetone twice weekly for 70 weeks. Rats in the control groups were treated with DMSO alone or acetone alone, and an additional group was left untreated. Sterigmatocystin was isolated from a culture of *Bipolaris* sp. and was 99% pure. At 40 weeks, one rat in the sterigmatocystin–DMSO group developed a papilloma-like lesion. By 70 weeks, all treated rats had either papillomas or squamous cell carcinomas. Liver lesions were found in 17/20 sterigmatocystin-treated rats. Hepatocellular carcinomas were observed in 7/10 rats treated with sterigmatocystin in DMSO and 5/10 rats treated with sterigmatocystin in acetone. No hyperplastic reaction was seen in the controls (Purchase & van der Watt, 1973).

Newborn (BALB/c × DBA/2)F1 mice received within 24 hours of birth a single subcutaneous injection of sterigmatocystin in 1% gelatine suspension at 0, 0.5, 1 or 5 mg/kg bw (56 animals per dose). When the animals were killed 1 year after the exposure, a high incidence of lung and liver adenomas was observed. An apparent dose–response relationship was found in the incidence of total tumours. The incidences of both lung and liver tumours in mice at 5 mg/kg bw were statistically significant, and the incidences of lung tumours in female mice and of liver tumours in male mice at 1 mg/kg bw were also statistically significant compared with tumours in control mice. The incidence of lung tumours in mice at 5 mg/kg bw was higher (male, 33%; female, 27%) than that of other experimental groups (0–20%). The incidence of liver tumours in treated male mice was 37%

at 5 mg/kg bw, 27% at 1 mg/kg bw and 15% at 0.5 mg/kg bw compared with 6% in the control group. A dose–response relationship was clearly demonstrated in the incidence of liver tumours in males (but not in females). Other tumours were induced in treated mice (two malignant lymphomas and one adenoma of the submaxillary gland), in contrast to none in control mice (Fujii et al., 1976).

Terao (1978) showed that sterigmatocystin induced mesothelioma when injected intraperitoneally. Male Wistar rats were given sterigmatocystin, *O*-acetylsterigmatocystin or dihydrosterigmatocystin via intraperitoneal injection once a week for 23 weeks (total dose of 20–25 mg) and observed for a period of 80 weeks after the first injection. The origin and the purity of the toxins are not known. The rats (90–110 g at the beginning of the study) were divided into four groups. A control group of 30 rats received only the solvent (dimethylformamide) via intraperitoneal injection. A group of 40 rats was treated with sterigmatocystin at 4 mg/kg bw, a group of 20 rats was treated with *O*-acetylsterigmatocystin at 4 mg/kg bw and a group of 30 rats was treated with dihydrosterigmatocystin at 4 mg/kg bw. Mesotheliomas were observed in 20/40 rats given sterigmatocystin, with the first mesothelioma observed at week 40. No mesotheliomas were observed in rats given *O*-acetylsterigmatocystin or dihydrosterigmatocystin. Two histological types of mesotheliomas, epithelioid and mesenchymal types, were induced by sterigmatocystin. Hepatocellular carcinomas were found in one rat treated with sterigmatocystin and in five rats treated with *O*-acetylsterigmatocystin. Nodules were observed in the liver of almost all the rats treated with *O*-acetylsterigmatocystin and in 17/40 rats treated with sterigmatocystin. No tumours were seen in the controls (Terao, 1978).

2.2.4 Genotoxicity

The genotoxicity of sterigmatocystin has been studied in vitro in bacteria and mammalian and human cells and in vivo in mice, rats and fish. In many cases, it was tested together or in comparison with other mycotoxins, especially with AFB_1. There are also studies on substances that are structurally related to sterigmatocystin.

(a) In vitro

A number of studies reported positive results for mutagenicity in bacterial systems in the presence of S9, using sterigmatocystin that was obtained mostly from commercial sources or from other researchers (as gifts), and was generally dissolved in DMSO. In the absence of S9, two tests were negative, one of which included *S. typhimurium* strain TA98, and one test using TA98 only was weakly positive (Table 12).

Table 12
Results of in vitro genotoxicity tests with sterigmatocystin

Test system	Test organism	Concentrations tested	Result	Reference
Bacterial cells				
Reverse mutation	*S. typhimurium* (TA98)	0.1 µg/plate +S9	Positive	McCann et al. (1975)
	S. typhimurium (TA100)	0.1–50 µg/plate +S9 or human liver extract	Positive	Tang & Friedman (1977)
	S. typhimurium (TA1538)	0.1–100 µg/plate +S9	Positive	Kuczuk et al. (1978)
		−S9	Negative	
	S. typhimurium (TA98)	1–100 µg/plate +S9	Weakly positive	Ueno et al. (1978)
		−S9	Positive	
	S. typhimurium (TA98, TA100, TA1535, TA1537)	1–10 µg/plate +S9	Positive only in TA100	Wehner et al. (1978)
		−S9	Negative	
	S. typhimurium (TA98, TA100)	100 nmol/plate + S9	Positive in TA100	Mori et al. (1986)
Rec assay	*Bacillus subtilus*	1, 2.5, 5, 10, 20 or 100 µg/disc, pH 6–8	Positive only at low doses (1, 2.5 and 5 µg/disc)	Ueno & Kubota (1976)
SOS chromotest	*Escherichia coli* (PQ37 strain)	With and without metabolic activation	Positive	Krivobok et al. (1987)
SOS repair	*S. typhimurium* (TA1535)/ pSK1002	10 µmol/L + human or rat liver extract	Positive	Baertschi et al. (1989)
Yeast				
Reverse mutation	*S. cerevisiae* (D3)	50 µg/mL +S9	Positive	Kuczuk et al. (1978)
Mutation frequency	*S. cerevisiae* (KY118) expressing cytochrome P450 CYP2B1	40–160 µg/mL	Positive	Black et al. (1992)
Mammalian cells				
Induction of 8-azaguanine-resistant mutations	Chinese hamster lung (V79) cells	1.0, 2.0, 5.0 µg/mL without metabolic activation	Dose-dependent increase of induced mutations	Noda, Umedia & Ueno (1981)
		0.1, 0.2, 0.5 µg/mL with S15		
Mutation frequency	Mouse mammary carcinoma FM3A cell line	0.032, 0.1, 0.32 µg/mL	Positive	Umeda, Tsutsui & Saito (1977)
DNA single-strand break		3.2 µg/mL	Positive (weak)	
Chromosome aberration		0.1, 0.32, 1.0 µg/mL	Positive	
HPRT	Chinese hamster lung V-79 fibroblasts	0.01–0.50 µmol/L without metabolic activation	Negative	Reiners et al. (1983)
		with metabolic activation (newborn SENCAR keratinocytes)	Weakly positive	
	Mouse mammary carcinoma FM3A cell line	1.0×10^{-6} mol/L + S15	Positive	Morita, Umeda & Ogawa (1991)

Test system	Test organism	Concentrations tested	Result	Reference
Unscheduled DNA synthesis	ACI rat hepatocytes (primary culture)	10^{-6}, 10^{-5} mol/L	Positive	Mori et al. (1984)
	C3H/HeN mouse hepatocytes (primary culture)	2×10^{-6}, 2×10^{-5} mol/L	Positive	
	ACI rat hepatocytes (primary culture)	10^{-7}–10^{-5} mol/L	Positive	Mori et al. (1986)
Micronucleus	Chinese hamster liver (primary culture of LiC2 cells)	0–1 µg/mL +S9 −S9	 Positive Negative	Ellard et al. (1991); Ellard & Parry (1993)
	Normal or genetically engineered immortal V79 Chinese hamster cell lines expressing rat liver CYP1A1, 1A2 and 2B1 cDNAs	0.1–1 µg/mL (±S9 tested only for normal V79)	Positive	
Human cells				
Unscheduled DNA synthesis	Skin fibroblasts derived from normal or *Xeroderma pigmentosum* patients	6×10^{-5} mol/L ±S9 or extracts of various organs from 5 species	Positive	Stich & Laishes (1975)
Chromosome aberration		6×10^{-6} mol/L	Positive	
Micronucleus	Human lymphoblastoid $TK^{+/-}$ MCL-5 cell line expressing a relatively high level of native CYP1A1; four other human cytochromes (CYP1A2, CYP2A6, CYP3A4 and CYP2E1); and microsomal epoxide hydrolase, carried as cDNAs in plasmids	0.01, 0.05, 0.10 µg/mL	Positive	Crofton-Sleigh et al. (1993)
	AHH-1 cells, the parent line that constitutively expresses CYP1A1, but does not contain the genetically engineered human cytochromes or epoxide hydrolase	0.02–0.50 µg/mL	Positive	
	Human adenocarcinoma lung cells (A549)	2 µmol/L	Positive	Jaksic et al. (2012)
Comet assay	Immortalized human gastric epithelial cells (GES-1)	0.075–3 µmol/L	Positive	Zhang et al. (2013)
	Immortalized human oesophageal epithelial cells (Het-1A)	6, 12, 24 µmol/L	Positive	Wang et al. (2013)
	Primary cultured human oesophageal epithelial cells (EPC)	24 µmol/L	Positive	Wang et al. (2015)

Table 12 (continued)

Test system	Test organism	Concentrations tested	Result	Reference
	Human hepatoma cell line (HepG2)	1.5, 3, 6 µmol/L	Positive	Gao et al. (2015)
	Immortalized human bronchial epithelial cells (BEAS-2B)	24 µmol/L	Positive	Huang et al. (2014)
	Human adenocarcinoma lung cells (A549)	24 µmol/L	Positive	
	Human adenocarcinoma lung cells (A549)	2 µmol/L	Positive	Jaksic et al. (2012)
Sister chromatid exchange	Human hepatocellular carcinoma cells (Hep3B)	10^{-8} to 10^{-12} µmol/L	Positive, dose-related response	Anninou et al. (2014)

CYP: cytochrome P450; HPRT: hypoxanthine-guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; S15: 15 000 × *g* supernatant fraction from rat liver homogenate

Results using a variety of end-points for gene mutation and chromosome aberration or damage conducted in several different types of non-human mammalian cells, including primary liver cells or liver cell lines, were nearly all positive or weakly positive. Similar studies in human cells were all positive.

In the Wang et al. (2013) study, the expressions of both *hMLH1* and *hMSH2* were upregulated at the messenger RNA (mRNA) and protein levels, indicating that sterigmatocystin initiated mismatch repair in response to DNA damage in Het-1A cells.

In the study of Gao et al. (2015), the increase in DNA strand breaks reported in human hepatoma cell line HepG2 was accompanied by increases in intracellular reactive oxygen species and in the expression of 8-hydroxydeoxyguanosine, indicating the involvement of oxidative stress. A significant increase in acridine orange fluorescence intensity was also observed, suggesting that the genotoxic effects on HepG2 cells could involve both oxidative stress and lysosomal leakage.

(b) In vivo

The results of in vivo studies on sterigmatocystin are shown in Table 13. All studies were positive.

(c) DNA adducts

Essigmann et al. (1979) studied the in vitro reaction of sterigmatocystin incubated with calf thymus DNA in the presence of phenobarbital-induced rat liver microsomes. The adduct formed was identified as 1,2-dihydro-2-(N^7-guanyl)-1-hydroxysterigmatocystin, the guanine and hydroxyl moieties being in a *trans* configuration. The structure and stereochemistry of this adduct indicated that the

Table 13
Results of in vivo genotoxicity tests with sterigmatocystin

Test system	Species	Dose of sterigmatocystin	Result	Reference
Sister chromatid exchanges	Female Swiss albino mice (femoral bone marrow)	0.06–6 mg/kg bw i.p. + negative (DMSO) and positive controls, 3 mice/group killed 2 h after i.p. injection	Positive, dose-related response	Curry et al. (1984)
Chromosome aberrations	Female Long–Evans rats (femoral bone marrow)	0.000 312 to 31.2 mg/kg bw i.p. + negative controls, 5 rats/group killed 1–96 h after i.p. injection	Positive, dose-related response	Ueda et al. (1984)
Chromosome aberrations	*Oreochromis niloticus* (Nile tilapia) (kidneys)	1.6 µg/kg bw, twice a week for 4 weeks, intragastric administration in corn oil, 1 group of untreated control and treated with corn oil alone, 8 fish/group (2 fish exposed to sterigmatocystin died)	Positive	Abdel-Wahhab et al. (2005)
Micronucleus	*Oreochromis niloticus* (Nile tilapia) (red blood cells)		Positive	

bw: body weight; DMSO: dimethyl sulfoxide; i.p.: intraperitoneal injection

exo-sterigmatocystin-1,2-oxide was the metabolite that reacted with DNA, and the quantitative yield of adduct indicated that this metabolite was a major product of the in vitro metabolism of sterigmatocystin. A component chromatographically identical to 1,2-dihydro-2-(N^7-guanyl)-1-hydroxysterigmatocystin was detected in isolated rat livers perfused with sterigmatocystin (Essigmann et al., 1980).

Baertschi et al. (1989) also observed guanyl-N^7 adducts generated in vitro by incubation of sterigmatocystin with calf thymus DNA in the presence of human liver microsomes, as did Raney et al. (1992) using chemically synthesized sterigmatocystin-1,2-oxide. No evidence for other adducts was observed. Baertschi et al. (1989) found that the sterigmatocystin-induced guanyl-N^7 adducts were more potent in the bacterial SOS repair assay than those induced by AFB_1, but Raney et al. (1992) found that the adducts had similar mutagenic efficiency.

In vivo, dose-dependent formation of DNA adducts was found in the liver of male Fischer 344 rats (140–160 g) treated via intraperitoneal injection with synthetic sterigmatocystin (purity unknown, dissolved in DMSO) at doses of 0.33, 1.0, 3.0 or 9 mg/kg bw (3–4 rats per dose) and killed 2 hours after dosing. A group of control rats received, also via intraperitoneal injection, DMSO alone. In addition, a group of rats given a 9 mg/kg bw dose was followed for up to 105 days to characterize the time course of the DNA adducts. The DNA adducts were found to persist for up to 105 days after treatment at a level of 0.5% of the 2-hour value. Loss of these adducts from liver DNA exhibited a triphasic profile: rapid loss during the first 24 hours ($t_{½}$ = 12 hours) followed by a slower decline from days 1 to 14 after dosing ($t_{½}$ = 7 days) and an extremely slow decline from days 14 to 105 post-treatment ($t_{½}$ = 109 days). DNA adducts were analysed using ^{32}P (Reddy, Irvin & Randerath, 1985).

A linear dose–response relationship in the formation of DNA adducts was also observed in the liver of male Fischer rats (100–150 g) treated via intraperitoneal injection with sterigmatocystin (extracted from *A. versicolor*, purity unknown, dissolved in DMSO) at doses of 1, 4, 8 or 16 mg/kg bw ($n = 4$ per group) and killed 24 hours after dosing (Olson & Chu, 1993a). Kinetic analysis showed that 73% of the adducts were eliminated 12 hours after exposure.

2.2.5 Reproductive and developmental toxicity

The reproductive and developmental toxicity of sterigmatocystin in mammalian species has not been investigated so far.

2.2.6 Special studies

(a) Toxicity in vitro

Cytotoxicity / cell viability

Bunger et al. (2004) tested the cytotoxicity of synthetic sterigmatocystin (purity ≥ 90%, dissolved in 10% glycerol) in four cell lines: A-549 (human lung cancer cell line with properties of alveolar type II pneumocytes), Hep-G2 (epithelium-like cell line from human hepatocellular carcinoma), L-929 cells (murine fibroblasts from areolar and adipose connective tissue) and Neuro-2a (a neuronal cell line from murine neuroblastoma). A-549 lung cells showed the highest susceptibility, with toxicity 80-fold higher than Hep-G2 liver cells. Neuro-2a cells were 7-fold more susceptible than Hep-G2. Median inhibitory concentrations (IC_{50}) were 3.7 µmol/L in A-549, 40.1 µmol/L in Neuro-2a, 163.3 µmol/L in L-929 and 286.1 µmol/L in Hep-G2 cells (Bunger et al., 2004).

Using isolated rat liver mitochondria, Kawai et al. (1984) showed that sterigmatocystin (isolated from *Chaetomium thielavioideum*, purity unknown) and 5,6-dimethoxysterigmatocystin affected adenosine triphosphate synthesis in a manner different from that of AFB_1, causing the uncoupling of oxidative phosphorylation. These effects may be part of the molecular mechanism for the respective cytotoxicities of sterigmatocystin and AFB_1. *O*-Methylsterigmatocystin (OMSTC) did not exhibit uncoupling activity at the limited concentrations tested (due to its low solubility in an aqueous system) (Kawai et al., 1984).

Sterigmatocystin and secosterigmatocystin extracted from a fermentation extract of fungus strain F2611 showed high cytotoxicity against Vero cells (monkey kidney epithelial cells), with IC_{50} values of 0.06 and 0.97 µmol/L, respectively (Almeida et al., 2014).

Xu et al. (2013) evaluated the potential anticancer activity of sterigmatocystin extracted from an endophytic fungal strain, *Emericella* sp. AST0036, using a panel of five tumour cell lines. Sterigmatocystin showed moderate cytotoxicity, with no apparent selectivity. The IC_{50} values were 3.41 µmol/L for

NCI-H460 (human non-small cell lung cancer), 2.96 μmol/L for SF-268 (human central nervous system cancer; glioma), 3.63 μmol/L for MCF-7 (human breast cancer), 3.23 μmol/L for PC-3M (metastatic human prostate adenocarcinoma) and 2.75 μmol/L for MDA-MB-231 (human breast adenocarcinoma) cell lines.

Despot et al. (2016) reported that sterigmatocystin was cytotoxic to human lung A549 cells (IC_{50} of 4.2 μmol/L [1.3 μg/mL]) and THP-1 macrophage-like cells (IC_{50} of 1.9 μmol/L [0.6 μg/mL]). The IC_{50} for this cell line confirms the value previously reported by Bunger et al. (2004).

Jaksic et al. (2012) reported an IC_{50} of 181 μmol/L for 5-methoxysterigmatocystin in A549 cells.

In immortalized ovarian cells (CHOeK1), IC_{50} values of sterigmatocystin ranged from 25.0 to 12.5 μmol/L after 24–72 hours. Sterigmatocystin was less cytotoxic than beauvericin (10.7–2.2 μmol/L) and patuline (2.9 μmol/L) (Zouaoui et al., 2016).

In Hep3B, a human hepatocellular cancer line, sterigmatocystin was more cytotoxic (IC_{50} values of 58 μmol/L and 22 μmol/L after 24 and 48 hours, respectively) than ochratoxin A (IC_{50} values of 104 and 45 μmol/L after 24 and 48 hours, respectively) and citrinin (IC_{50} values of 124 and 77 μmol/L after 24 hours and 48 hours, respectively) (Anninou et al., 2014).

In HepG2 cells, the IC_{50} of sterigmatocystin was 7.3 μmol/L. Sterigmatocystin was more cytotoxic than AFB_1, apparently due to its low water solubility, which makes it easy for it to diffuse into cells (Liu, Du & Zhang, 2014).

Sterigmatocystin inhibited aerobic glucose consumption of proliferating porcine kidney tubular epithelial (PK-15) cells at a low exposure concentration of 0.1 μg/mL (0.3 μmol/L), but had no effect on monolayer PK-15 cells (up to 40 μmol/L) or on boar sperm motility (up to 62 μmol/L) (Mikkola et al., 2015).

Effects on cell cycle

In primary monkey epithelial cells, sterigmatocystin induced a reduction in the mitotic count, a decrease in the number of cells per field, an increase in cell size together with nuclear and nucleolar changes and gradual cell degeneration (Engelbrecht, 1970). According to EFSA (2013), at 2 mg/mL, sterigmatocystin completely blocked mitosis and caused nuclear changes, and inhibited the incorporation of ^{3}H-labelled thymidine into DNA as well as that of ^{3}H-labelled uridine in RNA (Engelbrecht & Altenkirk, 1972).

The inhibitory effect of sterigmatocystin isolated from *Bipolaris* sp. (99% pure) at an intraperitoneally administered dose of 50 mg/kg bw on liver RNA synthesis was shown in vivo in Wistar rats. Sterigmatocystin inhibited the incorporation of orotic acid in liver RNA, with the maximum inhibition (60%)

1 hour after treatment and recovery to the control values within 4 hours after treatment (Nel & Pretorius, 1970).

In Hep3B cells, sterigmatocystin significantly reduced the mitotic index at concentrations greater than 10^{-8} mol/L and the proliferation rate index at concentrations greater than 10^{-1} mol/L (Anninou et al., 2014).

In immortalized human gastric epithelial cells (GES-1), sterigmatocystin has been shown to cause DNA damage and subsequently trigger cell cycle arrest in G2 and apoptosis (Xing et al., 2011; Zhang et al., 2013). In a subsequent study by the same team of researchers, it was observed that the initial G2 arrest was followed by G2 phase checkpoint adaptation driven by inactivation of checkpoint kinase Chk1, and entry of cells into mitosis despite damaged DNA (increased number of oγH2AX foci, a marker of DNA double-strand breaks). A small fraction of cells that had undergone checkpoint adaptation were able to survive and proliferate, which may potentially promote genomic instability and result in tumorigenesis (Jiang et al., 2017). As a member of the PI3K family, ataxia telangiectasia mutated (ATM) kinase is an important sensor activated in the response to DNA damage. ATM is triggered by double-strand breaks in DNA and initiates a signalling cascade to regulate the cell cycle. Once activated, ATM phosphorylates various downstream molecules including the checkpoint kinase Chk2 and the tumour suppressor protein p53. Sterigmatocystin was found to induce the activation of ATM and its downstream molecules, Chk2 and p53, in GES-1 cells. Chk2 plays an essential role in the maintenance rather than the initiation of G2 arrest (Zhang et al., 2013). The deregulation of cyclin B1, Cdc2 and Cdc25C was also reported to be involved in the G2 arrest induced by sterigmatocystin in GES-1 (Xing et al., 2011).

In human oesophageal epithelial cells, sterigmatocystin induced G1 arrest in primary cells (EPC) but induced G2 arrest in immortalized cells (Het-1A) (Wang et al., 2013, 2015). Activation of the ATM–Chk2 pathway was involved in the G1 phase arrest in EPC cells, whereas the p53–p21 pathway activation was involved in the G2 phase arrest in Het-1A cells. Het-1A is a noncancerous epithelial cell line immortalized by SV40LT, and Wang et al. (2015) suggested that SV40LT may disturb cell cycle progression by inactivating some of the proteins involved in the G1/S checkpoint. In EPC cells, the expression of CDK4, cyclin D1, CDK2, cyclin E1, Rb and E2F1 at the protein level was significantly decreased for all proteins after treatment, and the phosphorylation level of Rb (Ser-811) also decreased. According to Wang et al. (2015), G1 phase arrest might be the real response to sterigmatocystin–DNA damage in human oesophageal epithelial cells. The interference of SV40LT with cell cycle processes should be taken into account when interpreting such studies (Wang et al., 2013, 2015).

In human immortalized bronchial epithelial cells (BEAS-2B) and human lung cancer cells (A549), the effects of sterigmatocystin on cell cycle arrest were

complex and dependent on the tested concentration and cell type (Huang et al., 2014). Low concentrations of sterigmatocystin (6 and 12 μmol/L) arrested BEAS-2B cells in the G2/M phase and A549 cells in the S phase, whereas at a high concentration (24 μmol/L) both cell lines were arrested in S and G2/M phases. The modulation of cyclins and CDK expression showed concomitant changes with cell cycle arrest in BEAS-2B and A549 cells. BEAS-2B cells are normal human bronchial epithelial cells immortalized by SV40LT, while A549 cells are of alveolar origin. Among the cell cycle–related proteins, the expression of cyclin B1 was increased in both BEAS-2B and A549 cells. Huang et al. (2014) proposed that the high expression of cyclin B1 induced by sterigmatocystin might be involved in lung carcinogenesis. The deregulated expression of cyclin B1 is suggested to be closely associated with early events in neoplastic transformation and poor prognosis, and cyclin B1 may function as an oncogene and is overexpressed in a variety of cancers (Huang et al., 2014).

Xie et al. (2000) reported that sterigmatocystin induced G2/M phase arrest in primary murine embryonic fibroblasts via the failure of p53-mediated G1 checkpoint, mediated by induction of MDM2 proteins.

In HepG2 cells exposed to sterigmatocystin, adenosine triphosphate and DNA contents were decreased while reactive oxygen species and matrix metalloproteinase contents were increased with increasing treatment concentrations (4 doses between 0.5 and 7 μmol/L). Sterigmatocystin caused cell cycle arrest, decreasing mitochondria membrane potential and apoptosis in a dose-dependent manner. Most cells stayed at the G0/G1 phase, indicating that DNA synthesis was almost completely inhibited, especially at a high dose, which is consistent with the decreased DNA content. Sterigmatocystin increased the expression of apoptosis-related proteins Bax, Caspase-3 and p53 and decreased the expression of Bcl-2 (Liu, Du & Zhang, 2014).

(b) Neurotoxicity

In an in vitro receptor binding assay, sterigmatocystin showed antagonistic activity against the dopamine active transporter with a K_i value of 2.23 μmol/L (Almeida et al., 2014).

(c) Immunotoxicity

Sterigmatocystin showed immunotoxic and immunomodulatory properties in in vitro and in vivo studies.

In macrophages derived from human monocytic (THP-1) cells, sterigmatocystin increased the expression of proinflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin (IL)-1β. The half-maximal effective concentrations (EC_{50}) were 10.3 μmol/L for TNF-α and 11.5 μmol/L for IL-1β

mRNA response. Sterigmatocystin also caused disruption of cell and nuclear membranes in about half of the treated cells (Korkalainen et al., 2017).

Wang et al. (2014) used an in vivo model – the *Tg(mpx:GFP)*^{i114} zebrafish line, in which neutrophils are labelled with green fluorescent protein (GFP) – to screen a natural product library for compounds that can affect neutrophil migratory behaviour. Among 1040 fungal extracts screened, two were found to inhibit neutrophil migration completely. Subfractionation of these extracts identified sterigmatocystin and antibiotic PF1052 as the active components. Using the EZ-TAXIScan chemotaxis assay (an in vitro mammalian assay), both compounds were also found to have a dosage-dependent inhibitory effect on murine neutrophil migration (Wang et al., 2014).

In BALB/c male mice (six per dose) exposed to sterigmatocystin via a single intraperitoneal injection (0, 3, 30, 300 or 3000 µg/kg bw) and killed 24 hours after treatment, the proportion of FoxP3$^+$ regulatory T cells and the FoxP3 expression level were significantly increased in peripheral blood mononuclear cells, the spleen and the thymus (mainly for treatments ≥30 µg/kg bw). No dose-dependent change was observed in CD4$^+$ and CD8$^+$ T cells. The number of plasmacytoid dendritic cells and expression of pDCs marker-CD123 and BDCA2 significantly decreased in the thymus but increased in the spleen (Liu et al., 2012).

Zhang et al. (2012) also exposed BALB/c male mice to sterigmatocystin by a single intraperitoneal injection at a dose of 3 mg/kg bw. Mice were killed 2, 6, 12 and 24 hours after treatment (n = 8 at each time point) and compared with solvent and untreated controls (32 mice per group). Blood samples from four mice in each group were collected for further peripheral blood mononuclear cell separation, while the other four samples were used for serum separation. In treated mice, the TNF-α and IL-12p35 showed decreased expression in the murine peripheral blood mononuclear cells. The expression of IL-6 mRNA first showed an increase at 2 and 6 hours after treatment and then a decrease at 12 and 24 hours, with the lowest levels found in the 24-hour treatment group. In the peritoneal macrophages, a decrease in the levels of all three cytokines, TNF-α, IL-6 and IL-12p35, was observed. The serum TNF-α and IL-6 levels were also decreased.

Richard et al. (1978) showed that sterigmatocystin, given orally by capsule to guinea-pigs for 2 weeks (4.2 mg/animal), induced a significant reduction of complement activity (see section 2.2.2(c) for a full description of the study). No effects on the total serum protein were observed, but sterigmatocystin significantly reduced α_2-globulin in guinea-pig serum. As sterigmatocystin induced hepatotoxicity (diffuse fatty degeneration of hepatocytes and focal necrosis), it is not possible to conclude if sterigmatocystin has a specific effect on complement activity or is immunotoxic.

EFSA (2013) also described a number of papers published in Chinese:

A series of papers on in vitro experiments with human peripheral blood lymphocytes and murine peritoneal macrophages showed that [sterigmatocystin] inhibited the expression and secretion of IL-12 (Xing et al. (2005) and Huang et al. (2002), cited in Zhang et al., 2012) and affected IL-2, interferon-γ and IL-4 expression and secretion in murine spleen cells (Xing et al. (2005), cited in Zhang et al., 2012). In addition, Xing et al. (2005) demonstrated that [sterigmatocystin] reduced the expression of human leukocyte antigen (HLA) class I at the level of both RNA and protein. HLA-class I antigens have been associated with numerous immunological reactions and autoimmune diseases as well as with cellular apoptosis and development of neoplastic diseases, and the findings that [sterigmatocystin] can reduce HLA-1 expression suggest the immunomodulatory effects of [sterigmatocystin]. It should be noted, however, that these results were obtained with rather high concentrations ranging between 0.5 and 2 mg/L, and that the relevance of these findings for the in vivo exposure remains to be elucidated.

(d) Other types of study

Tong et al. (2013) studied the effects of sterigmatocystin in an experimental model of reflux oesophagitis developed in rats that had undergone a cardiectomy and partial pylorus ligation. This design was selected to investigate the potential role of sterigmatocystin in increased incidence of oesophageal cancer in patients with reflux oesophagitis. Thirty male Wistar rats (4 weeks old) were randomly divided into three groups (n = 10): a control group, treated with placebo and surgery; an oesophagitis model group, which did not receive sterigmatocystin treatment; and a reflux oesophagitis group that was treated with sterigmatocystin. Seven days after the surgery, rats received intraperitoneal injection with saline or sterigmatocystin at 30 μg/kg bw once a day for 7 days. Twenty-four surviving rats (n = 6, 8, 10, respectively) were killed 5 or 11 weeks after the seventh day of treatment. Both the oesophagitis group and the sterigmatocystin group exhibited mucosal congestion of the oesophageal mucosa with oedema, erosion and ulcers. At weeks 5 and 11, the hyperplasia severity score and the proliferating cell nuclear antigen index, but not the inflammation scores, were statistically higher in the sterigmatocystin-treated group than in the oesophagitis model group. The expression levels of the transporter associated with antigen processing 1 (TAP1) and the low molecular weight protein 2 (LMP2) in the cytoplasm of oesophageal epithelial cells were reduced in rats with reflux oesophagitis, and further decreased in the sterigmatocystin group. Thus, the downregulation of TAP1 and LMP2 proteins by sterigmatocystin may directly affect tumour immunity by allowing transformed cells to escape the host immune surveillance, thereby promoting oesophageal cancer (Tong et al., 2013).

In order to investigate if sterigmatocystin could be a causal agent of gastric cancer, Kusunoki et al. (2011) studied the effects of long-term exposure on stomach tissues in aged male Mongolian gerbils (75 weeks old at the beginning of the study). Thirty-one animals were divided into three groups: a control group of 11 gerbils and two groups of treated gerbils exposed to commercial sterigmatocystin (purity > 98%) in drinking-water for 24 weeks at a concentration of 100 µg/L (n = 7) or 1000 µg/L (n = 13), equal to 0.007–0.015 mg/kg bw per day for the low-dose group and 0.088–0.132 mg/kg bw per day for the high-dose group. The results of the histopathological investigations are shown in Table 14. According to the authors, the histological changes in treated groups suggest a precancerous condition and are similar to those previously observed with *Helicobacter pylori* (Kusunoki et al., 2009). Immunohistochemical staining in the affected areas of the gastric mucosa showed a high index of proliferating cell nuclear antigen (a marker of cell proliferation), p53 (tumour suppressor gene, a marker of transformation to a malignant tumour) and MDM2 (cellular inhibitor of p53). Note that most of the effects were not dose-dependent (incidence was higher in the low-dose group than in the high-dose group) (Kusunoki et al., 2011).

Some studies focused on the potential initiation or promotion of gastric cancers by sterigmatocystin. In particular, the co-exposure to *H. pylori* (one of the most common gastric infections) and sterigmatocystin was studied in Mongolian gerbils. Sterigmatocystin was given with the diet at a concentration of 100 or 1000 mg/kg feed to Mongolian gerbils (n = 196, divided into five treatment groups) for 27 months. According to EFSA (2013), in the presence of an *H. pylori* infection, dietary sterigmatocystin exposure enhanced the development of intestinal metaplasia of the gastric mucosa (Ma et al., 2003).

2.2.7 Sterigmatocystin and aflatoxins or other mycotoxins

The acute toxicity of sterigmatocystin is 10 or more times lower than that of AFB_1. In rats, oral LD_{50} values for AFB_1 are 7.2 mg/kg bw in males and 17.9 mg/kg bw in females (Butler, 1964); for sterigmatocystin, LD_{50} values are 120 mg/kg bw in males and 166 mg/kg bw in female rats (Purchase & van der Watt, 1969). In vervet monkeys, LD_{50} values were 3.7 mg/kg bw for intraperitoneally administered AFB_1 and 32 mg/kg bw for sterigmatocystin (van der Watt & Purchase, 1970a).

Short-term toxicity of sterigmatocystin in the rat showed extensive histopathological changes in the liver leading to necrosis, but it has been noted that bile duct proliferation was not nearly as extensive as that following AFB_1 treatment (van der Watt & Purchase, 1970b).

The carcinogenic potency of sterigmatocystin in comparison with that of AFB_1 has been considered. From hepatic tumour yields in the same strain of Wistar-derived rats, Purchase & van der Watt (1970b) estimated that AFB_1 is no

Table 14
Histological changes in the stomach in male Mongolian gerbils exposed to sterigmatocystin in drinking-water

Concentration in drinking-water (mg/L) / dose (mg/kg bw per day)	Total no. of gerbils	Incidence (*n*)[a]			
		Active gastritis	Erosion event	Hyperplastic polyps	Intestinal metaplasia
0	11	2	1	0	0
0.1 / 0.007–0.015	7	7	7	5	7
1 / 0.088–0.132	13	13	12	8	2

bw: body weight; no.: number
[a] Results expressed as number of gerbils showing the histological change.
Source: Kusunoki et al. (2011)

more than 10 times as potent as sterigmatocystin; after receiving AFB_1 at 105 µg/week for 50 weeks, 6/7 rats developed hepatomas in 80 weeks, whereas 60% and 80% of the rats receiving sterigmatocystin at 750 and 1500 µg/week, respectively, for 52 weeks developed tumours or hyperplastic nodules by week 123. Purchase & van der Watt (1970b) also noted the absence of bile duct proliferation and of cholangiocarcinomas after sterigmatocystin administration, in contrast to the extensive bile duct reaction produced by AFB_1.

There have been no studies in which purified AFB_1 and sterigmatocystin have been given in combination.

With respect to genotoxicity, sterigmatocystin was found to be less mutagenic in *S. typhimurium* in the presence of S9 (McCann et al., 1975; Tang & Friedman, 1977; Kuczuk et al., 1978; Ueno et al., 1978; Wehner et al., 1978; Mori et al., 1986). However, inconsistent results were observed with human liver extract, with sterigmatocystin being sometimes less and sometimes more mutagenic (Tang & Friedman, 1977).

In the rec assay with *B. subtilis*, AFB_1 exhibited a clear dose–response relationship, but not sterigmatocystin, which was positive only at doses between 1 and 5 µg/disc (not at higher doses up to 100 µg), without differences in the diameter of the inhibition zone (Ueno & Kubota, 1976).

Baertschi et al. (1989) used the bacterial SOS repair assay with *S. typhimurium* (TA1535) in which the *umu*C gene is linked to a *lac*Z reporter gene in plasmid pSK1002 to compare the mutagenicity of sterigmatocystin and AFB_1. The *umu* response was higher for sterigmatocystin than for AFB_1 when either of the toxins were activated enzymatically (with rat or human liver extract) or the synthetic epoxides were added directly to the bacteria. The quantification of guanyl-N^7 adducts generated in vitro in isolated calf thymus DNA in the presence of human liver microsomes showed a slightly higher level for AFB_1 than

for sterigmatocystin. No evidence for other adducts were observed. This order of genotoxicity – the higher *umu* response per molecule of guanyl-N^7 DNA adduct for sterigmatocystin than for AFB_1 – was unexpected because AFB_1 has been reported to be a more potent hepatocarcinogen than sterigmatocystin (Baertschi et al., 1989).

In a subsequent study by the same team, the quantification of guanyl-N^7 adducts showed similar results, that is, the yield of adducts was slightly higher with synthetic epoxide of AFB_1 than with those of sterigmatocystin. However, this time when *S. typhimurium* TA98 was treated with the synthetic epoxides of AFB_1 or sterigmatocystin, the number of revertants by plate was higher for aflatoxin than for sterigmatocystin oxides. The authors concluded that the adducts had similar mutagenic efficiency (Raney et al., 1992).

In tests for unscheduled DNA synthesis in mouse hepatocytes, the level for sterigmatocystin was lower than for AFB_1 (Mori et al., 1984, 1986). However, in the study of Stich & Laishes (1975) in human skin fibroblasts, the unscheduled DNA synthesis level of sterigmatocystin was higher than that of AFB_1, with or without S9.

In the mouse mammary carcinoma FM3A cell line, sterigmatocystin induced 8-azaguanine-resistant mutations (Umeda, Tsutsui & Saito, 1977) and 6-thioguanine-resistant mutations in the presence of S15 fraction (Morita, Umeda & Ogawa, 1991). Compared with AFB_1, sterigmatocystin induction of 8-azaguanine-resistant mutations and the level of chromosome aberrations were higher. The aberrations were both chromatid and chromosome types. Both toxins showed a weak induction of DNA single-strand breaks (Umeda, Tsutsui & Saito, 1977).

Other than carcinogenicity and genotoxicity effects, the few in vitro and in vivo studies indicate that the combined effects of sterigmatocystin and AFB_1 are additive on end-points such as apoptosis in a human liver cancer cell line, weight gain, reduction in complement activity and reduction in serum globulins. There was no additive effect for sterigmatocystin and AFB_1 on diffuse fatty degeneration of hepatocytes or focal necrosis in the liver (Richard et al., 1978; Liu et al., 2014).

Liu, Du & Zhang (2014) showed in HepG2 cells an additivity of sterigmatocystin and AFB_1 on cell apoptosis–related toxicity end-points in vitro (disruption of the integrity of mitochondria, increased expression of apoptosis-related proteins Bax, Caspase-3 and p53 and decreased expression of Bcl-2).

Effects of sterigmatocystin, alone at 4.2 mg/animal or in combination with aflatoxin (mixture of B_1 and G_1) at 0.01 mg/animal, were studied in guinea-pigs after 2 weeks of daily exposure. Sterigmatocystin (purity unknown) was extracted from *A. versicolor* and aflatoxin from *A. parasiticus* (37.8% pure). The toxins were given orally in capsules and a control group received no treatment.

At the beginning of the experiment, the animals weighed 475–520 g. Each group consisted of 10 animals; however, two animals treated with sterigmatocystin alone died before the end of the experiment. In comparison with controls, which gained weight over the 2 weeks of the study, weight loss was significant in guinea-pigs treated with sterigmatocystin alone or in combination with aflatoxin; in those given aflatoxin alone, there was weight gain, but it was significantly less than in the controls. The overall effect of the combination of toxins on decreased weight gain was additive. The effects of the two toxins in combination on lowering α_2-globulin and β-globulin were additive, and each toxin alone had a marked effect on lowering α_2-globulin. None of the other serum proteins were markedly changed. The combination of aflatoxin and sterigmatocystin significantly reduced complement activity in an additive effect. Synergism or additivity between sterigmatocystin and aflatoxin was observed in their effect on increasing serum albumin (neither of the toxins alone had a significant effect). The adverse effects induced by sterigmatocystin in the liver (diffuse fatty degeneration of hepatocytes and focal necrosis) were not affected by the combination with aflatoxin (Richard et al., 1978).

The cytotoxic interactions of sterigmatocystin, beauvericin and patulin were assessed on immortalized ovarian cells (CHOeK1) by the isobologram method. At low fraction affected, mycotoxin combinations were synergistic, whereas at higher fraction affected, the combinations showed an additive effect (Zouaoui et al., 2016).

The combination of sterigmatocystin, ochratoxin A and citrinin on Hep3B (a human hepatocellular cancer line) showed, in general, similar-to-additive or antagonistic genotoxic and cytotoxic effects (Anninou et al., 2014). Sister chromatid exchanges, mitotic divisions (mitotic index), cell cycle delays (proliferation rate index) and MTT reduction served as end-points of genotoxicity, cytotoxicity, cytostaticity and metabolic activity (cell viability), respectively. Using the coefficient of drug interaction index, an antagonistic interaction on cytotoxicity was revealed at concentrations ranging from 10^{-8} to 10^{-6} mol/L. At concentrations of 10^{-12} mol/L, all the possible mycotoxin combinations tended to show synergism in their MTT-promoting effect at 48 hours. In the case of the rates of sister chromatid exchanges, a slight though statistically significant antagonistic effect was shown at concentrations of 10^{-8} mol/L or less. No such effects (antagonism or synergism) were observed on mitotic index or proliferation rate index, as interaction was additive. According to Anninou et al. (2014), the three mycotoxins sterigmatocystin, ochratoxin A and citrinin share a specific biological pathway to affect cell viability, and this is probably the reason why they share a complicated interaction profile in vitro. Citrinin leads to cell death after causing disruption through selective loss of cell membrane permeability. Sterigmatocystin probably leads to cell deregulation and death through DNA

modification. Ochratoxin A preserves a complex mode of action in comparison with the other toxins. It can affect cell viability by disrupting metabolism and reducing glyconeogenesis or by causing cell deregulation and affecting membrane permeability (Anninou et al., 2014).

2.3 Observations in domestic animals/veterinary toxicology

In dairy cattle, a case of poisoning in a farm in the USA was reported in relation to feed contamination by several fungal strains, dominated by *A. versicolor* and *A. candidus*. The concentration of sterigmatocystin was 7.75 mg/kg feed. The animals exhibited bloody diarrhoea, loss of milk production and death, in some cases (Vesonder & Horn, 1985).

Experimental studies in pigs and poultry in which sterigmatocystin was administered by the oral route confirm that the liver is a major target organ (Salam & Shanmugasundaram, 1983; Sayed 1993; Kovalenko et al., 2011) (see section 2.2.2(e)).

2.4 Observations in humans

Some clinical observations, summarized below, were described in EFSA (2013). The Committee did not see the original publications.

One study reported on the concentrations of sterigmatocystin and sterigmatocystin–DNA adducts in biological material of patients with liver and stomach cancer diagnosed in the Chinese Academy of Medical Sciences Cancer Hospital in Beijing, China, and in healthy volunteers. The concentration of sterigmatocystin was measured in 28 samples of cancerous and pericancerous tissues of patients; 20 urine samples of patients and 10 of healthy people; and 14 blood samples of patients and 13 of healthy people. Using indirect competitive ELISA with an LOD of 20 µg/kg, sterigmatocystin was found in the blood of four out of 13 patients (range 65–113 µg/kg blood) and in one out of 14 healthy people (68 µg/kg blood). In urine, sterigmatocystin concentrations were below the LOD in all samples tested. Sterigmatocystin–DNA adducts were found in 14 out of 28 tissues of tumours collected from 12 patients (Tian, Lou & Du, 1995, in Chinese; cited in EFSA, 2013).

More recently, the concentration of sterigmatocystin in blood serum (measured by HPLC) was determined together with markers of liver function in 166 human patients, divided into three groups comprising 55 controls, 58 patients with liver cirrhosis and 53 patients with hepatocellular carcinoma. Sterigmatocystin was detected in 26.2% of all samples, with a statistically significantly ($P < 0.001$) higher prevalence in patients with liver cirrhosis and

liver cancer. Sterigmatocystin concentration varied between 0.01 and 0.005 ng/mL in blood in control subjects and reached values up to 2.02 ng/mL in blood and 9.39 ng/mL in urine in patients with hepatocellular carcinoma. In addition, a strong correlation between the presence of α-fetoprotein (a tumour marker) and sterigmatocystin was found in patients with liver cancer. The authors suggested that these findings may indicate a role of sterigmatocystin in the pathogenesis of liver cancer (Hutanasu et al., 2011, in Romanian; cited in EFSA, 2013).

Lou et al. (1995; cited in Scott, 2004) found that both the contamination rate and the content of sterigmatocystin in grains were significantly higher in high incidence areas for stomach and liver cancers than in a low incidence area in China. The contamination rate was 45% for levels above 20 μg/kg grains in high incidence areas compared with 15% in the low incidence area. Average sterigmatocystin concentrations were 20 μg/kg in high contamination areas and 12 μg/kg in the low contamination area.

Norback et al. (2016) investigated the association between sick building syndrome (ocular, nasal, throat and dermal symptoms, headache and tiredness) in 462 students aged 14–16 years from eight schools in Malaysia and fungal DNA in dust collected in 32 classrooms. Total fungal DNA in swab samples was associated with self-reported rhinitis (P = 0.02), ocular symptoms (P = 0.009) and tiredness (P = 0.001). There were positive associations between *A. versicolor* DNA (producing sterigmatocystin) in Petri dish samples, ocular symptoms (P = 0.02) and tiredness (P = 0.001). Sterigmatocystin was detected in two swab samples (6%) and verrucarol (a mycotoxin produced by *Stachybotrys chartarum*) in four samples (12%).

3. Analytical methods

3.1 Chemistry

Sterigmatocystin is a polyketide-derived mycotoxin with CAS No. 10048-13-2; IUPAC name (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3′,2′:4,5]furo[2,3-c]xanthen-7-one (see Fig. 1). The molecular formula of sterigmatocystin is $C_{18}H_{12}O_6$ and the molecular weight 324.28 g/mol.

Structurally, sterigmatocystin is closely related to aflatoxin (see Fig. 2), consisting of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is a yellow powder with a melting point at 245–246 °C. It is soluble in organic solvents, particularly chloroform, and stable in chloroform at 4 °C and −20 °C for 30 days (Septien et al., 1993; Veršilovskis & De Saeger, 2010).

Hatsuda & Kuyama (1954) first isolated the compound from the mycelial mat of *A. versicolor* and named it sterigmatocystin. Bullock, Roberts & Underwood (1962) proposed its chemical structure with a *bis*-dihydrofuran ring system in the molecule. Related compounds of sterigmatocystin, including 5-methoxysterigmatocystin, dihydrosterigmatocystin, dihydrodemethylsterigmatocystin, demethylsterigmatocystin and sterigmatin, which are xanthone derivatives with a *bis*-dihydrofuran or a dihydro *bis*-dihydrofuran ring system, were also isolated from *A. versicolor*, and their structures were determined by elemental and spectroscopic methods (Bullock et al., 1963; Holker & Mulheirn, 1968; Elsworthy et al., 1970; Hatsuda et al., 1972; Hamasaki et al., 1973).

Versicolorins A, B and C and other anthraquinone derivatives containing a *bis*-dihydrofuran or a dihydro *bis*-dihydrofuran ring system were also isolated from *A. versicolor*, and their structures deduced (Bullock et al., 1963; Hamasaki et al., 1965a,b; Hamasaki, Renbutsu & Hatsuda, 1967; Elsworthy et al., 1970; Hatsuda et al., 1971). *A. versicolor* was also found to produce a 6-carbon side-chain polyhydroxyanthraquinone derivative, norsolorinic acid (Hamasaki, Renbutsu & Hatsuda, 1967), averantin (Birkinshaw, Roberts & Roffey, 1966) and averufin (Pusey & Roberts, 1963; Holker et al., 1966).

3.1.1 Sterigmatocystin as precursor of aflatoxins and biosynthetic pathway

The pathway of sterigmatocystin and aflatoxin synthesis is shown in Fig. 3. Other closely related compounds such as OMSTC may exist and occur naturally. Sterigmatocystin and OMSTC are, respectively, the penultimate and ultimate precursors of aflatoxin. Although these precursors are chemically and structurally very similar, their accumulation differs at the species level for aspergilli.

Acetate is the primary precursor to form the polyketide. It is transformed via a series of intermediates by multistep oxidation–reduction reactions into sterigmatocystin. Studies of the biosynthetic pathway of sterigmatocystin using ^{14}C- and ^{13}C-labelled acetate indicate that sterigmatocystin originates from the acetate–malonate pathway (Holker & Mulheirn, 1968; Seto Cary & Tanabe, 1974; Steyn et al., 1975; Pachler et al., 1976). Several enzymatic reactions are involved in a complex polyketide pathway that converts acetate and malonate to sterigmatocystin (Townsend, Christensen & Trautwein, 1984; Cleveland et al., 1987; Bhatnagar, Ehrlick & Cleveland, 1992; Wunch, Bennett & Bhatnagar, 1992; Keller, Kantz & Adams, 1994; Brown, Adams & Keller, 1996; Kelkar, Keller & Adams, 1996; Yabe et al., 1998; Watanabe & Townsend, 2002; Bhatnagar et al., 2006).

The sterigmatocystin and aflatoxin biosynthetic pathway has been elucidated through a series of research studies involving the use of *Aspergillus* mutants blocked in aflatoxin production, feeding experiments, enzyme inhibition

Fig. 3
Sterigmatocystin and aflatoxin biosynthesis pathway

Fig. 3 (continued)

AFB_1: aflatoxin B_1; AFB_2: aflatoxin B_2; AFG_1: aflatoxin G_1; AFG_2: aflatoxin G_2; AVF: averufin; AVN: averantin; AVNN: averufanin; DHDMSTC: dihydrodemethylsterigmatocystin; DHOMSTC: dihydro-*O*-methylsterigmatocystin; DHSTC: dihydrosterigmatocystin; DMSTC: demethylsterigmatocystin; HAVN: 5-hydroxyaverantin; OAVN: oxoaverantin; OMSTC: *O*-methylsterigmatocystin; NOR: norsolorinic acid; STC: sterigmatocystin; VAL: versiconal; VERA: versicolorin A; VERB: versicolorin B; VHA: versiconal hemiacetal acetate
Source: Yu et al. (2004)

studies and biochemical characterization of enzymatic activities. The generally accepted pathway proposed for the synthesis of sterigmatocystin and aflatoxin from these studies is as follows: polyketide precursor → norsolorinic acid →

averantin → averufanin → verufin → versiconal hemiacetal acetate → versiconal → versicolorin B → versicolorin A → demethylsterigmatocystin → sterigmatocystin → OMSTC → AFB_1 and AFG_1 (Keller, Kantz & Adams, 1994; Yu et al., 2004). A branch point in the pathway at versiconal hemiacetal acetate leading to production of AFB_2 and AFG_2 and other metabolic grids providing an alternative pathway to aflatoxin biosynthesis was established (Cleveland et al., 1987; Bhatnagar, Ehrlick & Cleveland, 1992; Yabe, Nakamura & Hamasaki, 1999).

Wild-type *A. nidulans* and *A. versicolor* are apparently unable to transform sterigmatocystin into OMSTC, the direct precursor of AFB_1 and aflatoxin G_1 (AFG_1). Food matrices infected by these fungi can contain high levels of sterigmatocystin, whereas substrates infected by *A. flavus* and *A. parasiticus* may contain low amounts of sterigmatocystin because most of it will be converted to aflatoxin (Fig. 3; Sweeney & Dobson, 1999; Yu et al., 2004; Carbone et al., 2007). Notable examples are *A. nidulans* synthesizing only sterigmatocystin; *A. flavus* synthesizing predominantly aflatoxin; and *A. parasiticus* producing aflatoxin or OMSTC depending on the strain.

3.2 Description of analytical methods

3.2.1 Introduction

Sterigmatocystin has been reported to be produced by many phylogenetically and phenotypically different fungal genera including more than two dozen species each of *Aspergillus* and *Emericella*, and one or more species of *Bipolaris, Botryotrichum, Chaetomium* (*Humicola*), *Monocillium, Moelleriella* (*Aschersonia*), *Podospora* and a unique species of *Penicillium, P. inflatum*, now known as *A. inflatus* (Rank et al., 2011; Jurjević et al., 2013; Samson et al., 2014; Hubka et al., 2016). The names in parentheses are no longer in use.

Klich (1993) elucidated micro- and macro-morphological features of 24 fungal species belonging to *Aspergillus* section *Versicolores* and related species to assist in identifying these taxa. Barnes et al. (1994) reported that seven species of *Aspergillus*, including four strains of *A. versicolor,* one of *Bipolaris* and two of *Chaetomium*, were able to produce sterigmatocystin on a chemically defined medium and on three food commodities. Two species of *Aspergillus* in the section *Ochraceorosei*, *A. ochraceoroseus* and *A. rambellii*, produce both sterigmatocystin and aflatoxin (Rank et al., 2011). Although morphologically distinct from *A. nidulans,* molecular characterization of *A. ochraceoroseus afl*/*stc* genes and physiological characteristics of aflatoxin/sterigmatocystin production indicated that *A. ochraceoroseus* is more closely related to *A. nidulans* than to *A. flavus* (Cary et al., 2009).

Rank et al. (2011) re-examined all available culture collection fungal strains of the original producers, in addition to ex type and other strains of each species reported to produce sterigmatocystin and biosynthetically derived aflatoxins. They also screened strains of all available species in *Penicillium* and *Aspergillus* for sterigmatocystin and aflatoxin. They reported six new sterigmatocystin-producing fungi, *A. asperescens, A. aureolatus, A. eburneocremeus, A. protuberus, A. tardus and P. inflatum,* and one new aflatoxin producer, *A. togoensis* (= *Stilbothamnium togoense*). Sterigmatocystin was confirmed in 23 *Emericella*, four *Aspergillus*, five *Chaetomium*, one *Botryotrichum* and one *Humicola* species grown on a selection of secondary metabolite–inducing media and using multiple detection methods. OMSTC was found in *Chaetomium cellulolyticum, C. longicolleum, C. malaysiense* and *C. virescens*, but aflatoxin was not detected from any *Chaetomium* species (Rank et al., 2011).

Jurjević et al. (2013) found nine newly described *Aspergillus* species isolated from various matrices, *A. amoenus, A. creber, A. cvjetkovicii, A. fructus, A. jensenii, A. puulaauensis, A. subversicolor, A. tennesseensis* and *A. venenatus* in section *Versicolores,* that produce sterigmatocystin in liquid media.

3.2.2 Screening tests

Screening tests for sterigmatocystin range from TLC to rapid test kits based on antibodies.

Prior to the mid-1990s only a very limited number of research papers were published on qualitative and semiquantitative methods of sterigmatocystin screening.

Many immunoassays and antibodies have been developed, and manufacturers continue to offer test kits based on ELISA or immuno-chromatographic devices as screening tests for mycotoxins. Few such kits are available for routine screening of samples for sterigmatocystin in food, however. Some of these kits are rapid and portable and less expensive, but have limitations in terms of selectivity and reproducibility. Detection results should be confirmed with quantitative methods.

Little information is available on quantification of *A. versicolor* and sterigmatocystin-producing fungi in foods. However, Rodriguez et al. (2012) used two real-time quantitative polymerase chain reaction (qPCR) methods to quantify sterigmatocystin-producing fungi in raw material, food ingredients and pre-processed foods, with a minimum LOD of 1 $\log_{10}$ cfu/g. They opined that the qPCR method would be very useful for monitoring sterigmatocystin-producing fungi in hazard analysis and critical control point (HACCP) programmes to prevent accumulation of the toxin in processed foods.

3.2.3 Quantitative methods

Stack & Rodricks (1971, 1973) reported a validated official method for analysing sterigmatocystin based on TLC, with an LOQ of 30 µg/kg in maize and 100 µg/kg in wheat and barley. Stroka et al. (2004) developed a simple TLC-based method with reagent-free derivatization for routine analysis of sterigmatocystin in cereal-based products. With effective extraction and clean-up (solid-phase extraction column conditioned with methanol and water) steps, low limits of LOD or LOQ can be achieved for analysis of sterigmatocystin with methods such as TLC. Some laboratories still rely on TLC as a simple, cost-effective semiquantitative method for determining sterigmatocystin and even other mycotoxins. A TLC-based method has been officially validated by the Association of Official Analytical Chemists (1995).

Methods based on gas chromatography were used for quantifying sterigmatocystin in cereals with an LOD of 5–50 µg/kg (Manabe, Minomisawa & Matsuura, 1973; Salhab et al., 1976).

Veršilovskis & De Saeger (2010) determined sterigmatocystin from different food and feed matrices by TLC with fluorescence detection and reported LODs ranging from 2 to 140 µg/kg.

Further advances in the analysis of sterigmatocystin have been achieved by applying mass spectrometry detection systems to determine multiple mycotoxins, including sterigmatocystin. Stack et al. (1976) reported using HPLC to determine sterigmatocystin as a single analyte method; others subsequently published similar reports (Schmidt et al., 1981; Abrahamson & Thorsteinson, 1989; Scudamore, Nawaz & Hetmanski, 1998; Tanaka et al., 2007; Veršilovskis, Bartkevičs & Miķelsone, 2008). HPLC was applied for multi-mycotoxin assays in different food matrices with LODs ranging from 0.3 to 100 µg/kg (Hurst et al., 1987; Scudamore, Nawaz & Hetmanski, 1998; Tanaka et al., 2007; Tangni & Pussemier, 2007).

Veršilovskis, Bartkevičs & Miķelsone (2008) analysed 95 samples of Latvian grains obtained in 2006 and 120 samples obtained in 2007 using an LC-MS/MS method with an LOQ of 0.15 µg/kg and an LOD of 0.030 µg/kg. They found that 14% of the 2006 samples were contaminated with sterigmatocystin at concentrations from 0.7 to 83 µg/kg, and 35% of the 2007 samples were contaminated with sterigmatocystin at 1–47 µg/kg. A combined total for both years showed 26% of the samples contained sterigmatocystin above the LOD. The highest concentrations were found in wheat and barley and the lowest in oats and rye, with buckwheat in the middle.

LC-MS/MS methods with low LODs are available to determine sterigmatocystin in food matrices. Scudamore et al. (1997) described the determination of sterigmatocystin at levels less than 5 µg/kg in cheese, bread

and maize products by HPLC with atmospheric pressure ionization mass spectrometric detection. For cereals, a sensitive LC–electrospray ionization (ESI) MS/MS method was developed and validated for sterigmatocystin determination with the lowest validation level at 0.5 µg/kg (Veršilovskis, Bartkevičs & Miķelsone, 2007). This LC-MS/MS method was modified and applied to the analysis of sterigmatocystin in cheese samples by Veršilovskis, Van Peteghem & De Saeger (2009) with an LOD of 0.03 µg/kg.

LODs of sterigmatocystin ranging from 0.4 to 10 µg/kg in pistachios, sweet peppers, food supplements, beer and cereal grains have been reported in several multi-mycotoxin LC-MS methods (Sulyok, Krska & Schuhmacher, 2007; Di Mavungu et al., 2009; Monbaliu et al., 2009; Martos, Thompson & Diaz, 2010; Zachariasova et al., 2010).

Spanjer, Rensen & Scholten (2008) developed a method for the simultaneous detection of 33 mycotoxins, including sterigmatocystin, in peanuts, pistachios, wheat, maize, cornflakes, raisins and figs. The mycotoxins were extracted with an acetonitrile/water mixture, diluted with water and then directly injected into an LC-MS/MS system. The mycotoxins were separated by reversed-phase HPLC and detected using an electrospray ionization (ESI) interface and tandem mass spectrometry, using multiple reaction monitoring in the positive ion mode, to increase specificity for quality control. The mycotoxins were analysed in a single 30-minute run and the LOQ ranged from 1 to 200 µg/kg.

Significant developments in detecting sterigmatocystin have been achieved by applying LC-MS/MS as a single or multi-analyte method using an immunoaffinity column (IAC) for sample clean-up prior to analysis by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) and LC-MS/MS. To avoid the cross-reactivity of aflatoxin with sterigmatocystin that appears in some of the commercially available IACs used for sample clean-up prior to analysis, Sasaki et al. (2014) developed a method for analysing sterigmatocystin in grain using an IAC and LC-MS. The average percentage recovery (RSD%) of sterigmatocystin in the grains was 95.8 (3.3) at 5.0 µg/kg and 97.8 (1.5) at 100 µg/kg. The LOD was 2.5 pg (1 µg/kg), average recovery of wheat spiked at 5 µg/kg was 99.3% and the measurement uncertainty of the method was 10.2% ($k = 2$).

Using an LC-MS equipped with an electrospray ionization interface, Jurjević et al. (2013) reported detection of sterigmatocystin from *Aspergillus* species in mycological broths isolated from date fruit, indoor air, coffee berry and toxic dairy feed with an LOD of 3 ng/mL and LOQ of 10 ng/mL.

Marley et al. (2015) spiked cereals (wheat, oats, rye, maize and rice), sunflower seeds and animal feed with sterigmatocystin at 0.75–50 µg/kg to establish method performance. With IAC clean-up followed by HPLC, recoveries ranged from 68% to 106%, with repeatability from 4.2% to 17.5%. The LOQ with ultraviolet detection in these matrices was 1.5 µg/kg. For beer and cheese spiked at

5.0 μg/kg, the recoveries were 94% and 104%, and precision (average percentage recovery; RSD%) was 1.9% and 2.9%, respectively. When using a method based on LC-MS/MS to analyse sterigmatocystin in beer and cheese, the LOQs were 0.02 and 0.6 μg/kg, respectively. The IAC system for analysis of sterigmatocystin was demonstrated to provide an efficient clean-up of various food matrices to enable this mycotoxin to be determined by either HPLC-UV or LC-MS/MS.

Yogendrarajah et al. (2013, 2014a,b) used LC-MS/MS to detect multi-mycotoxins in spices in Sri Lanka and Belgium, employing a method based on a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach developed in-house. A simple extraction and clean-up process involved extracting the toxins with acetonitrile, acid and salt. The researchers analysed 168 spice samples collected from different regions of Sri Lanka during 2011–2012 and found that 43% of the black peppers (15.4 ± 9.8 μg/kg) and 45% of the white peppers were contaminated with sterigmatocystin with an LOQ less than 49 μg/kg.

Biancardi & Dall'Asta (2015) used an LC-MS/MS method based on a solid–liquid extraction and a dilute-and-shoot approach to analyse sterigmatocystin in cereals and feed. The mean overall recovery (n = 24; LOQ included) was 99%, with a confidence interval of 0.8% and a coefficient of variance of 1.9%. The method was then applied to 14 naturally incurred feed samples. Sterigmatocystin was detected at lower concentrations (0.2–2.2 μg/kg) and AFB_1 in the range of 28.7–240.1 μg/kg. They reported that this method might represent a valuable choice, ensuring a high level of accuracy and precision as well as high-throughput performance. They concluded that the method meets the recommendations, as expressed by EFSA, in terms of availability of fast and sensitive methods (recommended LOQ of 1.5 μg/kg) in order to increase data collection to allow for the assessment of dietary exposure (EFSA, 2013).

Because of a lack of suitable data on the occurrence of sterigmatocystin in food that could be used to characterize its risk for human health, the EFSA CONTAM Panel called for a proposal to generate such data (EFSA, 2013). Mol et al. (2015) presented analytical results from 247 food samples submitted by two Member States; all were below the LOD or LOQ. The EFSA-commissioned survey was conducted by four independent laboratories that used broadly similar methods for extraction, clean-up, detection and quantification of sterigmatocystin (Mol et al., 2015); all used LC-MS/MS as well as some different chromatographic conditions. The MS/MS measurement was performed using positive electrospray ionization. Quantification was based on multilevel calibration with solvent standards of sterigmatocystin concentrations of 0.2–10 μg/kg and internal standards at 1.5 μg/kg. The laboratories reported LODs of 0.05–0.15 μg/kg and an LOQ of 0.5 μg/kg.

The lowest validated levels of sterigmatocystin in food matrices, as reported in the literature, varied from 1 to 5 μg/kg (compared with 0.5 μg/kg in

the EFSA survey; Mol et al., 2015). From recent published information, it can be concluded that LC-MS/MS is more sensitive and has a better validation criterion with linearity of not more than 20%; recovery of 50–120% for 0.5 μg/kg and 70–110% for 5 μg/kg; and precision with repeatability (RSD_f) not exceeding 30% for 0.5 μg/kg and 23% for 5 μg/kg. These were derived from European Commission Regulation (EC) No. 401/2006 for AFB_1 for analysis of sterigmatocystin in cereal grain, cereal products (including beer) and nuts with LODs in the range of 0.05–0.15 μg/kg for grain, cereal products and nuts and 0.005–0.01 μg/kg for beer. Methods published in scientific literature in the past decade indicate that LC-MS/MS is a fast, accurate and reproducible technique for quantification of sterigmatocystin in foods and feeds; many of the methods used were similar in the extraction and clean-up procedures prior to quantification, with reported LODs of 0.2–1 μg/kg and LOQs of 0.3–3 μg/kg.

Nonchromatographic methods to detect and quantify sterigmatocystin have been reported. Yao et al. (2006) developed a biosensor constructed by multi-walled carbon nanotubes to detect sterigmatocystin. Chen et al. (2010) also developed a rapid and highly sensitive electrochemical biosensor for the detection of sterigmatocystin based on a new enzyme, named aflatoxin oxidase, cloned from fungus *Armillariella tabescens* and expressed in yeast *Pichia pastoris*.

Certified reference materials for quantification of sterigmatocystin in food matrices are unavailable. Tanaka et al. (2008) prepared a reference material for sterigmatocystin in brown rice containing a colouring agent, Food Red 106, as an indicator compound to monitor the homogeneity of the mixed material. There are no proficiency testing or quality assurance interlaboratory schemes for the analysis of sterigmatocystin in food or feed.

3.2.4 Quantitative methods for modified sterigmatocystin

There is no published information on reactions leading to modified, hidden, bound or masked sterigmatocystin in food and feed.

4. Sampling protocols

Because no sampling protocols specific to sterigmatocystin were found in the published literature, sampling plans for mycotoxins in general and for aflatoxins in particular (if available) were studied.

Mycotoxins are known to be unevenly distributed in batches of food. To obtain a representative sample, it is necessary to take large numbers of small incremental samples from different parts of a consignment. Sampling is done at various points in the food chain including primary production, storage, produce

for export, wholesale, processing and retail. The European Commission (EC No. 401/2006) has provided official procedures for sampling food for mycotoxin analysis (European Commission, 2006, 2010). Samples should be taken from the same lot with the same batch code and placed in a clean, dry and leak-proof container that can be securely sealed and stored in a controlled environment prior to analysis. Incremental samples of the appropriate weight should be taken at various points throughout the lot. The samples should be combined in one container to make up the aggregate sample for analysis.

An effective sampling plan is a prerequisite for control of mycotoxins in food and feed due to the heterogeneous distribution of mycotoxins in food and feed. FAO has developed a mycotoxin sampling tool for 26 mycotoxin–commodity combinations that provides support in analysing the performance of sampling plans and determining the most appropriate plan to meet the user's defined objectives (FAO, 2014).

Wesolek & Roudot (2014) used a Monte Carlo simulation as a prerequisite for validating a sampling plan. They presented a mathematical approach for sampling performance assessment for AFB_1 in pistachio nuts. They developed an operating characteristic curve that showed risk for both the consumer and the producer. The European Union sampling plan, which consists of testing two samples of 10 kg each, gave a consumer risk with a probability of acceptance at 5% for a lot mean concentration of 75.34 μg/kg and a producer risk with a probability of acceptance at 5% for a lot mean concentration of 1.62 μg/kg (Wesolek, Ramirez-Martinez & Roudot, 2014).

Following alignment with the Codex sampling plan, the EU Commission Regulation (EC No 178/2010) sampling size for aflatoxins in ready-to-eat tree nuts including peanuts, other oilseeds and apricot kernels was reduced from 3 × 10 kg to 2 × 10 kg, with each sample testing below the maximum level. The sampling size for aflatoxins in produce for further processing was reduced from 1 × 30 kg to 1 × 20 kg. This sampling plan might also be applicable to sterigmatocystin in foodstuffs.

An extensive survey of sterigmatocystin in food, commissioned by EFSA, was conducted to gain insight into its occurrence in some food commodities consumed in the European Union. The sampling plan was largely consumer oriented and involved sampling in different countries, namely Cyprus, Germany, Greece, Italy, the Netherlands and the United Kingdom (Mol et al., 2015). Samples were taken by official governmental bodies (Greece, the Netherlands, the United Kingdom), third parties (Food Allergens Laboratory, Greece/Cyprus), cereal trade bodies and food industry collaborators. At the retail level, bulk products were sampled with sampling probes or suction devices. The amount of aggregate sample varied from 1 to 20 kg depending on the type of product and the size of

the lot sampled. A total of 259 samples of cereals and cereal products, nuts and beer were analysed for sterigmatocystin (Mol et al., 2015).

5. Effects of processing

5.1 Sorting and cleaning

Because sorting and cleaning procedures specific to sterigmatocystin were not found in the literature, the effects of sorting and cleaning on aflatoxins in raw materials were examined.

Cleaning methods, such as sieving broken kernels from bulk materials and sorting by physically removing diseased or contaminated kernels, have been shown to reduce aflatoxin and fumonisin levels in corn (Afolabi et al., 2006; Pietri, Zanetti & Bertuzzi, 2009). However, there is no information on such processes reducing sterigmatocystin in food commodities.

5.2 Dry and wet milling

Milling and baking processes have been reported to decrease sterigmatocystin concentrations. Takahashi et al. (1984) reported that the levels of sterigmatocystin in brown rice were reduced by about one third during milling. They concluded that since milling detoxifies the rice intended for food, it is important to establish the safety of rice bran when used as a source of rice oil or livestock feed.

5.3 Thermal processing

Bokhari & Aly (2009) investigated the effect of roasting on sterigmatocystin content in coffee beans and reported a reduction by 70% after 15 minutes. These results were similar to those reported earlier by Levi, Ternk & Yeransian (1975) and Stoloff (1976). However, Veršilovskis & Bartkevičs (2012) investigated the stability of sterigmatocystin during the bread-making process in Latvia and found the toxin to be stable during baking.

Sterigmatocystin has a degradation temperature of 246 °C and is relatively insoluble in water. These physical properties will make it difficult for the toxin to degrade during cooking. However, as roasting of coffee beans at 200 °C for 20 minutes destroyed 68% of added sterigmatocystin under laboratory conditions, roasting may be an effective means of sterigmatocystin control (Levi, Ternk & Yeransian, 1975).

5.4 Alkaline treatment

No published reports on the alkaline hydrolysis of sterigmatocystin in food matrix were identified. However, it has been reported that hydrolysed forms of mycotoxins could be involved in binding with different compounds in the food matrix (Cortez-Rocha et al., 2002).

5.5 Fermentation

Fermentation of sterigmatocystin-contaminated products may reduce or eliminate the toxin.

Abd Alla (1994) reported a reduction of sterigmatocystin from sterigmatocystin-contaminated milk during cheese making depending on the type of starter culture, storage time and type of milk. Abd Alla et al. (1996) and Metwally et al. (1997) reported 80% reduction of sterigmatocystin in the curd and 20% in the whey during cheese making, indicating low solubility of the toxin in aqueous media.

Aly & Elewa (2007) reported that propolis, a strongly adhesive, resinous substance produced by worker honeybees, at a concentration of 1000 ppm completely inhibited growth of *A. versicolor* and production of sterigmatocystin during ripening of Ras cheese.

Veršilovskis, De Saeger & Miķelsone (2008) examined 26 Latvian barley-based beer samples and reported that two were contaminated, indicating that sterigmatocystin can survive the brewing process. In contrast, Matumba et al. (2014) found no sterigmatocystin in maize-based traditionally brewed beer in Malawi.

In conclusion, food processing such as milling, roasting, bread making and cheese making may result in the decrease of sterigmatocystin in foods, but the extent of the decrease depends on the type of food and processing conditions.

5.6 Modified, hidden and bound sterigmatocystin

No published reports on modified, hidden or bound sterigmatocystin in food or feed were identified.

6. Prevention and control

Sterigmatocystin is produced by a variety of fungi, namely *Aspergillus*, *Emericella*, *Bipolaris*, *Botryotrichum*, *Chaetomium*, *Moelleriella*, *Monocillium* and a unique

species of *P. inflatum* closely related to *A. tardus*. The majority of these fungi are storage fungi; fungi present on plants before harvest are referred to as field fungi. Typically these include species of *Cladosporium*, *Alternaria*, *Epicoccum*, *Verticillium*, *Fusarium* and phytopathogenic fungi (Magan & Lacey, 1984; Lacey, 1989). Pitt & Hocking (1997, 2009) reported the occurrence of *A. versicolor* in field crops of wheat and barley but noted that it occurs much more commonly in stored products. In drawing the distinction between field and storage fungi, Christensen & Kaufmann (1969) stated that at harvest the great majority of wheat kernels are free of external contamination by xerophilic storage fungi, which can be isolated on malt salt agar, and that they may be contaminated to some extent by such fungi after harvesting and before arriving at terminal warehouses.

Alternaria and the associated *Gontatobotrys, Cochlioboluts* and *Nigrospora,* which are carried within seeds from the field, gradually disappear in storage and are replaced by *A. versicolor* as well as other species of *Aspergillus, Penicillium, Absidia, Chaetomium* and *Rhizopus* (Sinha & Wallace, 1965; Sinha, Wallace & Chebib, 1969). In a study of barley grains stored in an unrestricted air supply, Hill & Lacey (1983) reported that *A. versicolor* and various *Penicillium* species became dominant at a_w 0.85–0.90 (moisture content 17.2–20.0% at 35 °C and 15–45% germination rate). At a_w between 0.90 and 0.95 (moisture content 20.0–25.3% at 50 °C and 0–15% germination rate), these species were replaced by more thermotolerant species including *A. nidulans* and *Chaetomium* spp.

With rare exceptions, sterigmatocystin is a storage toxin. Fungal and sterigmatocystin contamination of crops on the farm and during harvest and storage can be prevented by good agricultural, handling and hygiene practices in the whole supply chain. Field management practices will prevent contamination of the crop with sterigmatocystin. Maintaining a dry and clean storage facility that is well ventilated and pest- and water-proof will minimize mycotoxin contamination in stored grains. If food commodities are contaminated with sterigmatocystin, several decontamination strategies can be applied by various physical, chemical and biological means. The application and integration of the preventive and control strategies at appropriate stages along the crop value chain from "farm to fork", based on HACCP principles, yield better results. Although applying all these control methods either singly or in combination is effective in reducing mycotoxins to safe limits, it is impossible to obtain absolutely mycotoxin-free food or feed.

6.1 Preharvest control

6.1.1 Tillage and soil types

No papers were found that reported on the direct effects of tillage on contamination of crops with sterigmatocystin.

6.1.2 Planting resistant cultivars

No papers were found that reported on breeding plant cultivars resistant to sterigmatocystin.

6.1.3 Prevention of sterigmatocystin production in fungi

An approach that offers great potential for sterigmatocystin regulation is endowing transgenes with the ability to inhibit production.

6.1.4 Sowing date

No information was found on the appropriate planting date to avoid contamination of crops with sterigmatocystin.

6.1.5 Biocontrol of sterigmatocystin-producing fungi

As mentioned earlier, no specific biocontrol measures that reduce sterigmatocystin contamination were identified from the literature.

6.1.6 Fungicides and inhibitors of sterigmatocystin synthesis

Although there are studies suggesting that various botanicals might be useful as aids to prevent the growth of storage fungi, these have not been widely used.

6.1.7 Harvesting

No information was found on the effect of harvest conditions on contamination of crops with sterigmatocystin. However, the code of practice for reduction of mycotoxins in cereals (FAO/WHO, 2016) recommends that grains be harvested at full maturity and at low moisture content. The implements used for harvesting must be clean and dry and must not be in contact with soil to avoid contamination of grains with mycotoxigenic fungi from the soil. Harvesting methods and equipment should not cause mechanical damage to the grain.

6.2 Postharvest control

6.2.1 Storage of food products and commodities

A. versicolor is able to grow on substrates with a low water activity (a_w between 0.75 and 0.95) and in a temperature range between 4 and 40 °C. The optimum

temperature for toxin production occurs between 23 and 29 °C (Betina, 1989). Cereals and cereal products have been found to contain sterigmatocystin as a result of fungal infestation during storage, transport and processing. However, the toxin has been detected in other plant products including green coffee beans, spices and beer. Sterigmatocystin has also been detected in animal products, for example, cheese. Spores of sterigmatocystin-producing fungi may accumulate in the environment during storage and/or processing, and failure to thoroughly clean the premises could lead to subsequent contamination and production of sterigmatocystin. Adequate storage, cleaning, hygiene and monitoring would eliminate or minimize contamination and subsequent production of sterigmatocystin and enable long-term storage. In closed storage environments such as granaries, warehouses and processing units (e.g. for cheese making), control of relative humidity and temperature and general cleanliness are essential prerequisites in preventing fungal production of sterigmatocystin. Mills (1989) reported detailed accounts of the characteristics of storage of specific commodities to prevent, detect and control sterigmatocystin and other mycotoxins in agricultural products.

Sterigmatocystin contamination during storage can be prevented when the harvested crop is stored under conditions that do not favour toxin production. Using a predictive mathematical model that they validated with independent data, Yogendrarajah et al. (2016) estimated that the minimum temperature for the growth of *A. flavus* and *A. parasiticus* was 11–16 °C and the minimum water activity 0.73–0.76; the researchers then went on to prove that sterigmatocystin is not produced by these fungi in black peppercorn except under these conditions. Mycelial growth and toxin synthesis by sterigmatocystin-producing fungi are depressed when CO_2 concentration is at 40%, but a level of 90% CO_2 is required to completely inhibit production of the toxin (Weidenborner, 2013). According to the same review, decreasing O_2 concentration to 2% depresses production of sterigmatocystin but does not affect fungal growth, whereas at 0.2% O_2, growth and toxin production are completely inhibited. Therefore, application of controlled atmospheres with increased CO_2 (>10%) and decreased O_2 (≤2%) can be used to retard fungal growth, while sterigmatocystin growth and production can be completely inhibited at controlled atmospheric conditions of 90% CO_2 and 0.2% oxygen. Since sterigmatocystin is produced at high temperature (37 °C), storage at cooler temperatures is effective for control of the toxin (FAO/WHO, 2016). Tanaka et al. (2007) reported that preserving rice in warehouses with moisture content 13–14% (a_w 0.65–0.70, humidity of warehouse controlled to 70–75% and temperature of warehouse also controlled and maintained at less than 15 °C) has prevented postharvest mycotoxin contamination in rice in Japan.

6.3 Decontamination

6.3.1 Irradiation

Two reports were found on the effectiveness of irradiation in detoxifying crops from sterigmatocystin. Kume et al. (1983) reported that the growth of *A. versicolor* and production of sterigmatocystin were completely abolished in dry states by gamma irradiation at 0.7 mrad (7 μGy) and 52 mrad (0.52 mGy), respectively. The radiosensitivity of the fungi and toxin to gamma radiation was higher in the wet states. Aziz & Refai (1989) exposed dairy cattle feed contaminated with *A. versicolor* and sterigmatocystin to doses of gamma radiation increasing from 100 rad (1 Gy) to 1000 rad (10 Gy). The feeds were completely free of *A. versicolor* and sterigmatocystin at lower doses of 400 rad (4 Gy) and 800 rad (8 Gy), respectively.

6.3.2 Chemical detoxification

Various chemicals including acids, bases (ammonia, sodium hydroxide), oxidizing reagents (hydrogen peroxide, ozone), reducing agents (bisulfite, sugars), chlorinating agents (chlorine), salts and other reagents such as formaldehyde have been tested for their abilities to degrade mycotoxins. Results show varying levels of effectiveness in the degradation of different toxins, with ammoniation and ozonation given the greatest attention (Abdel-Wahhab & Kholif, 2008). No information on chemical detoxification of specifically sterigmatocystin was found.

6.3.3 Microbial degradation

No data were available on microbial degradation of sterigmatocystin in harvested crops.

6.3.4 Enzymatic degradation

Except for a report on the metabolism of sterigmatocystin in mammalian systems (Pfeiffer, Fleck & Metzler, 2014), there are no reports on the application of enzymes in the elimination of sterigmatocystin in contaminated agricultural produce.

6.3.5 Prevention of absorption of sterigmatocystin in gastrointestinal tract by adsorbents

The use of nonnutritive physical materials referred to as binders, such as adsorbents, to prevent absorption of mycotoxins in the guts of animals is an effective control strategy against mycotoxin exposure. Abdel-Wahhab et al. (2005) demonstrated that the sterigmatocystin adsorption capacity of the binder montmorillonite was between 93.1% and 97.8% in vitro. The montmorillonite–

sterigmatocystin adsorption complex formed was stable at pH 2, 7 and 10 at 37 °C, indicating that the binder would be effective in the stomach, small and large intestines and rumen, and during sharp changes in pH in the gut.

In an in vivo study, the same researchers used the binder to prevent toxicity and clastogenicity in Nile tilapia fish administered sterigmatocystin intragastrically.

6.4 HACCP and integrated mycotoxin management system

One possible approach to managing the risks associated with mycotoxin contamination is the use of an integrated system based on the HACCP approach in the whole supply chain (Lopez-Garcia, Park & Phillips, 1999).

There are no data on integrated sterigmatocystin management systems.

7. Levels and patterns of contamination in food commodities

Data on sterigmatocystin contamination come from governments (FAO database) and from around 50 papers published mainly after 2000. The first observation concerns the low number of data and the low level of contamination (occurrence and contamination) in the last years: 6% of detected data over 4229 measurements in the FAO database. The two products with detection are feed and sorghum. Generally the only food products tested are the cereals and cereal-based products. Very little information relating to other food groups is available.

7.1 Surveillance data

7.1.1 Contamination in WHO regions

Table 15 shows food data obtained from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database.

(a) African Region

The African Region has the highest occurrence of sterigmatocystin, with 1083 data points noted in the database. They came from three countries: Burkina Faso, Ethiopia and Mali. The only product analysed was sorghum, with 233 positive samples. The LOD and LOQ values are 1.25 and 2.5 µg/kg for all the data, which are quite low and can explain the high occurrence. However, around 10% of the

Table 15
Description of the food data from GEMS/Food contaminants database by WHO region

	Concentration data (µg/kg)				
	African Region	Region of the Americas	European Region	Eastern Mediterranean Region	Western Pacific Region
Cereals					
N	1 083	1 941	239	450	51
<LOD (%)	78.5	98.6	100	97.1	100
Mean LB	12.6	0.04	0	0.18	0
Mean UB	38.9	0.63	5.21	2.60	0.1
Food for infants					
N		450			
<LOD (%)		96.7			
Mean LB		0.11			
Mean UB		0.41			
Legumes					
N		3			
<LOD (%)		100			
Mean LB		0			
Mean UB		0.4			
Nuts and oilseeds					
N		1			
<LOD (%)		100			
Mean LB		0			
Mean UB		0.4			
Snacks and desserts					
N			6		
<LOD (%)			100		
Mean LB			0		
Mean UB			3.6		
Starchy roots					
N		5			
<LOD (%)		100			
Mean LB		0			
Mean UB		0.4			

GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; <LOD (%): percentage of the values below the LOD; mean LB: mean concentration (LB approach); mean UB: mean concentration (UB approach); *N*: number of data points in the database; UB: upper bound; WHO: World Health Organization

positive samples have a contamination higher than or equal to 100 µg/kg, with a maximum of 1130 µg/kg for Burkina Faso, 1189 µg/kg for Ethiopia and 255 µg/kg for Mali.

(b) **Region of the Americas**

The database contains 2400 data points for cereals and cereal-based products (1941), food for infants (450) and, to a lesser extent, starchy roots (5), nuts (1) and legumes (3). The only contributing country is Canada. The LOD is between 0.4 and 3 µg/kg and the LOQ between 5 and 9 µg/kg. Forty-two samples were positive (1.75%).

(c) **European Region**

Three countries (Czech Republic, the Netherlands and the United Kingdom) contributed 245 data points. Only two groups were sampled: cereals and cereal-based products (239) and snacks and desserts (6). The LODs are 3 µg/kg and 6 µg/kg, and the LOQs between 10 µg/kg and 20 µg/kg. No positive samples were found.

(d) **Eastern Mediterranean Region**

The only contributing country is Sudan with 450 data points from sorghum flour. Of these, 13 were positive with a maximum of 8.8 µg/kg. The LOD was always 1.25 µg/kg and the LOQ 2.5 µg/kg.

(e) **Western Pacific Region**

The only contributing country is Japan with 241 data points from feed (190) and from cereals and cereal-based products (51). The only positive samples came from feed (29 positive samples) with a maximum contamination of 3 µg/kg. The LOD is in the range of 0.3–0.5 µg/kg and the LOQ in the range of 0.5–1 µg/kg.

No other WHO region is represented in the database.

7.1.2 Contamination in countries as reported in published papers

All results are summarized in Table 16.

(a) **African Region**

Burkina Faso

One paper reported a low level of contamination of maize, groundnuts and other food products (<2.5 µg/kg for maize and 8.6 µg/kg for other food). No occurrence was found in groundnuts.

Cameroon

Different products were analysed in a 2013 paper, with high levels of detection but low levels of contamination (maximum at 9 µg/kg for groundnuts).

Table 16
Occurrence and contamination of food by sterigmatocystin

Region / Country	Sample	No. of samples	>LOD (%)	Min. (µg/kg)	Mean[a] (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
African										
Burkina Faso	Maize	26	8	2.2	2.3 (med)	2.5	2	–	LC-MS/MS	Warth et al. (2012)
	Groundnut	9	0	–	–	–	–	–		
	Others	30	7	4.8	6.7 (med)	8.6	–	–		
Cameroon	Maize	37	27	–	2	3	0.15	–	LC-MS/MS	Abia et al. (2013)
	Groundnut	35	51	–	5	9	0.15	–		
	Groundnut soup	15	33	–	0.6	1	0.1	–		
	Kuru-kuru[b]	6	50	–	1	1	–	–		
	Dagwa[b]	8	75	–	1.4	3	–	–		
Malawi	Maize beer	9	0	–	–	–	–	–	LC-MS/MS	Matumba et al. (2014)
Mozambique	Maize	13	8	2.7	–	–	2	–	LC-MS/MS	Warth et al. (2012)
	Groundnuts	23	5	9.7	–	–	–	–		
	Other	7	29	3	26.1 (med)	49.2	–	–		
Nigeria	Rice	38	44	–	18.99 (med: 0.75)	124.95	0.01	–	LC-MS/MS	Rofiat et al. (2015)
	Stored maize	70	37	0.4	3	17	–	0.4	LC-MS/MS	Adetunji et al. (2014)
Americas										
Brazil	Brazil nut	5	20	5.9	–	–	1.5	–	LC-MS/MS	Freitas-Silva et al. (2011)
Mexico	Maize	2	100	–	6.5	–	–	–	LC-MS/MS	Peña Betancourt et al. (2015)
South-East Asia										
Sri Lanka	Chilli	86	–	–	–	–	–	–	LC-MS/MS	Yogendra-rajah et al. (2014b)
	Black pepper	82	43	–	15.4	–	–	–		
	Chilli						–	–	LC-MS/MS	Yogendra-rajah et al. (2014a)
	pod	18	28	<LOQ	34.3	31.8				
	flake	26	39	<LOQ	<LOQ	<LOQ				
	powder	42	43	<LOQ	13	13.6				
	Pepper						–	–	LC-MS/MS	Yogendra-rajah et al. (2014c)
	black	82	43	<LOQ	15.4	49				
	white	11	45	<LOQ	15.4	49				
	Red chilli	10	40	<LOQ	–	<LOQ	–	11	LC-MS/MS	Yogendra-rajah et al. (2013)
	Black pepper	10	50	<LOQ	–	<LOQ	–	8		
	White pepper	10	80	<LOQ (for 4 samples)	24 (mean of 4 samples)	–	–	16		

Table 16 (continued)

Region / Country	Sample	No. of samples	>LOD (%)	Min. (µg/kg)	Mean[a] (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
European										
Belgium	Cheese	13	15	0.52	–	1.23	0.03	0.1	LC-MS/MS	Veršilovskis, Van Peteghem & De Saeger (2009)
	Chilli	35	28	<LOQ	<LOQ	<LOQ	–	–	LC-MS/MS	Yogendra-rajah et al. (2014b)
	Pepper	27	40	7.7	–	77.1	–	–	LC-MS/MS	Yogendra-rajah et al. (2014a)
	Cereals	14	100	1.1	1.3 (med)	3.2	0.1	0.3	HPLC	Tangni & Pussenier (2007)
Germany	Paprika	50	–	–	Traces	–	3	–	LC-MS	Reinholds, Pugajeva & Bartkevics (2016)
Italy	Wheat	46	0	–	–	–	0.25	1.5	LC-MS/MS	Alkadri et al. (2014)
	Chestnut			–	–	–	0.1	0.3	LC-MS/MS	Bertuzzi, Rastelli & Pietri (2015)
	fresh	32	0							
	dried	25	0							
	flour	25	0							
	Durum wheat	74	0	–	–	–	3	5	LC-MS/MS	Juan et al. (2016)
Latvia	Wheat	50	18	>0.25	–	<200	–	–	LC-MS/MS	Veršilovskis, Bartkevičs & Miķelsone (2008; using 2006 data)
	Barley	10	20	>0.25		<25				
	Oat	15	0							
	Buckwheat	10	20	>0.25		<25				
	Rye	10	0							
	Wheat	20	40	>0.25		<200			LC-MS/MS	Veršilovskis, Bartkevičs & Miķelsone (2008; using 2007 data)
	Barley	25	44	>0.25		<200				
	Oat	25	24	>0.25		<25				
	Buckwheat	25	36	>0.25		<200				
	Rye	25	32	>0.25		<25				
	Beer	26	7	4 (µg/L)	–	7.8 (µg/L)	0.26 (µg/L)	0.68 (µg/L)	HPLC-UV	Veršilovskis, De Saeger & Miķelsone (2008)
	Cheese	8	0	(<LOQ)	–	–	0.03	0.1	LC-MS/MS	Veršilovskis, Van Peteghem & De Saeger (2009)

Region / Country	Sample	No. of samples	>LOD (%)	Min. (µg/kg)	Mean[a] (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
	Bread	29	17	2.4	3.74	7.1	–	–	LC-MS/MS	Veršilovskis & Bartkevičs (2012)
Spain	Brewed coffee	169	16	7.65	22.63	63.19	1	2.04	LC-MS/ MS-IT	García-Moraleja et al. (2015a)
	Roasted coffee	3	66	23.77	–	36.54	1	2.04	LC-MS/MS	García-Moraleja et al. (2015b)
	Instant coffee	3	0	–	–	–	–			
	Cheese			–	–	–	20	–	TLC	Lopez-Diaz et al. (1996)
	manchego	12	0							
	blue	10	0							
Europe	Wheat soft	169	6	0.06	–	0.65	0.05–0.15	0.5 µg/kg	LC-MS/MS	Mol et al. (2015)
	Wheat hard	52	4	0.27		0.79				
	Rye	35	6	0.27		0.75				
	Maize	33	6	0.64		1.3				
	Rice crop	28	96	0.14		5.5				
	Barley	59	2	1.9						
	Oat	51	22	0.17		33				
	Spelt	2	0							
	Grain to be processed	125	5	0.05		0.66				
	Rice to be processed	89	21	0.06		2.2				
	Pasta	115	5	0.12		0.3				
	Bread	143	7	0.17		1.7				
	Breakfast cereals	97	19	0.12		1				
	Baked goods	90	7	0.23		0.50				
	Infant cereal	54	7	0.20		0.90				
	Beer	53	0							
	Peanuts	28	0							
	Hazelnuts	36	0							
Eastern Mediterranean										
Egypt	Spices (20 types)						–	–	TLC	El-Kady, El-Maraghy & Eman Mostafa (1995)
	Red pepper	5	60	10	–	23				
	Caraway	5	60	14	–	18				
	Cumin	5	60	–	11	–				
	Marjoram	5	20	–	17	–				
	Peanut seed								TLC, ELISA	Youssef et al. (2008)
	untreated	20	–	–	–	–				
	roasted	20	15	12.2	–	16.8				
	roast salted	20	5	12.2	–	–				
	Ras cheese	100	35	10	22.23	37.7			TLC	Abd Alla et al. (1996)

Table 16 (continued)

Region / Country	Sample	No. of samples	>LOD (%)	Min. (µg/kg)	Mean[a] (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
United Arab Emirates	Date fruit	16	0	–	–	–	–	–	TLC-UV	Shenasi, Candlish & Aidoo (2002)
Saudi Arabia	Coffee beans	30	6.6	11	40% decrease over 10 min roasting time; 65% over 12 min; 70% over 15 min	13	–	–	TLC	Bokhari & Aly (2009)
Syrian Arab Republic	Wheat	40	10	1.2	1.4	1.6	0.25	1.5	LC-MS/MS	Alkadri et al. (2014)
Western Pacific										
China		1 580							ELISA	Tian & Liu (2004)
	Wheat		98		68.9					
	Corn		89		32.2					
	Rice		72		13.9					
	Sesame butter	30	10	1.5	2.7	5.1	0.05 ng/mL	0.15 ng/mL	LC-MS/MS	Liu et al. (2014)
Japan	Rice	48	0						GC-MS; LC-MS; LC-MS/MS; LC-UV	Tanaka et al. (2007)

ELISA: indirect enzyme-linked immunosorbent assay; GC-MS: gas chromatography–mass spectrometry; HPLC: high-performance liquid chromatography; HPLC-UV: high-performance liquid chromatography with ultraviolet detection; LC-MS: liquid chromatography–mass spectrometry; LC-MS/MS: liquid chromatography–tandem mass spectrometry; LC-MS/MS-IT: ion trap liquid chromatography–tandem mass spectrometry with ion trap; LC-UV: liquid chromatography with ultraviolet detection; LOD: limit of detection; LOQ: limit of quantification; Min.: minimum; Max.: maximum; med: median; No.: number; TLC: thin-layer chromatography; TLC-UV: thin-layer chromatography with ultraviolet detection

[a] Mean from quantified measurement.

[b] Dagwa and kuru-kuru are traditional snacks made of maize and groundnuts.

Malawi

One study of maize beer found no occurrence of sterigmatocystin.

Mozambique

A study reported contamination of maize, groundnuts and other food products with low occurrence (generally one positive sample).

Nigeria

Two publications were found on rice and stored maize with high occurrence (44% for rice and 37% for maize) and high levels of contamination (up to 125 µg/kg for rice and 17 µg/kg for maize).

(b) **Region of the Americas**

Brazil

One study examined contamination of five samples of Brazil nuts; one sample was contaminated (5.9 µg/kg).

Mexico

One paper was published on two samples of maize. Both were contaminated at an average level of 6.5 µg/kg.

(c) **South-East Asia Region**

Sri Lanka

The occurrence of contamination is high (between 28% and 80%), but generally at less than the LOQ.

(d) **European Region**

Belgium

Four papers were published on pepper, chilli, cheese and cereals. Occurrence could be high (between 15% and 100%), but the levels of contamination were low (maximum 3.2 µg/kg) except for pepper (77.1 µg/kg).

Germany

The only paper on sterigmatocystin contamination was on paprika, where only traces were found.

Italy

Three recent papers on wheat and chestnuts reported no positive samples detected.

Latvia

Five papers on cereals, beer, cheese and bread reported that occurrence can be high (maximum 40% for wheat) but contamination levels, when indicated, are low.

Spain

Three papers reported on coffee and cheese. There were no positive samples on cheese whereas the occurrence in coffee was high (maximum 66%) and there was a high level of contamination (maximum 63.19 µg/kg).

Europe

An external scientific report by RIKILT for EFSA took into account food from all over Europe (Mol et al., 2015). Occurrence of sterigmatocystin was found to be quite low except for rice (96%), oats (22%) and breakfast cereals (19%). Mean contamination was low except for oats (33 µg/kg).

(e) **Eastern Mediterranean Region**

Egypt

Two studies (1995 and 1996) on spices and Ras cheese reported high levels of contamination (maximum 37.7 µg/kg for cheese) and high occurrence (60% in five samples of spices). Another study of peanuts reported a lower occurrence (maximum 15%).

Saudi Arabia

One paper reported low occurrence (6.6%) of contamination of coffee beans, decreasing with duration of roasting.

Syrian Arab Republic

One paper was published on wheat, with a low level of contamination (around the LOQ).

United Arab Emirates

One paper on date fruits reported no occurrence.

(f) **Western Pacific Region**

China

One paper reported on a high occurrence of sterigmatocystin in cereals and high levels of contamination (up to 68.9 µg/kg on average), and another on sesame butter, with low occurrence (3 samples) and low concentrations (lower than 5.1 µg/kg).

Japan

One publication reported no occurrence in rice.

7.1.3 Contamination in feed

Sterigmatocystin feed contamination, summarized in Table 17, varies depending on geographical area, from very low occurrence in Europe to very high occurrence and high concentrations in the Russian Federation based on few samples; relatively low occurrence and contamination levels in Africa; and very high occurrence and contamination levels in Argentina and Japan based on few samples. Thus, it is difficult to clearly see the real situation based on the published results in comparison with the GEMS/Food database, where feed contamination was listed only for Japan (with low contamination).

7.1.4 Co-occurrence of sterigmatocystin with other mycotoxins

Abramson et al. (1999) investigated the production of mycotoxins in hulless barley at different moisture contents and reported simultaneous occurrence and development of ochratoxin A, citrinin and sterigmatocystin in the substrate. These mycotoxins reached mean levels of 24, 38 and 411 µg/kg, respectively, by 20 weeks in 19% moisture of hulless barley. LODs for ochratoxin A, citrinin and sterigmatocystin were approximately 2, 10 and 20 µg/kg, respectively. The authors stated that the study was the first report on the development of these three mycotoxins simultaneously in the same food substrate.

Co-occurrence of sterigmatocystin and aflatoxin is also reported (EFSA, 2013). Using a validated multi-mycotoxin LC-MS/MS method for simultaneous detection of mycotoxins, including sterigmatocystin, in 367 grain samples intended for use as animal feed in Belgium, Monbaliu (2010) found 11 samples of wheat, maize and barley contaminated with sterigmatocystin at concentrations ranging from 6.9 to 574 µg/kg. Three maize samples showed co-occurrence of sterigmatocystin (7 and 574 µg/kg) with AFB_1 (24 and 503 µg/kg) and with AFB_2 (4.3 and 43 µg/kg).

Yogendrarajah et al. (2014a,b) reported co-occurrence of sterigmatocystin with other mycotoxins in spices consumed in Sri Lanka. They further investigated co-occurrence of sterigmatocystin and other mycotoxins in 121 chilli samples collected from various markets in Sri Lanka (n = 86) and Belgium (n = 35) in 2012–2013. All samples of chilli peppers from Belgium were imported retail products. Co-occurrence of different mycotoxins was as follows: AFB_1–ochratoxin A (36%); AFB_1–sterigmatocystin (28%); ochratoxin A–AFB_1–sterigmatocystin (17%); and AFB_1–AFB_2 (14%). Reinholds, Pugajeva & Bartkevics (2016) reported co-occurrence of sterigmatocystin with ochratoxin A and fumonisin B_1 in 16 paprika samples (32%) from the total of 50 samples of paprika grown in Brazil and China.

Table 17
Occurrence and contamination of sterigmatocystin in feed

Region / Country	Sample	No.	%	Min. (µg/kg)	Mean (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
African										
Burkina Faso	Feed	4	75	4.3	6.5 (med)	40.1	2	–	LC-MS/MS	Warth et al. (2012)
Mozambique	Feed	10	10	11	–	–	2	–	LC-MS/MS	Warth et al. (2012)
Americas										
Argentina	Feed (grass) (2011)	106	90	–	4.15 (med)	733	–	0.3	LC-MS/MS	Nichea et al. (2015)
	Feed (grass) (2014)	69	60	–	6.78	147	–	0.3	LC-MS/MS	Nichea et al. (2015)
European										
Belgium	Feed (sow feed, wheat, maize)	82	0	–	–	–	–	–	LC-MS/MS	Monbaliu et al. (2010)
Czech Republic and United Kingdom				–			–		LC-MS/MS	Zacharias-ova et al. (2014)
	Hay	4	0					2		
	Wheat	21			1	23		1		
	Barley	16	0					1		
	Maize	8	0					1		
	Oat	3	0					1		
	Soy meal	10	0					1		
	Sugar beet	6	0					1		
	Oil seed	14	0					2		
	Maize silage	11	0					1		
	Clover silage	12			0.6	6		1		
	Malt sprout	28	0					1		
	Brewer grain	28	0					1		
	Maize-based	71	0					0.5		
	Wheat-based	16	0					0.5		
Italy	Feed	14		0.7		2.2		1	LC-MS/MS	Biancardi & Dall'Asta (2015)
Netherlands	Feed	169	0						LC-MS/MS	Driehuis et al. (2008a)
Russian Federation	Forage (feed)								ELISA then LC-MS/MS	Kononenko et al. (2015)
	Grass	9	90	8	17	44				
	Clovergrass	15	85	8	65	600				
	Alfalfa-timothy mixture	5	85	16		200				
	Clover	15	80	8	97	32				
	Grass	9	80	8	15	20				
	Alfalfa	5	40	10	13	20				

Region / Country	Sample	No.	(%)	Min. (µg/kg)	Mean (µg/kg)	Max. (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Method	Reference
	Maize	140	0						LC-MS/MS	Driehuis et al. (2008b)
	Grass	120	0							
	Wheat silage	30	0							
United Kingdom	Maize	40	0	–	–	–	–	–		Scudamore, Nawaz & Hetmanski (1998)
	Maize products	27	0							
	Feed ingredients	186	0.5	18						Scudamore et al. (1997)
Eastern Mediterranean										
Egypt	Silage	40	5	–	–	–	–	–	TLC	El-Shanawany Mostafa & Barakat (2005)
Western Pacific										
Japan	Cattle feed (straw)	8	87.5	30		240			LC-MS/MS	Fushimi et al. (2014)
	Concentrate	6	0							

ELISA: indirect enzyme-linked immunosorbent assay; LC-MS/MS: liquid chromatography–tandem mass spectrometric detection system; med: median; LC-MS/MS: liquid chromatography–tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; med.: median; Min.: minimum; Max.: maximum; No.: number; TLC: thin-layer chromatography

8. Food consumption and dietary exposure estimates

8.1 Surveillance data

The analysis of surveillance data published in the GEMS/Food contaminants database (see Table 16) shows that the occurrence of sterigmatocystin in all foods is low. Quantified data were only reported on sorghum in Africa. For all other foods, no occurrence was reported. Using the published papers on occurrence and contamination shows a higher occurrence but very often on few samples. The larger studies (for instance, by RIKILT, Wageningen University & Research, the Netherlands) in Europe, on more than 1000 samples, show low occurrence and low contamination. The country with higher contamination is China. The one food product other than cereal that is more contaminated is coffee. Most analyses have been carried out on cereals, and data on other food products cannot be considered as representative because of the low number of samples. In these conditions – few food products tested, very low occurrence and great variability when detected – estimating dietary exposure is very difficult.

8.2 National estimates

Three studies have presented dietary exposure estimates for sterigmatocystin, in Spain, the Syrian Arab Republic and Sri Lanka. However, these studies calculated exposure only through one food product (coffee for Spain, wheat for the Syrian Arab Republic and spices for Sri Lanka). As contamination of cereals with sterigmatocystin seems to be more common than for other food commodities, results from the Syrian Arab Republic could be of particular interest. However, for all three studies the numbers of samples were small, with positive data for Spain coming from 10 samples, for the Syrian Arab Republic from four samples and for Sri Lanka from 35 samples. It seems unlikely that the sampling is representative of the national food supply.

Coffee consumption data from the Spanish Agency for Food Safety Survey for long-term exposure (chronic) food consumption statistics in g per day per kg body weight were selected. This 2009 survey obtained information from 1067 subjects (86 adolescents and 981 adults). Analytical data lower than the LOD or LOQ were assigned a value of zero for mean concentration calculation (lower bound; LB). For adults, the mean dietary exposure estimate was 0.049 ng/kg bw per day and the 95th percentile exposure estimate was 0.226 ng/kg bw per day; for adolescents, the mean dietary exposure estimate was 0.011 ng/kg bw per day and the 95th percentile exposure estimate was 0.099 ng/kg bw per day (García-Moraleja et al., 2015a,b).

A study in the Syrian Arab Republic considered sterigmatocystin exposure from consumption of wheat only (Alkadri et al., 2014). Based on 10 positive samples, three scenarios were considered for the level of wheat contamination. In the first case, exposure estimates were based on the average of positive samples; in the second case, all data were average, substituting zero for analytical results below the LOD; and in the third case, the highest observed sterigmatocystin concentration was used. Daily wheat intake was considered to be 420 g/day and an adult body weight of 60 kg was used. Dietary exposure to sterigmatocystin from wheat consumption was estimated to range between 0.7 and 10 ng/kg bw per day, the latter being a worst-case scenario.

A study carried out in Sri Lanka considered dietary sterigmatocystin exposure in adults from consumption of spices only. Details of the consumption data used were not given in the paper. In north Sri Lanka, mean LB and upper bound (UB) dietary exposure estimates were 0.05 and 0.15 ng/kg bw per day, respectively, and 95th percentile LB and UB dietary exposure estimates were 0.19 and 0.38 ng/kg bw per day, respectively. In south Sri Lanka, these estimates were 0.04, 0.11, 0.16 and 0.33 ng/kg bw per day, respectively (Yogendrarajah et al., 2014c).

8.3 International estimates

Five international estimates of dietary exposure were made using the GEMS/Food contaminants database and GEMS/Food cluster diets (Table 18). The WHO regions analysed were Africa (G13), Americas (G10), Europe (average of G07, G08, G11, G15), Eastern Mediterranean (G13) and Western Pacific (G10). These were the only WHO regions with contamination data. It was difficult to determine a global estimate of the exposure because most of the data came only from the cereals and cereal-based products. Very large left-censorship exists except for Africa (78.5%). The extremes were Europe and Western Pacific, with 100% data left-censored. Another point is the low number of samples. However, the LOD and LOQ were relatively low (very often between 1 and 3 µg/kg), which allowed an LB/UB evaluation with low differences.

With quantified data reported only for sorghum, exposure in the African Region is high because of the number of detected samples and because of the very high contamination levels found, higher than 100 µg/kg and up to 1200 µg/kg. The LOD and LOQ were low (1 and 2.5 µg/kg). This high intake is the main information of these calculations.

Data for the Region of the Americas came only from Canada and almost all samples were cereals. Because the LOD and LOQ are low, the UB estimate is quite low too.

Differences between the European Region and Western Pacific Region came from the different LOQs: 0.5 µg/kg for Japan (the only country for the Western Pacific Region) and around 5 µg/kg for the European Region. With 100% censorship, this explains these different intakes.

Only Sudan gave data for the Eastern Mediterranean Region, and only on sorghum. The difference with the African Region is a higher censorship and lower level of contamination: never higher than 10 µg/kg.

The estimates are very uncertain because of the high left-censorship (generally higher than 95%). However, generally low LOD and LOQ values allow UB estimates to be relatively low, with the exception of the African Region. This geographical area is the only one with a combination of high occurrence (21.5%) and high contamination in cereals (maximum: 1200 µg/kg). Further contamination studies are needed in order to evaluate the contamination of a larger number of food products.

Table 18
International exposure estimates[a] from GEMS/Food contaminants database and GEMS/Food cluster diets

Region	Mean exposure LB (ng/kg bw per day)	Mean exposure UB (ng/kg bw per day)	90th percentile exposure (ng/kg bw per day)	Left-censorship (%)[b]
African	16	17	34	78.5
Americas (Canada)	0.25	6.3	13	98.25
European	0	22	44	100
Eastern Mediterranean (Sudan)	0.3	3.5	7	97.1
Western Pacific (Japan)	0	0.47	1	100

bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; LOD: limit of detection; UB: upper bound
[a] Estimates are per capita based on a mean body weight of 60 kg for adults.
[b] Below the LOD.

9. Dose–response analysis and estimation of toxic/carcinogenic risk

The most consistent toxicity findings for sterigmatocystin were liver toxicity seen in acute, short-term and long-term studies and liver tumours in carcinogenicity studies and consistent, positive results, in vitro and in vivo, in genotoxicity studies. Carcinogenicity was therefore identified as the key end-point for risk assessment by benchmark dose (BMD) analysis.

9.1 General modelling considerations

In general, dose–response modelling of toxicological data is used to determine a point of departure for further risk assessment. Dose–response data were used to derive the 95% lower confidence limit of the benchmark dose (BMDL) for the observed effects on the liver.

9.1.1 Selection of data

Three mouse and five rat long-term toxicity/carcinogenicity studies were available. Most of these had limitations in terms of the small numbers of animals used, the use of only one or two doses, less-than-lifetime durations of exposure and/or use of fungal preparations or mouldy feed as test material in which other mycotoxins were likely present, with the Maekawa et al. (1979) study considered the most suitable for dose–response modelling. However, even this study had

limitations: only male animals were used; there were only 36 animals per treated group and 12 in the control group at study start; dosing did not commence until the animals were 11 weeks old; and the doses differed by factors of 10. It is therefore not optimal for modelling or for characterizing the shape of dose–response relationships that largely occur over a narrower range.

9.1.2 Measure of exposure

Rats were exposed to sterigmatocystin in the diet. The sterigmatocystin was isolated from *A. versicolor* and purified (no further details given), mixed with normal diet and made into pellets. Treated and control diet was fed from 11 weeks of age until natural death (58–122 weeks old).

9.1.3 Measure of response

There were four end-points in the Maekawa et al. (1979) study for which there was a clear relationship between dose and the frequency of response. The liver was the target organ in all cases. Two of these were carcinogenic end-points, total liver tumours and haemangiosarcomas, where the latter were a subset of the former. At lower doses, increased incidences of hyperplastic foci or areas and hepatocellular necrosis were also observed. The Committee concluded that only the data on haemangiosarcomas were relevant for BMD modelling and a benchmark response of 10% was used.

9.1.4 Selection of mathematical models

Dose–response modelling was conducted using United States Environmental Protection Agency (USEPA) Benchmark Dose Software version 2.6.1, with the standard set of models for quantal end-points. In addition, a model-average estimate using the methodology developed by Wheeler & Bailer (2007) was used. This estimate was computed using the default version of the software (Wheeler & Bailer, 2008) and including all models except the quantal-quadratic model, and using the Bayesian Information Criterion to compute the model-average weights.

9.2 BMD analysis

The modelling outputs for haemangiosarcomas are shown in Tables 19 and 20 and Fig. 4.

All models produced an acceptable fit, and the log-logistic model gave the lowest benchmark dose for a 10% response (BMD_{10}) and BMDL for a 10% response ($BMDL_{10}$). The estimated BMD_{10} and $BMDL_{10}$ ranged from 0.36 to 0.50 mg/kg bw per day and from 0.16 to 0.34 mg/kg bw per day, respectively.

Table 19
Reported data for haemangiosarcomas

Doses of sterigmatocystin (mg/kg bw per day)	No. of rats	No. of rats with haemangiosarcomas
0	11	0
0.005	27	0
0.05	29	1
0.5	26	3

bw: body weight; no.: number

Table 20
BMD_{10} and $BMDL_{10}$ estimates for haemangiosarcomas

Model name	DF	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	*P*-value	AIC
Gamma	3	0.363 278	0.176 076	0.813 5	30.057 9
Logistic	2	0.487 447	0.344 290	0.567 3	32.759 6
Log-logistic	3	0.355 736	0.160 825	0.842 8	29.988 6
Log probit	1	0.496 929	0.284 527	0.248 8	34.991 0
Multistage	3	0.363 278	0.176 076	0.813 5	30.057 9
Probit	2	0.482 991	0.323 830	0.573 7	32.722 9
Weibull	3	0.363 278	0.176 076	0.813 5	30.057 9
Quantal-linear	3	0.363 278	0.176 076	0.813 5	30.057 9

AIC: Akaike information criterion; BMD_{10}: estimated benchmark dose for a 10% inhibition; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose for a 10% response; bw: body weight ; DF: degrees of freedom

In accordance with JECFA guidance on dose–response modelling, all models in the USEPA's Benchmark Dose Software were fitted to the data using default constraints for restricted models. The log-logistic model yielded the lowest estimate of the BMDL of 0.16 mg/kg bw per day. As a comparison, the BMDLs also were computed from unrestricted models; two of the models yielded somewhat lower BMDLs, with log-logistic again having the lowest estimate at 0.11 mg/kg bw per day. For further comparison, the Bayesian model-averaging BMDL estimates were the highest of the three calculations, yielding an estimate of 0.30 mg/kg bw per day.

Fig. 4
BMD estimation with the log-logistic model for haemangiosarcomas

BMD: benchmark dose for a 10% response; BMDL: 95% lower confidence limit of the benchmark dose for a 10% response; BMR: benchmark response

10. Comments

10.1 Biochemical aspects

In animal studies, the absorption of [^{14}C]sterigmatocystin administered orally could be as high as 77–100% in adult rats (Walkow et al., 1985) and 85% in vervet monkeys (Steyn & Thiel, 1976). In rats, peak plasma levels were reached between 3 and 12 hours after oral administration, with a plasma half-life of 61–130 hours; about 10% of an administered dose was eliminated in the urine, and 64–92% in the faeces (Walkow et al., 1985). The half-life of excretion was 44 hours (Wang et al., 1991). In the vervet monkey, 70% of an oral dose was excreted unchanged in the faeces, 15% was eliminated in the urine and 4.5% was eliminated in the bile as sterigmatocystin glucuronide (Thiel & Steyn, 1973; Steyn & Thiel, 1976).

The structures of phase I metabolites observed in vitro have not been completely identified, and there is no consensus in the literature about the pathways of metabolism. As sterigmatocystin forms DNA adducts and is

structurally related to aflatoxins, some authors have speculated that a transient reactive epoxide, *exo*-sterigmatocystin-1,2-oxide, may be formed (Essigmann et al., 1979, 1980; Cabaret et al., 2010, 2011), but this is not supported by others (Krol, 2011; Pfeiffer, Fleck & Metzler, 2014). Regarding phase II metabolites, in vivo studies in rats and vervet monkeys identified a glucuronide conjugate as the major metabolite in urine, and no sulfate conjugates were observed (Thiel & Stein, 1973; Steyn & Thiel, 1976; Olson & Chu, 1993b). In vitro studies also reported the formation of a glucuronide conjugate (Cabaret et al., 2010, 2011).

10.2 Toxicological studies

In acute toxicity tests, oral LD_{50} values for purified sterigmatocystin in rats were 120 mg/kg bw and above (Purchase & van der Watt, 1969). The main target organs for acute toxicity in both rat and vervet monkey following acute oral and intraperitoneal dosing were the liver and kidney, which showed haemorrhage and necrosis in both species, with bile duct proliferation in the vervet monkey (Purchase & van der Watt, 1969; van der Watt & Purchase, 1970a).

Short-term toxicity studies confirm that the liver is the main target organ for the toxicity of sterigmatocystin (Purchase & van der Watt, 1970s; van der Watt & Purchase, 1970b; Richard et al., 1978; Sivakumar et al., 2001). In the rat, a dose of purified sterigmatocystin equivalent to 5–10 mg/kg bw per day administered in the feed for 2–16 weeks caused extensive histopathological changes in the liver, leading to necrosis; hyperplastic nodules and bile duct proliferation were observed (van der Watt & Purchase, 1970b). A lower dose of sterigmatocystin of 0.2 mg/kg bw per day given in the diet (contaminated with *A. versicolor*) for 30 days also led to necrosis of the liver and caused depletion of cellular antioxidants and generation of reactive oxygen species, resulting in lipid peroxidation (Sivakumar et al., 2001). Guinea-pigs given sterigmatocystin in capsules for 2 weeks at 4.2 mg/animal per day also showed extensive liver damage (Richard et al., 1978). Vervet monkeys given sterigmatocystin by intragastric administration at a dose of 20 mg/kg bw per day every 2 weeks for 12 months showed progressive necrotic liver changes and chronic hepatitis, culminating in large hyperplastic nodules with pleomorphic nuclei (Purchase & van der Watt, 1970a).

In long-term studies, sterigmatocystin was carcinogenic after oral administration in mice (Zwicker, Carlton & Tuite, 1974; Enomoto et al., 1982), rats (Purchase & van der Watt, 1970b; Ohtsubo, Saito & Kimura, 1978; Terao, Aikawa & Kera, 1978; Maekawa et al., 1979) and monkeys (species not specified) (Thorgeirsson et al., 1994). Sterigmatocystin was also carcinogenic in experimental animals after intraperitoneal, subcutaneous or dermal administration (Dickens, Jones & Waynforth, 1966; Purchase & van der Watt, 1973; Fujii et al., 1976; Terao, 1978).

Mice fed intermittently (2 weeks on, 2 weeks off) for up to 58 weeks with a diet containing sterigmatocystin (commercial or from mouldy rice contaminated with *A. versicolor*) at a dose equivalent to 0.75 mg/kg bw per day showed increased incidences of pulmonary adenomas and adenocarcinomas in both males and females, with both forms of sterigmatocystin (Zwicker, Carlton & Tuite, 1974). Mice given purified sterigmatocystin in the diet at doses equivalent to 4.5 and 18 mg/kg bw per day for up to 55 weeks showed low incidences of hepatocellular adenoma and hepatocellular carcinoma, but high incidences of haemangioendotheliomas, hepatic angiosarcomas and brown fat angiosarcomas (Enomoto et al., 1982). The Committee noted that the results of this study show that sterigmatocystin has two targets for carcinogenicity – namely, the hepatocytes and the blood vessels.

Wistar-derived male and female rats were given purified sterigmatocystin for 52 weeks at a dose of 0.15, 0.3 or 1.5 mg/rat by gavage, 5 days a week, or continuously in the diet at doses equivalent to 0.5, 1 and 5 mg/kg bw per day for the first 6 months, increasing to 0.75, 1.5 and 7.5 mg/kg bw per day for the second 6 months. There was a high incidence of mortality in the high-dose dietary group. Hepatocellular carcinomas were seen at all doses with both methods of administration in 40–100% of survivors, with a clear dose–response relationship in the groups dosed by gavage (Purchase & van der Watt, 1970b). In another study in male Wistar rats given purified sterigmatocystin in the diet at a dose equivalent to 0.5 mg/kg bw per day for 54 weeks, 53% developed hepatocellular carcinomas (Terao, Aikawa & Kera, 1978).

In male Donryu rats exposed to sterigmatocystin for up to 101 weeks from feed to which mouldy rice contaminated with *A. versicolor* was added, giving doses equivalent to 0.25 and 0.5 mg/kg bw per day, a high proportion of test animals died as a result of infection. In the survivors, the incidence of hepatocellular carcinomas was 85% and 92% in the low- and high-dose groups, respectively (Ohtsubo, Saito & Kimura, 1978).

In male ACI/N rats given purified sterigmatocystin in the diet for more than 2 years at a dose equivalent to 0.005, 0.05 or 0.5 mg/kg bw per day, tumours in the liver, testis, adrenal gland and 12 other sites were found. The overall incidence of tumours of all types combined did not show a dose–response relationship, but the incidence of liver tumours was dose related (0/11, 0/27, 1/29 and 5/26 in control, low-dose, mid-dose and high-dose groups). One animal in the mid-dose group had a liver haemangiosarcoma; three animals in the high-dose group had haemangiosarcoma, and one had hepatocellular carcinoma. The Committee noted that the authors stated that there were five liver tumours in total in the highest-dose group, but the breakdown of the histological types accounts for only four tumours. There were clear dose–response relationships for other nonneoplastic histopathological changes in the liver, including central

necrosis (2/11, 7/27, 14/29 and 16/26 in control, low-dose, mid-dose and high-dose groups), hyperplastic foci or areas of hepatocellular alteration (1/11, 4/27, 9/29 and 21/26 in control, low-dose, mid-dose and high-dose groups) and hyperplastic nodules (0/11, 0/27, 0/29 and 3/26 in control, low-dose, mid-dose and high-dose groups) described by the authors as benign hepatic cell tumours (Maekawa et al., 1979). Although not all such lesions are preneoplastic and such lesions do not necessarily progress to carcinomas, hyperplastic foci and hyperplastic nodules are known to be increased in incidence after administration of hepatocarcinogens (Thoolen et al., 2010, 2012).

In 30 monkeys (species not specified) treated orally once a week with sterigmatocystin at either 1.0 or 2.0 mg/kg bw (equivalent to 0.14 and 0.29 mg/kg bw per day) for about 18 years, 33% of treated monkeys developed one or more hepatic tumours. Five animals in each dose group developed hepatic tumours; in total across the two dose groups, there were seven hepatocellular carcinomas, two cholangiocarcinomas and one cholangiosarcoma. One monkey developed a renal cell carcinoma. The incidence of tumours in each dose group was not given (Thorgeirsson et al., 1994).

Although there are a number of long-term studies in rodents, the majority of them have limitations with regard to their utility for risk assessment owing to small numbers of animals, use of only one sex, use of only one or two doses, less than lifetime durations of exposure, or use of fungal preparations or mouldy feed as test material in which other mycotoxins were likely to be present. It should be noted that the study by Maekawa et al. (1979) also has limitations (e.g. brief reporting, only male rats used, small control group, dosing not commencing until 11 weeks of age, and a discrepancy between the total numbers of liver tumours given in tables), but the Committee considered that it was the only study with data that are appropriate for dose–response analysis.

The Committee noted that the rat strain used in the Maekawa et al. (1979) study, ACI/N, did not appear to be as responsive as other strains tested in terms of the induction of hepatocellular tumours. Comparing the results from dietary administration of sterigmatocystin, Purchase & van der Watt (1970b) reported that 8/9 Wistar-derived rats developed hepatocellular carcinoma at the lowest dose tested of 0.5–0.75 mg/kg bw per day for 52 weeks, Terao, Aikawa & Kera (1978) reported that 8/14 Wistar rats developed hepatocellular carcinoma at the only dose tested of 0.5 mg/kg bw per day for 54 weeks, and Ohtsubo, Saito & Kimura (1978) reported that 11/13 Donryu rats developed hepatocellular carcinoma at the lowest dose tested of 0.25 mg/kg bw per day for up to 101 weeks. By contrast, in the study of Maekawa et al. (1979), only 1/26 ACI/N rats developed hepatocellular carcinoma at the highest dose tested of 0.5 mg/kg bw per day for up to 122 weeks, with slightly more hepatic haemangiosarcomas (3/26 and 1/29 in the high- and mid-dose groups, respectively). ACI/N rats also developed

proportionately more haemangiosarcomas than hepatocellular carcinomas, which is the opposite of the reported findings in the other two strains.

Extensive in vitro studies on the genotoxicity of sterigmatocystin are available, and almost all tests give positive results. Sterigmatocystin is mutagenic in vitro in bacterial cells after metabolic activation (McCann et al., 1975; Ueno & Kubota, 1976; Tang & Friedman, 1977; Kuczuk et al., 1978; Ueno et al., 1978; Wehner et al., 1978; Mori et al., 1986; Krivobok et al., 1987; Baertschi et al., 1989) and in mammalian cells (Umeda, Tsutsui & Saito, 1977; Noda, Umedia & Ueno, 1981; Reiners et al., 1983; Morita, Umeda & Ogawa, 1991). It induces chromosomal aberrations, micronuclei and chromosome damage (sister chromatid exchange, unscheduled DNA synthesis, comet assay) in vitro in mammalian cells, including human cells (Stich & Laishes, 1975; Umeda, Tsutsui & Saito, 1977; Mori et al., 1984, 1986; Ellard et al., 1991; Crofton-Sleigh et al., 1993; Ellard & Parry, 1993; Jaksic et al., 2012; Wang et al., 2013, 2015; Zhang et al., 2013; Anninou et al., 2014; Huang et al., 2014; Gao et al., 2015). In vivo, it causes chromosomal aberrations in rats, chromosomal aberrations and micronuclei in fish, and chromosome damage (sister chromatid exchange) in mice (Curry et al., 1984; Ueda et al., 1984; Abdel-Wahhab et al., 2005).

Sterigmatocystin forms guanyl-N^7 adducts in vitro with calf thymus DNA in the presence of rat or human liver microsomes (Essigmann et al., 1979; Baertschi et al., 1989) and forms dose-related DNA adducts in rat liver in vivo after intraperitoneal administration (Reddy, Irvin & Randerath, 1985; Olson & Chu, 1993a).

Taking account of the available information on genotoxicity, carcinogenicity and DNA adduct formation, the Committee concluded that sterigmatocystin is genotoxic and carcinogenic.

Studies on immunotoxic effects suggest that sterigmatocystin may have immunomodulatory activity (Richard et al., 1978; Liu et al., 2012; Zhang et al., 2012; EFSA, 2013; Wang et al., 2014; Korkalainen et al., 2017). However, firm conclusions cannot be drawn, because the in vivo data are difficult to interpret as specific immunotoxic effects, whereas the relevance of the in vitro data is difficult to interpret because these experiments were conducted with rather high concentrations of sterigmatocystin.

There are no data on the reproductive or developmental toxicity of sterigmatocystin in mammalian species.

10.2.1 Sterigmatocystin and aflatoxin B_1

Compared with AFB_1, the acute oral toxicity (LD_{50}) of sterigmatocystin in rats is 10 or more times lower (Butler, 1964; Purchase & van der Watt, 1969).

In a short-term toxicity study in which sterigmatocystin was administered orally to rats for up to 16 weeks with interim kills, there were extensive histopathological changes in the liver leading to necrosis, but the study authors noted that bile duct proliferation did not progress beyond that seen after 8 weeks of exposure and was not nearly as extensive as that following aflatoxin (probably AFB_1) treatment (van der Watt & Purchase, 1970b).

The carcinogenic potency of sterigmatocystin relative to that of aflatoxin (probably AFB_1) has been considered by Purchase & van der Watt (1970b). In their studies on Wistar-derived rats, administration of 105 µg of aflatoxin per week for 50 weeks resulted in 6/7 rats (86%) developing hepatomas in 80 weeks; in comparison, 60% or 80% of rats receiving 750 or 1500 µg sterigmatocystin per week, respectively, for 52 weeks developed tumours or hyperplastic nodules by week 123. From these data, Purchase & van der Watt (1970b) estimated that aflatoxin is "no more than 10 times" as potent as sterigmatocystin. The authors also noted the virtual absence of bile duct proliferation and complete absence of cholangiocarcinomas after sterigmatocystin administration, in contrast to the extensive bile duct reaction produced by aflatoxin. There have been no studies in which purified AFB_1 and sterigmatocystin have been given in combination.

Regarding genotoxicity, sterigmatocystin was found to be less mutagenic than AFB_1 in bacterial cells in the presence of metabolic activation (McCann et al., 1975; Ueno & Kubota, 1976; Tang & Friedman, 1977; Kuczuk et al., 1978; Ueno et al., 1978; Wehner et al., 1978; Mori et al., 1986). However, inconsistent results were obtained in bacterial cells using human liver extract for metabolic activation, with sterigmatocystin sometimes showing less and sometimes more mutagenicity (Tang & Friedman, 1977). In mouse hepatocytes, induction of unscheduled DNA synthesis was higher for AFB_1 than for sterigmatocystin (Mori et al., 1984, 1986). However, in human skin fibroblasts, the induction of unscheduled DNA synthesis with sterigmatocystin was higher than with AFB_1, with or without metabolic activation (Stich & Laishes, 1975). In a mouse mammary carcinoma cell line, the induction of 8-azaguanine-resistant mutations and the level of chromosomal aberrations were higher for sterigmatocystin than for AFB_1 (Umeda, Tsutsui & Saito, 1977).

10.3 Observations in domestic animals/veterinary toxicology

In dairy cattle, a case of poisoning in a farm in the USA has been reported in relation to feed contamination by several fungal strains dominated by *A. versicolor* and *A. candidus*. The concentration of sterigmatocystin was 7.75 mg/kg feed. The animals exhibited bloody diarrhoea, loss of milk production and death in some cases (Vesonder & Horn, 1985).

10.4 Observations in humans

Studies in China have suggested that there is a correlation between exposure to sterigmatocystin (contamination rate and content in grains) and the prevalence of stomach and liver cancers (Lou et al., 1995). A clinical study in China detected sterigmatocystin in the blood of 4/13 patients with liver and stomach cancer (range 65–113 μg/kg) and in 1/14 healthy persons (68 μg/kg), but sterigmatocystin concentrations in the urine were all below the LOD. Sterigmatocystin–DNA adducts were found in 50% of sampled tissues of tumours from 12 patients (Tian, Lou & Du, 1995).

10.5 Analytical methods

Screening tests for sterigmatocystin include TLC and rapid test kits based on antibodies. Prior to the mid-1990s, only a very limited number of research papers had been published on the screening of sterigmatocystin using qualitative and semiquantitative methods. ELISA or immunochromatographic devices as screening tests for mycotoxins are commercially available, but few are suitable to screen for sterigmatocystin in food. Positive results should be confirmed with specific and quantitative methods.

Analytical methods for the determination of sterigmatocystin in foodstuffs include TLC, gas chromatography, gas chromatography–mass spectrometry, HPLC-UV detection, HPLC with fluorescence detection, LC-MS, LC-MS/MS and ELISA; however, methods published in the scientific literature in recent years indicate that LC-MS/MS is a fast, accurate and reproducible technique for the detection and quantification of sterigmatocystin in foods and feeds (Marley et al., 2015; Mol et al., 2015). Chromatographic methods with an LOQ of 2 μg/kg for sterigmatocystin have been developed and validated (Stroka et al., 2004; Veršilovskis & De Saeger, 2010). Further advances in the analysis of sterigmatocystin have been achieved by applying multi-mycotoxin analysis using LC-MS/MS (Veršilovskis, De Saeger & Miķelsone, 2008; Veršilovskis, Van Peteghem & De Saeger, 2009).

Yao et al. (2006) developed a biosensor constructed by multi-walled carbon nanotubes for the detection of sterigmatocystin. Chen et al. (2010) also developed a rapid and highly sensitive electrochemical biosensor for the detection of sterigmatocystin based on an enzyme, aflatoxin oxidase.

Real-time qPCR methods have been proposed to quantify sterigmatocystin-producing fungi in foods with a minimum LOD of 10 colony-forming units per gram. It had been reported that the qPCR method would be useful for monitoring sterigmatocystin-producing fungi in HACCP programmes to prevent the accumulation of the toxin in foods during storage and processing.

No certified reference materials are available for the determination of sterigmatocystin in food matrices. Furthermore, no proficiency tests or quality assurance interlaboratory schemes for the analysis of sterigmatocystin in food or feed have been identified.

10.6 Sampling protocols

An effective sampling protocol is a prerequisite for the control of mycotoxins in food and feed. Although no sampling protocols specific to sterigmatocystin were found, there are some generic guidelines on sampling of mycotoxins available. The FAO sampling tool on sampling protocols, developed for both food analysts and regulatory officials, can be used (FAO, 2014), and there are sampling protocols available from the Codex Alimentarius Commission's standard CODEX STAN 193-1995 (FAO/WHO, 1995). Furthermore, the European Commission has sampling protocols for the purpose of official control of the levels of mycotoxins in foodstuffs, as described in Regulation (EC) No 401/2006 and its amendments (European Commission, 2006, 2010).

10.7 Effects of processing

Cleaning methods such as sieving fines or broken kernels from bulk materials and sorting, by physically removing contaminated kernels, have been shown to reduce mycotoxins; however, there is no information on such processes for the reduction of sterigmatocystin in food commodities. Milling and baking processes have been reported to decrease sterigmatocystin levels. Roasting of coffee beans at 200 °C for 20 minutes reduced the concentration of added sterigmatocystin by 68% under laboratory conditions (Levi, Ternk & Yeransian, 1975).

Sterigmatocystin levels were reported to decrease during food fermentation and in cheese making; a report showed 80% reduction of sterigmatocystin in the curd and 20% in the whey, indicating low solubility of the toxin in aqueous media. Sterigmatocystin has also been reported in beer, which indicates that the toxin can survive the brewing process (Veršilovskis, De Saeger & Miķelsone, 2008).

Food processing, such as milling, roasting, bread making, cheese making and fermentation, can result in decreased levels of sterigmatocystin in foods; however, the extent of the decrease depends on the type of food and processing conditions.

10.8 Prevention and control

Sterigmatocystin is produced during storage of food and feed, which means that prevention and control will focus on postharvest measures. However, specific management and control measures to prevent sterigmatocystin in food and feed were not identified. It is assumed that most of the strategies focusing on prevention of aflatoxin contamination postharvest may also be relevant for sterigmatocystin, as these two mycotoxins have a common biosynthetic pathway.

Several in vitro studies reported on prevention of fungal growth or production of sterigmatocystin by, for example, extracts of oregano, African pencil-cedar, tomato, onions and garlic (Kocic-Tanackov et al., 2011; Kouadio, Koffi & Dosso, 2013; Abdel Ghany, 2014; Lim, 2015).

The most important postharvest measure to prevent sterigmatocystin contamination is management of storage conditions. It was reported that storing grains at controlled temperature and water activity with elevated carbon dioxide and low oxygen concentrations resulted in no observable growth of or sterigmatocystin production by *A. flavus* and *A. parasiticus* (Weidenborner, 2013).

Also, no mycelial growth or sterigmatocystin production was detected after gamma irradiation of *A. versicolor* in vitro (Kume et al., 1983), and irradiation was shown to eliminate the occurrence of the fungus and toxin in dairy cattle feed (Aziz & Refai, 1989).

10.9 Levels and patterns of contamination

In total, 4419 data on sterigmatocystin occurrence were reported in the GEMS/Food contaminants database, with 94% censorship globally. The only food commodity analysed is cereals and cereal-based foods. Africa is the region that contributes the most positive data, with 21% positive samples, all being sorghum; 10% of the positive samples had concentrations in excess of 100 µg/kg. Data from the other WHO regions show lower prevalence: 1.75% for the Americas, 0% (0/51 food samples) for the Western Pacific, 2.9% for the Eastern Mediterranean and 0% (0/246 samples) for Europe. The range of LODs reported was 0.3–3 µg/kg.

Approximately 50 papers were found in the scientific literature with information on sterigmatocystin occurrence. Most of the publications employed multi-mycotoxin analysis and were not on sterigmatocystin specifically, and they were therefore difficult to interpret. Most information was found on cereals and cereal-based products. There were also positive detections at low concentrations (usually below 20 µg/kg) in cheese, chilli, pepper, coffee, beer and nuts.

Contamination of feed is generally low, with a few reports of high concentrations (maximum 733 µg/kg) in Japan, Argentina and the Russian

Federation. No reports were found on the occurrence of sterigmatocystin in animal products; therefore, it was not possible to evaluate the transfer of sterigmatocystin from feed to foods.

10.10 Food consumption and dietary exposure assessment

As a consequence of the limited information on occurrence, few dietary exposure evaluations were published. Dietary exposure through coffee consumption was estimated for Spain, with mean values of 0.049 ng/kg bw per day for adults and 0.011 ng/kg bw per day for adolescents (García-Moraleja et al., 2015a,b). Dietary exposure to sterigmatocystin from wheat consumption in the Syrian Arab Republic was estimated to range between 0.7 and 10 ng/kg bw per day, the latter being a worst-case scenario (Alkadri et al., 2014). Another estimated dietary exposure to sterigmatocystin was from consumption of spices in Sri Lanka, resulting in a mean range of 0.04–0.15 ng/kg bw per day for adults (lowest LB–highest UB) (Yogendrarajah et al., 2014c). These dietary exposure estimates were based on very limited data and cannot be considered as representative of national or international exposure.

10.10.1 International estimates

Considering the limited contamination data in published papers and the very high proportion of nondetected analytical results for sterigmatocystin in foods (from 78.5% for Africa up to 100% for Europe and the Western Pacific), an LB–UB approach was used by the Committee to calculate estimates only for WHO regions for which data on consumption and contamination were available in the GEMS/Food contaminants database and the GEMS/Food cluster diets (Table 21). The five WHO regions analysed were Africa (G13 cluster diet with sorghum), Eastern Mediterranean (G13 cluster diet with sorghum), Europe (average of G07, G08, G11 and G15 cluster diets with cereals, snacks and desserts), Western Pacific (G10 cluster diet with cereals) and the Americas (G10 cluster diet with cereals, food for infants, legumes and pulses, nuts and oilseeds, starchy roots).

The best refined international LB–UB mean (or high) exposure estimates for adults were 16–17 ng/kg bw per day (32–34 ng/kg bw per day) for Africa, 0.3–6.3 ng/kg bw per day (0.6–13 ng/kg bw per day) for the Americas, 0.3–3.5 ng/kg bw per day (0.6–7 ng/kg bw per day) for the Eastern Mediterranean, 0–22 ng/kg bw per day (0–44 ng/kg bw per day) for Europe and 0–0.5 ng/kg bw per day (0–1 ng/kg bw per day) for the Western Pacific.

These results are very uncertain because of the very high left-censorship (below LOD), equal to 100% in Europe and in Western Pacific, except for Africa, and the limited number of food commodities analysed.

Table 21
Exposure estimates[a] for WHO regions

Region	Mean exposure (LB–UB) (ng/kg bw per day)	High exposure (LB–UB)[b] (ng/kg bw per day)	Left-censorship[c] (%)
Africa (Burkina Faso, Mali, Ethiopia)	16–17	32–34	78.5
Americas (Canada)	0.3–6.3	0.6–13	98.25
Europe (Czech Republic, the Netherlands, United Kingdom)	0–22	0–44	100
Eastern Mediterranean (Sudan)	0.3–3.5	0.6–7	97.1
Western Pacific (Japan)	0–0.5	0–1	100

bw: body weight; LB: lower bound; LOD: limit of detection; UB: upper bound
[a] Estimates are per capita based on a mean body weight of 60 kg for adults.
[b] High estimates are equal to twice the mean.
[c] Below the LOD.

10.11 Dose–response analysis

The critical effect for sterigmatocystin is carcinogenicity, and the long-term rat study by Maekawa et al. (1979), using dietary administration of doses equivalent to 0, 0.005, 0.05 and 0.5 mg/kg bw per day, was considered the most suitable for dose–response modelling. The critical end-point selected was hepatic haemangiosarcoma in male rats. In accordance with JECFA guidance on dose–response modelling, all models in the USEPA's Benchmark Dose Software (version 2.6.1) were fitted to the data using the software's default constraints for restricted models. The log-logistic model yielded the lowest estimate of the $BMDL_{10}$, 0.16 mg/kg bw per day. For comparison (see Annex 1, reference *233*, section 2.1.1), the model-averaging software of Wheeler & Bailer (2008), which is available in source code as supplemental material, was used to compute the model-average estimate to compare the estimates based upon the log-logistic model. For this comparison, all models, except the quantal-quadratic, were included, and the Bayesian Information Criterion was used to compute the model-average weights. The $BMDL_{10}$ estimates using model-averaging yielded an estimate of 0.30 mg/kg bw per day.

The Committee selected the $BMDL_{10}$ of 0.16 mg/kg bw per day for hepatic haemangiosarcoma in male rats (Maekawa et al., 1979) from the restricted log-logistic model as the point of departure for use in the risk assessment.

11. Evaluation

As it is not appropriate to establish a health-based guidance value for substances that are genotoxic carcinogens, the Committee used a margin of exposure (MOE)

approach based on the $BMDL_{10}$ for sterigmatocystin of 0.16 mg/kg bw per day as the point of departure.

The Committee noted that there is a paucity of occurrence data, and what data were available to the Committee frequently were left-censored, thereby increasing the uncertainty in the exposure assessment.

The Committee calculated MOEs for mean and high estimates of dietary exposure to sterigmatocystin. The MOEs for adults ranged from 9400 to more than 530 000 for mean estimates based on UB and LB assumptions, respectively. For high estimates, MOEs for adults ranged from 4700 to 270 000. The lowest MOEs were observed for the African Region (from 4700 [UB] to 5000 [LB] for the high-exposure range, and from 9400 [UB] to 10 000 [LB] for the mean-exposure range). The Committee noted that these estimates, which are based only on adult populations and for which only one food commodity (sorghum) was considered, may indicate a human health concern. MOEs were not calculated for Europe or Japan, as sterigmatocystin was not detected in any samples. For all other regions, the Committee considered that the MOEs were not of human health concern even at the UB high exposure.

Overall, the Committee concluded that the data used for calculating the MOEs have considerable limitations, both for the dietary exposure estimate and for the toxicological point of departure. Limited data on occurrence in food were available, and analytical detection limits were high in some countries. The only long-term carcinogenicity study suitable for dose–response modelling used an uncommon strain of rat (ACI/N), and, in view of the low incidence of liver tumours in this animal model, it may not be the most appropriate for human risk assessment. Consequently, the derived MOEs should be considered only as crude estimates.

The Committee also noted that sterigmatocystin and AFB_1 have the same main target organ (the liver). The comparative animal data on carcinogenicity are very limited, but indicate that sterigmatocystin is less potent than AFB_1.

11.1 Recommendations

The Committee recommends improving the LOQs for sterigmatocystin, particularly when developing multi-mycotoxin methods.

The Committee recommends that more food commodities, especially stored crops, be analysed with appropriate analytical LODs that would allow refining the estimates of dietary exposure to sterigmatocystin from all regions.

The Committee encourages the development of suitable certified reference materials and proficiency tests to support the analysis of sterigmatocystin.

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Co-exposure of fumonisins with aflatoxins

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1. Explanation 880
2. Biological data 881
  2.1 Biochemical aspects of co-exposure 882
    2.1.1 Absorption, distribution and excretion 882
    2.1.2 Biotransformation 882
    2.1.3 Effects on enzymes and other biochemical parameters relevant to the mechanism of action 883
  2.2 Toxicological studies of co-exposure 885
    2.2.1 In vivo studies on combined toxicity of pure fumonisin with aflatoxin, partially purified fumonisin with aflatoxin, or fumonisins and aflatoxins in culture material 886
      (a) Pure fumonisin and pure aflatoxin in vivo 887
      (b) Culture material or partially purified fumonisin and pure aflatoxin in vivo 889
      (c) Culture material or partially purified fumonisin and aflatoxin in vivo 890
    2.2.2 In vitro studies investigating various aspects of combined FB_1 and AFB_1 cytotoxicity using pure toxins 891
  2.3 Observations of co-exposure in humans 892
    2.3.1 Biomarkers of exposure 892
    2.3.2 Biomarkers of effects 893
    2.3.3 Epidemiological studies 893
3. Co-occurrence of aflatoxins and fumonisins in foods 895
  3.1 Co-occurrence of aflatoxins and fumonisins in foods in general 896
  3.2 Co-occurrence of AFB_1 and FB_1 in foods 898

3.2.1 Evaluation of co-occurrence based on data from the GEMS/Food contaminants database 898
(a) Results not based on unique sample identification numbers for AFB_1 and FB_1 899
(b) Results by unique sample identification number for AFB_1 and FB_1 912
3.2.2 Evaluation of co-occurrence based on the literature 915
(a) Co-occurrence of AFB_1 and FB_1 918
(b) Co-occurrence of aflatoxins and fumonisins with other mycotoxins 924
3.3 Summary of the co-occurrence of aflatoxins and fumonisins in foods 925
4. Co-exposure to aflatoxins and fumonisins in the diet 926
4.1 Assessing co-exposure 926
4.2 Foods with high concentrations of AFB_1 and FB_1 927
4.3 Populations with high consumption of key food commodities 927
4.4 Commodities that are high contributors to dietary exposure 930
4.5 International estimates of dietary exposure 930
4.6 National estimates of dietary exposure 932
4.7 Summary of the evaluation of co-exposure to aflatoxins and fumonisins from the diet 934
4.8 Co-exposure to aflatoxins and fumonisins for infants 935
4.8.1 Breast milk 936
4.8.2 Infant formula 936
4.8.3 Infant foods 937
4.8.4 Summary of the evaluation of co-exposure to aflatoxins and fumonisins for infants 938
5. Dose–response analysis and estimation of toxic/carcinogenic risk 938
6. Comments 939
6.1 Biochemical aspects 939
6.2 Toxicological studies 940
6.3 Observations in humans 942
6.4 Co-occurrence of fumonisins and aflatoxins in foods 943
6.5 Co-exposure to aflatoxins and fumonisins in the diet 944
7. Evaluation 947
7.1 Recommendations 948
8. References 948
Appendix 1. Countries/regions and foods with no co-occurrence of AFB_1 and FB_1 from both the GEMS/Food contaminants database and the literature 957

1. Explanation

Fumonisins and aflatoxins are mycotoxins produced by fungi of *Fusarium* and *Aspergillus* species. Considering that fumonisins and aflatoxins are both frequent

contaminants in cereal (especially maize, rice, sorghum and wheat) and cereal-based foods and that aflatoxins are common contaminants in groundnuts and tree nuts, co-exposure to both mycotoxins is likely in areas where these foods are consumed as part of the routine diet.

As part of the evaluation of fumonisins at the seventy-fourth meeting (Annex 1, reference *205*), the Committee evaluated the toxicological data on the concurrent exposure to fumonisins and other mycotoxins. There were no human studies available showing toxicity associated with co-exposure. None of the co-exposure studies in animal models was considered adequate for use in the Committee's evaluation of fumonisins; the Committee noted that the interaction between aflatoxin B_1 (AFB_1), a compound with known genotoxic and hepatocarcinogenic properties, and fumonisins, which have the potential to induce regenerative cell proliferation in the liver, would be of concern. The Committee has not performed a full evaluation for the co-exposure of fumonisins and aflatoxins previously.

At the current meeting, the Committee evaluated updated toxicological and exposure data for fumonisins and aflatoxins separately (see "Aflatoxins (addendum)" and "Fumonisins (addendum)"). At the request of the Codex Committee on Contaminants in Foods (CCCF), the Committee also evaluated co-exposure to aflatoxins and fumonisins. The evaluation was based on a comprehensive literature search (University of Georgia Libraries Galileo databases, University of Saskatchewan Electronic Library, PubMed and Web of Science) for relevant publications from 2010 to 2016.

2. Biological data

Several comprehensive authoritative reviews of the data have described the biological activity, toxicology and human epidemiological studies of either fumonisins or aflatoxins individually (IPCS, 2000; IARC, 2002; Eaton et al., 2010; Annex 1, references *77*, *122*, *131*, *152*, *188* and *205*). However, only a few reviews have explored the implications of co-exposure in humans, for example, in hepatocellular carcinoma (IARC, 2012), chronic liver disease (Torres et al., 2015), gut health and impaired growth (Smith, Stoltzfus & Prendergast, 2012).

This monograph will briefly summarize the existing mechanistic, toxicological and human observations published since 2011 that describe possible effects related to co-exposure in animal and human studies.

The literature search conducted to identify all available biological data published since 2010 is described in "Aflatoxins (addendum)" (pages 3–279) and "Fumonisins (addendum)" (pages 415–573).

2.1 Biochemical aspects of co-exposure

2.1.1 Absorption, distribution and excretion

There were no studies describing the toxicokinetics of AFB_1 and fumonisin B_1 (FB_1) absorption or distribution following co-exposure in animals or humans. There is some evidence that co-exposure to pure AFB_1 and FB_1 can affect urinary excretion of aflatoxin M_1 (AFM_1) (Mitchell et al., 2014).

2.1.2 Biotransformation

Several in vitro and in vivo studies have shown that cytochrome P450 (CYP) activity can be altered as a result of the inhibition of ceramide synthase by fumonisin (Annex 1, reference *152*). More recently, Chuturgoon, Phulukdaree & Moodley (2014) showed that treatment of human hepatoma cells with 200 µmol/L FB_1 significantly downregulated expression of microribonucleic acid (miRNA) miR-27b, while CYP1B1 messenger ribonucleic acid (mRNA) and protein expression were significantly upregulated. The authors concluded that FB_1-induced modulation of miR-27b may contribute to hepatic neoplastic transformation. However, it should be noted that the dose was the median inhibitory concentration (IC_{50}) for cell viability. The authors suggested that FB_1 may be able to indirectly affect biotransformation of other compounds, but there are no convincing data showing that FB_1 modulates metabolic pathways involved in aflatoxin carcinogenicity.

Nonetheless, there is some indirect evidence that co-exposure to pure AFB_1 and FB_1 might affect the metabolism of AFB_1. To test the efficacy of orally administered calcium montmorillonite clay to reduce AFB_1 and FB_1 exposure individually and in combination, Mitchell et al. (2014) divided 60 male Fischer 344 rats (6 weeks old; 131 g) between 10 treatment groups (n = 6/group). This summary only describes the results of the positive control groups given a single oral gavage dose of AFB_1 (125 µg/kg body weight [bw]) or FB_1 (25 mg/kg bw) or a combination of the two. Urinary excretion of AFM_1 and FB_1 was monitored at intervals for up to 72 hours after dosing. Serum AFB_1–albumin adduct levels were measured at 72 hours.

AFM_1 excretion was reduced by approximately 65% in co-exposed animals compared with those exposed to AFB_1 only. At the same time, AFB_1–albumin adduct levels were significantly increased in the co-exposed group compared with rats given only AFB_1: relative amounts were approximately 1100 and 600 pg adduct per mg albumin, respectively. Mitchell et al. (2014) speculated that modification of aflatoxin metabolism during co-exposure might occur through induction of CYP enzymes by FB_1, as has been shown in rat liver and rat hepatoma cells treated with FB_1 (Martinez-Larranaga et al., 1996; Chuturgoon,

Phulukdaree & Moodley, 2014), or altered phase II metabolism. In any event, the results suggested that FB_1 altered AFB_1 metabolism/excretion.

A possible explanation given for the increased serum AFB_1–albumin adduct levels would be FB_1-induced increased production of the reactive AFB_1-8,9-epoxide intermediate. The Committee noted that if FB_1 co-exposure induced an increased production of the reactive AFB_1-8,9-epoxide intermediate, co-exposure could, in principle, increase the risk of hepatocarcinogenicity of AFB_1. However, no changes in DNA adduct formation were observed in rainbow trout exposed for 1 week to dietary FB_1 prior to AFB_1 injection using a protocol known to induce liver tumours (Carlson et al., 2001). The lack of changes in the formation of hepatic AFB_1–DNA adducts by FB_1 pretreatment suggests that CYP-mediated AFB_1 bioactivation was not altered after 1 week on the FB_1 diets.

2.1.3 Effects on enzymes and other biochemical parameters relevant to the mechanism of action

Mechanistic considerations provide a plausible explanation for how co-exposure to AFB_1 and FB_1 could potentiate the risk of chronic liver disease in populations consuming diets contaminated with high levels of both toxins. The biochemical aspects of aflatoxins and fumonisins are described in detail in their separate monograph addenda and only briefly here.

The toxicity of AFB_1 and AFG_1 is a consequence of the presence of an oxidizable 8,9-double bond in both AFB_1 and AFG_1 and their metabolism to the AFG_1- and AFB_1-*exo*-8,9-epoxide. The mutagenicity and carcinogenicity of AFB_1 involve covalent binding of the *exo*-8,9-epoxide to DNA and other cell macromolecules. Metabolism of AFB_1 is key to its mechanism of action, and the balance between biologically active or detoxified metabolites produced during phase I metabolism is critical for determining the toxicological outcome with regard to both hepatotoxicity and hepatocarcinogenicity.

FB_1 causes decreased ceramide biosynthesis through inhibition of ceramide synthases (CerS 1–6). Fumonisin inhibition of ceramide synthase 2, the predominant ceramide synthase in liver and kidney, results in decreased biosynthesis of ceramide and increased sphingoid bases and sphingoid base 1-phosphates in liver, kidney and blood. Many studies have shown that decreased ceramide synthase activity and increased sphingosine kinase activity (Fig. 1) are closely associated with tumour development and progression in many human tumours (Espaillat et al., 2015; Reimann et al., 2015; Suh & Saba, 2015), and it has been proposed that sphingosine kinase 1 and 2 are oncogenes (Vadas et al., 2008; Pyne et al., 2016). Additional support for fumonisin-induced ceramide synthase inhibition as a contributing factor in hepatocarcinogenicity is the fact that ablation of ceramide synthase 2 in mice results in a large reduction in C-22

Fig. 1
The sphingolipid rheostat

In the presence of fumonisin, inhibition of ceramide biosynthesis shifts the balance of ceramide and sphingoid base 1-phosphates in tissues and blood. Sphingoid base 1-phosphates and ceramides have opposite effects on cell survival. Sphingosine 1-phosphate and sphinganine 1-phosphate interact with extracellular receptors (see Fig 2. in Annex 1, reference *206*) that are part of signalling pathways that regulate cell proliferation, cell migration and vascularization, all processes involved in cancer progression (Watterson et al., 2003). The balance between bioactive sphingolipid metabolites regulating cell death and survival/proliferation is termed the "sphingolipid rheostat".

and C-24 ceramides and an increase in C-16 ceramides and sphinganine in the liver (Pewzner-Jung et al., 2010a). These same ceramide synthase 2–null mice show elevated levels of apoptosis and proliferation in the liver and upregulation of cell cycle–related genes, and spontaneously develop liver tumours at about 10 months of age (Pewzner-Jung et al., 2010a,b). Many of the changes and effects seen in liver of the ceramide synthase 2 knock-out mice are reminiscent of changes and effects reported in studies where ceramide synthases are blocked using FB_1.

At the seventy-fourth meeting in 2011, the Committee considered the interaction between DNA-reactive AFB_1 and the potential for FB_1 to induce regenerative proliferation to be of concern (Annex 1, reference *205*). More recently, the International Agency for Research on Cancer (IARC) Scientific Publication No. 158 stated that "...given the interactions found experimentally, the co-contamination of crops by aflatoxins and fumonisins, and the fact that both toxins occur in populations with a high prevalence of [hepatitis B virus] infection, a role for fumonisins in [hepatocellular carcinoma] is plausible" (IARC, 2012). Specifically, the concern of the IARC Working Group stemmed from the

fact that AFB_1 is a potent mutagen and DNA-reactive carcinogen whereas FB_1 is an effective promoter of liver cancer and preneoplastic liver lesions in animal models (Gelderblom et al., 1988, 2002; Carlson et al., 2001) with a nongenotoxic mechanism of action (IPCS, 2000; IARC, 2012; Annex 1, references *152* and *205*).

Aflatoxins and fumonisins are frequent contaminants in maize, and aflatoxins are also common contaminants in groundnuts. Thus, co-exposure to both mycotoxins is likely in areas where maize and groundnuts are dietary staples (reviewed in "Aflatoxins (addendum)" (pages 3–279) and "Fumonisins (addendum)" (pages 415–573)). Given that their mechanisms of action are quite different, it is not unreasonable to assume that co-exposure is likely to at least additively enhance hepatotoxicity and hepatocarcinogenicity. It must be recognized that a number of dietary and environmental factors, especially co-exposure to hepatitis B virus (IARC, 2012), modulate AFB_1 hepatocarcinogenicity in humans. In this regard, the known ability of fumonisin to inhibit ceramide biosynthesis and negate the efficacy of chemotherapeutic agents (Mullen, Hannun & Obeid, 2012) and the proven over-expression of sphingosine kinases (proposed oncogenes; Vadas et al., 2008; Pyne et al., 2016) in tumour tissues provide a reasonable basis for how dietary exposure to FB_1, a potent ceramide synthase inhibitor, could contribute to chronic liver diseases in humans consuming high levels of foods contaminated with both fumonisins and aflatoxins. In support of this hypothesis is the human study conducted in Guatemala (Riley et al., 2015) that found a positive and statistically significant correlation between urinary fumonisin B_1 (UFB_1; a biomarker of exposure) and the blood levels of the sphingosine kinase product sphinganine 1-phosphate and the sphinganine 1-phosphate/sphingosine 1-phosphate ratio (biomarkers of effect) in humans consuming diets containing high levels of fumonisins. This result is consistent with the hypothesis that daily intake of high levels of fumonisin is likely to result in inhibition of ceramide synthase (mediator of apoptosis) and elevation of sphinganine and its phosphorylated metabolite sphinganine 1-phosphate (mediator of cell survival) in humans, similar to what has been described in many animal studies.

2.2 Toxicological studies of co-exposure

This section summarizes the results of toxicological co-exposure studies in animals conducted since the two previous Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluations and briefly describes the toxicological studies reviewed in the two previous evaluations. The purpose of this review of toxicological studies is to identify and evaluate all available new studies addressing the adverse effects in animals co-exposed to aflatoxins and fumonisins B_1, B_2 and B_3, individually or in combination, under controlled conditions. In

addition, as in previous evaluations, studies conducted using diets prepared using naturally contaminated maize or fungal culture material with known amounts of fumonisins and aflatoxins are also summarized.

Previous JECFA evaluations (Annex 1, references *152* and *205*) described studies documenting the ability of FB_1 to induce precancerous lesions in a more than additive fashion in rats orally dosed with pure FB_1 (Gelderblom et al., 2002). FB_1 was also shown to induce liver cancer and preneoplastic lesions with other DNA-reactive compounds (diethylnitrosamine [Gelderblom et al., 1988] and 7,12-dimethylbenz[a]anthracene [Takasaki et al., 2000]). In 2011, other oral gavage or feeding studies using pure FB_1 and AFB_1, or FB_1 and AFB_1 from fungal culture material, also showed adverse effects, suggesting a more than additive interaction in liver and/or kidney (reported in Annex 1, reference *205*) in studies in rats and rabbits (McKean et al., 2006; Orsi et al., 2007; Theumer et al., 2008).

In the sole chronic dose–response feeding study on the carcinogenic interaction of AFB_1 and FB_1, FB_1 was found to not be a complete carcinogen (Carlson et al., 2001). Rainbow trout fed diets with 3–104 parts per million (ppm) of pure FB_1 for 34 weeks did not develop tumours. However, when the researchers fed pure FB_1 to AFB_1-initiated fish (with a 30-minute immersion in 100 parts per billion [ppb] AFB_1 at 3 months of age) for 42 weeks, FB_1 caused a dose-related increase in liver tumour incidence, from 39% (for 3 ppm FB_1) to 74% (for 104 ppm FB_1). Liver cancer incidence in the FB_1-unexposed, AFB_1-initiated group was 35%. Furthermore, histological examinations revealed that a higher percentage of tumours found in the high-dose FB_1 treated group were malignant (58%) than in FB_1-unexposed, initiated fish (28%). Promotion was specific for the liver since no tumours were found in other tissues and increased tumour incidence was correlated with dose-related increases ($P < 0.05$, at 23 and 104 ppm FB_1 in the diet) in hepatic concentrations of free sphinganine and sphingosine (Carlson et al., 2001). It should be noted that FB_1, when fed to the fish for 1 week at up to 104 ppm in a second experiment, did not affect the incorporation of [^{3}H]-AFB_1 (a predictor of liver cancer initiation) into hepatic DNA. The liver cancer promotional activity of FB_1 was further verified in an experiment in which it was fed to trout that had been initiated with *N*-methyl-*N*′-nitro-nitrosoguanidine (MMNG).

2.2.1 In vivo studies on combined toxicity of pure fumonisin with aflatoxin, partially purified fumonisin with aflatoxin, or fumonisins and aflatoxins in culture material

In the present evaluation, all the newly available in vivo studies of combined effects of co-exposure to aflatoxins and fumonisins were evaluated qualitatively for evidence suggestive of interactions. Where possible, interactions were classified as suggesting or appearing to be less than additive, additive (no interaction)

or more than additive, based solely on the arithmetical sum of the observed individual and combined responses. The errors and pitfalls of this approach have been critiqued (Chou, 2010). Combined cytotoxicity (in vitro) studies are also briefly summarized; as with in vivo studies, there are many pitfalls in reaching conclusions about interactions between/among mycotoxins during co-exposure (Alassane et al., 2016; Smith et al., 2016).

(a) Pure fumonisin and pure aflatoxin in vivo

Mice

In an oral gavage study, 80 female Balb/c mice were divided between eight groups (n = 10/group) and treated for 14 days with the following: control; *Lactobacillus paracasei* only; AFB_1 only; FB_1 only; AFB_1 plus FB_1; AFB_1 plus *L. paracasei*; FB_1 plus *L. paracasei*; and AFB_1 plus FB_1 plus *L. paracasei*. The AFB_1 and FB_1 doses were 80 µg/kg bw and 100 µg/kg bw, respectively. After 14 days, all the animals were killed and the spleens removed, weighed and analysed for selected inflammatory cytokine mRNA, and selected antioxidant enzymes. The thymus was removed and analysed for apoptosis.

Only the AFB_1 and FB_1 toxicology results are summarized in detail here. The relative weights of the spleens were significantly increased to the same extent in the AFB_1, the FB_1 and the AFB_1-plus-FB_1 treatment groups (less than additive). The mRNA expression of the measured cytokines was significantly affected (up or down), and the effect of combined treatment was possibly additive for interleukin 10. Some enzymes indicative of oxidative stress were also significantly elevated or decreased in what appeared to be an additive fashion, and caspase 3 activity was significantly increased to the same extent in the AFB_1, FB_1 and AFB_1-plus-FB_1 treatment groups (less than additive). Overall, the combined exposure resulted in effects that were less than additive or additive for some of the measured parameters. The *L. paracasei* treatment was protective (Abbès et al., 2015).

The Committee concluded that co-exposure resulted in some effects suggesting additivity.

Rats

In a rat feeding study, Qian et al. (2016) placed 62 male F344 rats (6 weeks old; 150 g) in five groups and fed them as follows: negative control group (n = 10) fed American Institute of Nutrition (AIN)-76 diet for 56 days; AFB_1 group (n = 13) fed AIN-76 diet containing 150 µg/kg pure AFB_1 for 14 days followed by control diet for 42 days; FB_1 group fed control diet for 35 days followed by AIN-76 diet containing pure FB_1 at 250 mg/kg diet for 21 days; and AFB_1-plus-FB_1 group (n = 13) fed diets with AFB_1 at 150 µg/kg diet for 14 days followed by control diet for 21 days and the FB_1-containing diet for 21 days. A positive control

group (n = 13) was initiated with diethylnitrosamine (single intraperitoneal dose on day 1) and placed on an AIN-76 diet containing the tumour promoter 2-acetylaminofluorene at 150 µg/kg diet on day 14 for 21 days. The positive control rats were killed on day 35, and the rats in all other groups were killed on day 56. The positive control was added to ensure that the initiation/promotion protocol would induce preneoplastic lesions in the liver. The AFB_1 and FB_1 doses were approximately equivalent to 15 and 25 mg/kg bw per day, respectively.

The final body weights of both the FB_1 and the AFB_1-plus-FB_1 groups were significantly reduced to a similar extent compared with the negative control group or the AFB_1 group, which did not differ. Compared with the negative control group, feed consumption was significantly reduced to a similar extent in the AFB_1, FB_1 and AFB_1-plus-FB_1 groups. There were many statistically significant treatment-related differences in serum biochemistry, some of which were suggestive of being additive or more than additive. For example, compared with the negative control group, aspartate transaminase and alanine transaminase activities in the AFB_1 group were not significantly different, but the activities in the FB_1 group were double those of the negative control and AFB_1 groups. Aspartate transaminase and alanine transaminase activities in the AFB_1-plus-FB_1 group were more than 3-fold higher than in the negative control and AFB_1 groups. The most prominent treatment-related histological alterations were increased number of dysplasia and apoptotic cells. For example, the mean numbers (± standard deviation [SD]) of apoptotic cells per slide view (400× magnification) in the AFB_1, FB_1 and AFB_1-plus-FB_1 groups were 2.5 ± 2.1, 3.4 ± 1.6 and 26.5 ± 7.8, respectively. The frequency and area of glutathione *S*-transferase placental form positive (GST-P^+) foci also showed an increase, suggesting a more than additive effect. For example, the average numbers of GST-P^+ foci per cm^2 in the AFB_1, FB_1 and AFB_1-plus-FB_1 groups were 1.6 ± 0.6, 0.9 ± 1.0 and 11.6 ± 4.7, respectively. Similar relative treatment-related differences were seen in the foci area. (Note that although in this rat strain and sex the kidney is the primary target for FB toxicity, effects on kidney were not reported (Qian et al., 2016).)

The results of this study are important because they confirm the findings of the study by Gelderblom et al. (2002), which are consistent with the hypothesis that FB_1 promotes AFB_1 hepatocarcinogenesis in rats. In fact, the protocol for AFB_1 and FB_1 treatment in this study was a modification of that used by Gelderblom et al. (2002). The primary difference between the two protocols was that Gelderblom et al. (2002) administered aflatoxin by oral gavage, whereas Qian et al. (2016) incorporated it into the diet.

The Committee concluded that the effects from co-exposure suggest that they might be more than additive.

In a rat feeding study, Hassan et al. (2015) divided 80 female Sprague Dawley rats (3 months old; 100–120 g) between eight groups (n = 10/group) and

fed them as follows for 12 weeks: control group fed basal diet only; *Panax ginseng* group fed basal diet plus 20 mg/kg bw per day *P. ginseng* extract; AFB_1 group fed basal diet and treated orally with 80 µg/kg bw per day pure AFB_1; FB_1 group fed basal diet and treated orally with 100 µg/kg bw per day pure FB_1; AFB_1-plus-FB_1 group fed basal diet with 80 µg/kg bw per day pure AFB_1 and 100 µg/kg bw per day pure FB_1; and three additional groups treated with either one or both mycotoxins plus the *P. ginseng* extract. After 12 weeks on the diets, the rats were killed and liver and kidney were collected and analysed for markers of lipid peroxidation, glutathione, expression of some antioxidant enzymes and DNA fragmentation.

Statistically significant changes in some parameters were indicative of additive or less-than-additive effects on lipid peroxidation, glutathione content, antioxidant enzyme expression and DNA fragmentation in liver and kidney. The *P. ginseng* extract treatment was partially protective (Hassan et al., 2015).

The Committee concluded that co-exposure could have resulted in some additive effects. However, the description of the dosing and the experimental design were inadequate to draw any firm conclusion.

(b) Culture material or partially purified fumonisin and pure aflatoxin in vivo

In a rat feeding study, 24 male Wister rats (6–8 weeks old; 220 g) were divided between four groups and fed the following diets for 90 days: control group ($n = 6$) fed control experimental diet; FB_1 group ($n = 6$) fed control experimental diet containing 100 mg/kg diet of FB_1 (B_2 and B_3 were also present) from *Fusarium verticillioides* (MRC 826) culture material extract; AFB_1 group ($n = 6$) fed control experimental diet containing 40 µg/kg diet of pure AFB_1; FB_1-plus-AFB_1 group ($n = 6$) fed control experimental diet containing 100 mg/kg diet of FB_1 from *F. verticillioides* culture material extract plus 40 µg/kg diet of pure AFB_1. After 90 days on the diets, the rats were killed and spleens were removed. Spleen mononuclear cells were isolated and analysed for DNA damage (alkaline comet assay and micronucleus assay), lipid peroxidation, catalase and superoxide dismutase. These same assays were conducted on a subset of the spleen mononuclear cells cultured from the spleen cells collected at termination, except they were also dosed in vitro (20 and 10 µg/mL of pure AFB_1 and pure FB_1, respectively). The average total FB_1 intakes for the in vivo FB_1 group and the FB_1-plus-AFB_1 group were 980 and 742 mg/kg bw per 90 days, respectively, or 10.9 and 8.2 mg/kg bw per day. The average total AFB_1 intakes for the AFB_1 and the FB_1-plus-AFB_1 groups were 328 and 299 µg/kg bw per 90 days, respectively, or 3.6 and 3.3 µg/kg bw per day.

The results showed that, under the conditions of this experimental design, there was significant DNA damage based on both the alkaline comet assay and micronucleus assay in vivo and in vitro (Theumer et al., 2010). The Committee concluded that the DNA damage, lipid peroxidation and other

measured indicators of oxidative stress in the FB_1-plus-AFB_1 group suggest that most effects were less than additive, but sometimes possibly additive, compared with the groups exposed to FB_1 or AFB_1 alone.

In a rat feeding study, 60 female Sprague Dawley rats (3 months old; 155–160 g) were acclimated for 1 week and then divided between six groups (n = 10/group) to be fed the following diets for 12 weeks: control group fed basal diet only; *P. ginseng* group fed basal diet plus 150 mg/kg bw per day *P. ginseng* extract; AFB_1-plus-FB_1 group fed basal diet and a daily oral gavage dose of pure AFB_1 (17 µg/kg bw per day) in maize oil for 14 days, followed by 3 weeks on basal diet only and then 3 weeks on basal diet containing FB_1 at 250 mg/kg diet prepared from *F. verticillioides* culture material extract and finally by 4 weeks on basal diet only (total 12 weeks). There were three additional treatment groups but no FB_1 group. Thus, the study design makes it impossible to evaluate the interaction between AFB_1 and FB_1 with regard to their combined toxicity. Nonetheless, the sequential treatment with AFB_1 and fumonisins from culture material resulted in changes indicative of precancerous liver lesions. *P. ginseng* extract treatments did provide the rats significant protection based on serum biochemical parameters, selected serum lipids, tumour markers, antioxidant expression and lipid peroxidation at 12 weeks (Abdel-Wahhab et al., 2010).

The Committee concluded that an interaction could not be determined.

(c) Culture material or partially purified fumonisin and aflatoxin in vivo

Chickens (Hisex Brown layers; n = 168; 37 weeks old) were divided between seven treatment groups so that each cage containing four birds constituted an experimental unit and each treatment group contained six cages (Siloto et al., 2013). The treatment groups were as follows: control group fed diets with no mycotoxins or binder; AFB group fed a diet prepared with *Aspergillus parasiticus* (strain not given) culture material containing AFB_1 and AFB_2 to a final concentration of 1 mg/kg diet (estimated to be 0.125 mg/kg bw per day); fumonisin group fed a diet prepared with *F. verticillioides* culture material (strain not given) containing fumonisins (not characterized) to a final concentration of 25 mg/kg diet (estimated to be 3.125 mg/kg bw per day); AFB-plus-fumonisin group fed a diet prepared with both the *A. parasiticus* and *F. verticillioides* culture material; and three groups fed diets with a commercial mycotoxin binder prepared from yeast cell walls. The diets were analysed for aflatoxin, fumonisin and other mycotoxins (data not presented). The animals were fed the various diets for 56 days. They were then killed, their tissues weighed and the lengths of the small and large intestines measured. Blood samples were also collected from two birds from each cage, and selected plasma lipids and per cent liver fat were determined.

Statistical analyses indicated that, compared with the groups fed the diets prepared with only the *A. parasiticus* or *F. verticillioides* culture material, the combined exposure caused changes in plasma triglycerides, very low density lipoprotein levels and per cent total liver lipids that were indicative of an interactive effect. The fumonisin group and the AFB-plus-fumonisin group showed significantly reduced length of the small intestines compared with the control animals. The mycotoxin binder appeared to have a protective effect for some of the measured parameters (Siloto et al., 2013).

The Committee concluded that there were effects from co-exposure that suggested they were additive (no interaction) or less than additive.

2.2.2 In vitro studies investigating various aspects of combined FB_1 and AFB_1 cytotoxicity using pure toxins

The potential problems associated with the interpretation of the results of in vitro co-exposure studies are discussed in section 2.2.6(c) of "Fumonisins (addendum)". Since 2011, only a few in vitro published studies have addressed AFB_1 and FB_1 co-exposure.

The dose-dependent effects of FB_1 and AFB_1 show that both mycotoxins can induce oxidative stress in bovine peripheral mononuclear cells, but the effects of combined treatment were not reported (Bernabucci et al., 2011).

The effects of combined treatment were reported in a study using rat spleen mononuclear cells. Mary et al. (2012) showed that 20 μmol/L AFB_1 and 10 μmol/L FB_1 increased production of reactive oxygen species and that the production was greatest with the combined treatment, suggesting a less-than-additive effect. Individually, AFB_1 and FB_1 had no significant effect on superoxide anion radical generation relative to the control, but the combined treatment significantly increased its production by about 15% relative to the control. Effects on protein oxidation, lipid peroxidation and DNA oxidation were mostly suggestive of additivity in the combined treatment. The increased production of reactive oxygen species and superoxide anion radical generation was attributed to effects on mitochondrial function and pathways regulating the nicotinamide adenine dinucleotide phosphate (reduced) oxidase system, CYP and arachidonic acid metabolism.

In a study using the rat liver hepatoma cell line H4IIE, FB_1 alone or in combination with AFB_1 increased CYP1A transcription and CYP1A activity, as well as upregulated the aryl hydrocarbon receptor in a dose-dependent manner (Mary et al., 2015). The effects were greatest in the cells treated with the FB_1–AFB_1 mixture; in some cases, the effects were suggestive of being more than additive. The effects were seen at FB_1 and AFB_1 concentrations that were not cytotoxic. Freshly isolated rat spleen mononuclear cells were also tested; the nature of the effects on CYP1A mRNA expression was much more variable and the time dependence

differed compared with the effects in H4IIE cells. Nonetheless, effects in the rat spleen mononuclear cells were often suggestive of being more than additive.

The IC_{50} for AFB_1 and FB_1 inhibition of alkaline phosphatase isolated from shrimp was greatest when the two mycotoxins were combined (Pérez-Acosta et al., 2016). How the two mycotoxins inhibited alkaline phosphatase was not determined but appeared to be less than additive.

2.3 Observations of co-exposure in humans

2.3.1 Biomarkers of exposure

Urinary multi-biomarker analytical methods have recently been developed and are increasingly used to estimate human exposure to mycotoxins. These methods are typically capable of simultaneously measuring the concentration of more than five urinary mycotoxin biomarkers, including UFB_1 and urinary AFM_1 ($UAFM_1$).

The majority of the published studies that attempted to detect multiple urinary mycotoxin biomarkers concurrently provided only the frequency of positive samples for each individual mycotoxin without reporting the status of co-occurrence. For example, of 120 urine samples collected in northern Nigeria and analysed for eight mycotoxins using a multi-biomarker approach, $UAFM_1$ was detected in 14.2% of the samples and UFB_1 in 13.3%; however, no co-occurrence information was provided (Ezekiel et al., 2014). Also, when urinary mycotoxins were measured in 220 children from high mycotoxin contamination regions in Cameroon, 14% of the urine samples were positive for $UAFM_1$ and 11% for UFB_1, but the number of samples positive for both biomarkers was not reported (Njumbe Ediage et al., 2013). These types of studies provide very limited information on the co-exposure status.

Similarly, very few multi-biomarker analysis studies yielded information on urinary mycotoxin biomarker co-occurrence. In a study in Guatemala, of 602 urine samples collected from female residents, 48% (n = 287) were positive for UFB_1 and 15% (n = 90) were positive for $UAFM_1$. Of the 90 AFM_1-positive samples, 66 (73%) were also positive for UFB_1. This result was in concordance with the fact that the contamination of maize with fumonisins and aflatoxins is common in Guatemala (Torres et al., 2015).

None of the other published studies of mycotoxin co-occurrence were conducted in a population with high fumonisin and aflatoxin exposure from food. For example, multiple urinary biomarkers were determined in samples from 52 volunteers resident in Apulia region in southern Italy. While UFB_1 was detected in 56% (n = 29) of the samples, $UAFM_1$ was only detected in 6% (n = 3), indicating that co-exposure to aflatoxin and fumonisin is rare in this population (Solfrizzo, Gambacorta & Visconti, 2014). Similarly, in a comparative study using

samples from Bangladesh, Germany and Haiti, co-exposure was not observed because of the low frequency of detectable UFB_1 or $UAFM_1$ or both (Gerding et al., 2015).

One study in the United Republic of Tanzania determined the co-exposure status by measuring the urinary marker UFB_1 and the plasma aflatoxin–albumin (AF–alb) adduct in the same 148 children. UFB_1 was detected in 96% of the children and plasma AF–alb in 84% of the same children; 82% of the children had co-exposure. In addition, there was a significant positive correlation between levels of UFB_1 and AF–alb (Shirima et al., 2013).

2.3.2 Biomarkers of effects

There have been no human studies using biomarkers of effect for both FB_1 and AFB_1 concurrently.

2.3.3 Epidemiological studies

Although evidence in laboratory animals suggests that fumonisin and aflatoxin may act additively or synergistically in the development of hepatocellular carcinoma (Torres et al., 2015), no epidemiological data are currently available on such an association in humans.

Two epidemiological studies conducted in the United Republic of Tanzania investigated the association of fumonisin–aflatoxin co-exposure and childhood growth. One study was conducted in infants up to 5 months of age (Magoha et al., 2016), the other in infants and toddlers aged 6–14 months (Shirima et al., 2015).

In the Magoha et al. (2016) study, 143 infants were progressively recruited after birth and followed up at 1, 3 and 5 months of age. At each follow-up visit, the infants' weight and length were measured. Weight-for-age *z*-scores (WAZ) and length-for-age *z*-scores (LAZ) were computed according to the World Health Organization (WHO, 2006) child growth standards. Exclusive breastfeeding is rarely practised in the United Republic of Tanzania; 80% of the infants had started receiving complementary food at 3 months of age and 97% had at 5 months of age. The majority of the complementary food was prepared from maize flour or mixed cereal flours with maize as the primary constituent. For infants who had been introduced to maize foods (in the form of maize flour), a 24-hour dietary recall was used to estimate the amount of flour intake, and samples of maize flour from families were collected for mycotoxin analysis. Of these maize flour samples (n = 67), 39 had detectable aflatoxins (median: 6 μg/kg diet), 21 had detectable fumonisins (median: 124 μg/kg diet) and 15 had both aflatoxins and fumonisins. The infants' growth status at 5 months of age was compared with that at 3 months of age. The weight and length gains were slightly higher in exclusively

breastfed infants (n = 23) than in those who had received complementary foods (n = 92) regardless of mycotoxin contamination. Of the infants introduced to maize-based food (n = 67), 6% were underweight and 18% were stunted. Of these underweight and stunted infants, 39 had been exposed to aflatoxin alone (with 3% underweight and 15% stunting); 21 had been exposed to fumonisin alone (0 underweight and 5% stunting); and 15 had been exposed to both aflatoxin and fumonisin (0 underweight and 7% stunting). The likelihood of an association between exposure to fumonisins or aflatoxins (alone or combined) and growth abnormalities (underweight or stunted) was analysed using logistic regression. No statistically significant associations were found (Magoha et al., 2016).

Although the results of the study did not show a significant association between mycotoxin exposure and impairment of growth in this infant population, the Committee acknowledges two limitations in the study that may compromise the value of the results. First, growth impairment in infants is influenced by multiple factors, which were not controlled in the analysis of this study. Second, previous studies conducted in the same region of the United Republic of Tanzania indicated that breast milk was also an important source of exposure to mycotoxins. Magoha et al. (2014a,b) found that 100% of the breast milk samples collected from local lactating women were contaminated with AFM_1 and 44% with FB_1. Unfortunately, without taking into account the exposure from breast milk, the total exposure was not accurately estimated. The Committee noted that the total exposure measured by validated biomarkers would be useful in determining the association between mycotoxin exposure and child growth.

In the other Tanzanian epidemiological study, Shirima et al. (2015) used biomarkers to determine the total mycotoxin exposure in 166 seemingly healthy children, aged 6–14 months, randomly recruited from three villages, and subsequently followed up after 6 months and 12 months. At recruitment and each of the two follow-up visits, the height and the weight of each child were recorded, a dietary recall was performed, and the blood and urine samples for each child were collected for aflatoxin and fumonisin biomarker analysis. Using individual data, multiple regression analysis was performed to determine the association between mycotoxin biomarkers and childhood growth. All models were adjusted for village, breastfeeding, maternal education, socioeconomic status and protein/energy intakes. The results showed that UFB_1 and AF–alb were negatively associated with the LAZ score and length velocity (effect of AF–alb alone did not reach statistical significance). However, no interpretable results were found regarding the effect of fumonisin–aflatoxin co-exposure and stunting.

3. Co-occurrence of aflatoxins and fumonisins in foods

An evaluation of the co-occurrence of aflatoxins and fumonisins in foods was undertaken by the Committee.

Co-occurrence in foods can be defined as the presence of detectable concentrations of the mycotoxins in foods in general, within the same analytical sample, or in the foods that make up a whole diet. This evaluation focuses on the co-occurrence in food by reviewing analytical concentration data (from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme [GEMS/Food] contaminants database and the literature) both within foods in general and within the same analytical sample.

The information on the co-occurrence (also referred to as "coexistence") of a number of mycotoxins in foods in general is broader than the information on aflatoxin and fumonisin groups (Lopez et al., 2016; Smith et al., 2016). There is also information on the different aflatoxins and fumonisins in foods, either individually or as totals (outlined below). While this information is covered in general here, the focus of this evaluation is the co-occurrence of AFB_1 and FB_1. This is because the Committee previously noted (at its seventy-fourth meeting) that the co-exposure to AFB_1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation, is of concern.

IARC recently published a report on mycotoxin control in low- and middle-income countries that highlights the issue of co-occurrence and notes high concurrent exposure to aflatoxins and fumonisins in maize-eating populations in Africa, Latin America and parts of Asia (IARC, 2015). The report also noted that the recent development of analytical methods that can analyse for multiple mycotoxins in one analysis has raised the awareness of co-occurrence of aflatoxins and fumonisins. Much of the scientific literature reviewed by the Committee uses these methods.

The co-occurrence of aflatoxins and fumonisins in foods was evaluated in two ways. It was assessed, firstly, by analysing data in the GEMS/Food contaminants database and, secondly, by reviewing the literature for studies that have analysed for aflatoxins and fumonisins in the same foods. The aims of the analysis were to determine what foods have co-occurrence, the degree of co-occurrence and in which countries the co-occurrence exists.

Co-occurrence for this analysis was defined as where detected concentrations were found for *both* aflatoxins and fumonisins in food. Therefore, there was no co-occurrence where either aflatoxins or fumonisins were not detected (concentrations less than the limit of detection [LOD] or quantification [LOQ], depending on the data available).

While there may be aflatoxin and fumonisin co-occurrence in animal feed, the Committee did not consider animal feed for this review because it had previously noted that transfer of fumonisins from feed to animal products, either as parent compounds or as their hydrolysis products, in significant amounts is minimal; therefore, the occurrence of fumonisins in feed was considered not to be a human health concern (Annex 1, reference *205*).

The co-occurrence of aflatoxins and fumonisins in foods is likely under certain conditions. (These are outlined in detail separately for each of these mycotoxins in their respective monograph addenda.) Fungal growth and toxin production can occur in the field or preharvest (particularly for fumonisins but also for aflatoxins), in storage (aflatoxins) or in both (IARC, 2015). Favourable conditions for co-occurrence have been identified as temperatures between 25 and 30 °C and water activity between 0.8 and 0.99 (Bhat, Rai & Karim, 2010; Kamala et al., 2015). Levels of fumonisins and aflatoxins can be affected by the source of the food, where it was grown, the degree of contamination preharvest, postharvest conditions, cross-contamination and processing.

3.1 Co-occurrence of aflatoxins and fumonisins in foods in general

Many published studies on co-occurrence were not restricted to AFB_1 and FB_1. Co-occurrence may have been reported for aflatoxins or fumonisins as totals, or for a range of subtypes for the contaminants. The co-occurrence of aflatoxins and fumonisins was most prevalent in Africa, Asia and South America, with maize being the commodity most commonly contaminated with aflatoxins and fumonisins (Smith et al., 2016).

Co-occurrence of total aflatoxins and total fumonisins has been reported in a number of studies:

- In a study of corn infected by common smut in the USA (Abbas et al., 2015), using 5 kg bags of samples collected over 4 years, all three corn sample types (corn kernels from smutted ears of corn; corn kernels from control ears of corn; smut galls) contained both total aflatoxins and total fumonisins. Concentrations in smutted ears were higher than in non-smutted ears.
- Samples of maize flour–based foods for infants in the United Republic of Tanzania showed co-occurrence in 23% of samples (Magoha et al., 2016).
- In western Kenya, 4% of samples of maize from farm stores contained total aflatoxins (n = 488) and 41% total fumonisins (n = 233). Of samples of maize from mills, 41% contained total aflatoxins (n = 574) and 87% total fumonisins (n = 125) (Mutiga et al., 2015).

- Of 57 samples of maize, sorghum and pearl millet from Nigeria, 89% were co-contaminated with aflatoxins and fumonisins (Vismer et al., 2015).

In general, studies found that the majority of maize/corn samples have higher occurrence of fumonisins than of aflatoxins (Mutiga et al., 2015); this was also shown in maize flour–based foods for infants (Hove et al., 2016a; Magoha et al., 2016).

Some studies that analysed both AFB_1 and FB_1 did not report results at this level of detail or by food but included summaries at a less specific level. A study of maize in Argentina noted that the degree of co-occurrence of total aflatoxins, FB_1 and FB_2 in the same samples was 8.4% (Garrido et al., 2012). Mulunda et al. (2013) found both aflatoxins and fumonisins in maize-based foods (n = 40; 95% and 100%, respectively) and beans (n = 30; 80% and 83%, respectively) in the Democratic Republic of Congo. Rodrigues et al. (2011) found both aflatoxins and fumonisins in maize (n = 63; 35% and 84%, respectively), wheat and wheat bran (n = 32; 19% and 9%, respectively) and soybean (n = 33; 24% and 12%, respectively).

There is much variation in the degree of co-occurrence of aflatoxins and fumonisins between countries and/or between foods. This could be attributed to variety of the commodity, geographical region, climate, seasonal variations, grower knowledge, storage conditions, food preparation, food processing and time of sampling. The degree of co-occurrence has been shown to vary due to a number of factors, including the following:

- geographical region, mostly due to climatic conditions; for example, Mngqawa et al. (2016) found variations in the degree of co-occurrence of aflatoxins and fumonisins in maize in two areas of South Africa, as did Phuong et al. (2015) in two areas of Viet Nam;
- different growing seasons/years, the time of harvest and storage conditions, as found by Mngqawa et al. (2016) in South Africa;
- length of storage; for example, Mutinga et al. (2015) found that the occurrence of aflatoxins in maize was between 42% and 54% and of fumonisins was between 76% and 100%, depending on how long they were stored in mills in Kenya;
- food processing; for example, the concentration of FB_1 in the source maize was reduced by 99.4% (sum of FB_1 for all samples reduced from 21 845 to 123 μg/kg) by fermenting the cereal in the brewing of the Nigerian beverage kunu-zaki (Ezekiel et al., 2015).

3.2 Co-occurrence of AFB_1 and FB_1 in foods

As noted above, a key aim of this evaluation was to assess the co-occurrence of AFB_1 and FB_1 in foods. This was done by (1) analysing data in the GEMS/Food contaminants database, and (2) reviewing the literature for information on co-occurrence.

3.2.1 Evaluation of co-occurrence based on data from the GEMS/Food contaminants database

CCCF requested that JECFA evaluate the co-occurrence, in food and feed, of fumonisins with other mycotoxins at its seventy-fourth meeting. However, the evaluation could not be performed at that time because only aggregated data were available. The Committee noted at that meeting that levels of fumonisins and other mycotoxins must be available at the level of the individual analytical sample for such an assessment to be conducted. At the current meeting, the Committee used the GEMS/Food contaminants database, which contains information from samples at the individual level, to evaluate the co-occurrence of aflatoxins and fumonisins.

The concentration data available in the GEMS/Food contaminants database for aflatoxins and fumonisins are described in detail for each of these mycotoxins separately, as the Committee also evaluated them separately at the current meeting.

The co-occurrence of AFB_1 and FB_1 was evaluated in foods using data from the GEMS/Food contaminants database for the following:

- AFB_1 and FB_1 from all samples for each food, regardless of whether the foods have a unique sample identification number. (The database field descriptor for the sample identification number is "serial number", which refers to the unique number for each individual analytical sample. From here on, this monograph refers to this serial number as "sample identification number".) Therefore, this analysis did not necessarily include samples from the same country or study for the same food, and the number of samples of AFB_1 differed from the number for FB_1 for the same food.
- AFB_1 and FB_1 for only those samples where a unique sample identification number was provided and both contaminants were analysed in the same sample. This analysis is presented by country and by food.

The GEMS/Food contaminants database had over 474 600 rows of data for any type of aflatoxin or fumonisin, including total and specific types, from 41 countries and one WHO region (which, since 2016, have been included to

allow anonymous submissions by European Member States for submissions to the European Food Safety Authority).

Just over 8300 rows of data were excluded, as these were aggregated (summary) data and not for individual samples. Samples with high LODs or LOQs for FB_1 (n = 287) were excluded based on the criteria used in the dietary exposure assessment for fumonisins for this meeting (i.e. LOD >250 µg/kg and/or LOQ >750 µg/kg). No data were excluded by year. Where sample identification numbers were duplicated, the duplicates were evaluated and, if the results and all information for the sample were the same in all the database fields for all duplicates, the duplicates were removed so that only one value for the sample identification number was retained and used in the analysis. Where the results for the same sample identification number differed, both results were retained in the dataset and were assumed to be individual results. Some data points were also excluded where the results or metadata were evaluated and determined to be unreliable.

(a) Results not based on unique sample identification numbers for AFB_1 and FB_1

An evaluation was conducted based on the data in the GEMS/Food contaminants database for AFB_1 and FB_1 irrespective of unique sample identification number. Just over 106 500 rows of data for AFB_1 (>84 000) and FB_1 (>19 000) were used for this analysis from 38 countries and one WHO region.

This analysis determined the number of samples of a food that had a record for AFB_1, the number of samples in which AFB_1 was detected and the percentage of positive samples. This analysis was repeated for FB_1. Where there were positive results for a food for both AFB_1 and FB_1, it was determined that this food had co-occurrence of AFB_1 and FB_1. The results of this evaluation are shown for each food group by food in Table 1.

Both AFB_1 and FB_1 were detected in the majority of foods in the cereals and cereal-based products group, including barley, maize, millet, rice, rye, sorghum and mixed cereal products. Neither AFB_1 nor FB_1 was detected in oats or triticale. Many other food groups had a small number of foods with both contaminants. These included foods for infants and young children (which included cereal-based foods), snack foods, confectionery, fruit and fruit products, herbs and spices, and legumes. Neither AFB_1 nor FB_1 was detected in vegetables and vegetable products, starchy roots and tubers, milk and dairy products, eggs and egg products, fish and seafood, composite foods (meals/dishes) and tap water. This was either because (1) only AFB_1 or FB_1 was analysed (and therefore co-occurrence could not be assessed) or because (2) neither contaminant was detected.

Table 1

Occurrence of AFB_1 and FB_1 in foods based on the concentration data in the GEMS/Food contaminants database irrespective of country or sample identification number

	AFB_1				FB_1				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB_1	FB_1	Co-occurrence?
Alcoholic beverages											
Alcoholic beverage, nes	1	0	ND	ND	–	–	–	–	0	–	NA
Beer and beer-like beverage	717	341	ND	0.2	431	5	ND	4	47.6	1.2	Yes
Liqueur and spirits	2	0	ND	ND	–	–	–	–	0	–	NA
Wine	204	0	ND	ND	16	0	ND	ND	0	0	No
Wine-like drinks (e.g. cider, perry)	–	–	–	–	2	0	ND	ND	–	0	NA
Cereals and cereal-based products											
Barley	849	20	ND	11	244	1	ND	143	2.4	0.4	Yes
Bran, unprocessed, of cereal grain (except buckwheat, canihua, quinoa)	164	4	ND	3	77	9	ND	655	2.4	11.7	Yes
Bread and other cooked cereal products	2 931	315	ND	4 068	3 299	952	ND	2 270	10.7	28.9	Yes
Buckwheat	293	19	ND	21	99	2	ND	80	6.5	2	Yes
Cereal grains	1 409	124	ND	7	493	90	ND	35 400	8.8	18.3	Yes
Cereals and cereal-based products, nes	3 132	537	ND	30	4 140	1 597	ND	11 963	17.1	38.6	Yes
Job's tears	41	39	ND	8	–	–	–	–	95.1	–	NA
Maize	2 425	519	ND	97	4 760	2 458	ND	23 800	21.4	51.6	Yes
Millet	127	7	ND	2	127	2	ND	44	5.5	1.6	Yes
Oats	541	0	ND	ND	431	3	ND	331	0	0.7	No
Popcorn	324	6	ND	2	69	27	ND	6 601	1.9	39.1	Yes
Rice	3 819	798	ND	17	503	9	ND	253	20.9	1.8	Yes
Rye	245	5	ND	1	195	9	ND	78	2	4.6	Yes
Sorghum	1 621	113	ND	359	1 569	183	ND	3 419	7	11.7	Yes

Food group / Food	AFB_1 Total no. of samples[a]	AFB_1 No. of samples detected[b]	AFB_1 Minimum (µg/kg)	AFB_1 Maximum (µg/kg)	FB_1 Total no. of samples[a]	FB_1 No. of samples detected[b]	FB_1 Minimum (µg/kg)	FB_1 Maximum (µg/kg)	% samples detected[c] AFB_1	% samples detected[c] FB_1	Co-occurrence?
Sweet corn (corn on the cob)	61	2	ND	0.2	259	26	ND	793	3.3	10	Yes
Triticale	–	–	–	–	2	0	ND	ND	–	0	NA
Wheat	3 114	68	ND	2	952	17	ND	750	2.2	1.8	Yes
White bread	66	4	ND	0.2	98	1	ND	29	6.1	1	Yes
Wholemeal bread	19	0	ND	ND	3	0	ND	ND	0	0	No
Composite food (including frozen products)											
Beans-based meals	4	0	ND	ND	4	0	ND	ND	0	0	No
Cereal-based dishes	3	0	ND	ND	32	13	ND	727	0	40.6	No
Composite food, nes	26	6	ND	3	16	0	ND	ND	23.1	0	No
Egg-based meal (e.g. omelette)	16	0	ND	ND	–	–	–	–	0	–	NA
Fish and seafood-based meals	28	4	ND	7.3	–	–	–	–	14.3	–	NA
Meat-based meals	15	3	ND	2	12	0	ND	ND	20	0	No
Potato-based dishes	1	0	ND	ND	–	–	–	–	0	–	NA
Prepared salads	–	–	–	–	7	6	ND	243	–	85.7	NA
Ready-to-eat soups	1	0	ND	ND	24	0	ND	ND	0	0	No
Rice-based meals	31	13	ND	0.2	12	0	ND	ND	41.9	0	No
Vegetable-based meals	9	5	ND	0.2	5	0	ND	ND	55.6	0	No
Drinking-water (water without any additives except carbon dioxide; includes water ice for consumption)											
Tap water	8	0	ND	ND	8	0	ND	ND	0	0	No
Eggs and egg products											
Chicken eggs	14	0	ND	ND	–	–	–	–	0	–	NA
Eggs and egg products, nes	2	0	ND	ND	1	0	ND	ND	0	0	No
Fats and oils of animal and vegetable (excluding butter)											
Cottonseed oil, edible	–	–	–	–	2	2	7.2	15	–	100	NA
Fats and oils, nes	471	165	ND	12	25	3	ND	46	35	12	Yes
Lard (of pigs)	4	0	ND	ND	–	–	–	–	0	–	NA

Table 1 (continued)

	AFB$_1$				FB$_1$				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB$_1$	FB$_1$	Co-occurrence?
Palm oil, edible	2	1	ND	0.1	–	–	–	–	50	–	NA
Peanut oil, edible	13	0	ND	ND	1	0	ND	ND	0	0	No
Poultry, fats	4	0	ND	ND	–	–	–	–	0	–	NA
Rapeseed oil, edible	47	0	ND	ND	–	–	–	–	0	–	NA
Sesame seed oil, edible	25	0	ND	ND	7	0	ND	ND	0	0	No
Sunflower seed oil, edible	40	0	ND	ND	2	0	ND	ND	0	0	No
Fish and other seafood (including amphibians, reptiles, snails and insects)											
Carps	78	0	ND	ND	–	–	–	–	0	–	NA
Crustaceans	8	0	ND	ND	–	–	–	–	0	–	NA
Dried fish	8	0	ND	ND	–	–	–	–	0	–	NA
Fish, seafood, amphibian, reptile, snail or insect, nes	54	0	ND	ND	–	–	–	–	0	–	NA
Fishes	32	3	ND	6	–	–	–	–	9.4	–	NA
Molluscs, including cephalopods	1	0	ND	ND	–	–	–	–	0	–	NA
Oysters (including cupped oysters)	–	–	–	–	1	1	89.8	90	–	100	NA
Shrimps and prawns	1	0	ND	ND	–	–	–	–	0	–	NA
Trout	42	0	ND	ND	–	–	–	–	0	–	NA
Food for infants and small children											
Cereal-based food for infants and young children	1 651	41	ND	1	1 053	57	ND	1 110	2.5	5.4	Yes
Follow-on formula, liquid	1	0	ND	ND	–	–	–	–	0	–	NA
Follow-on formula, powder	118	0	ND	ND	7	0	ND	ND	0	0	No
Food for infants or children, nes	152	1	ND	0.1	124	13	ND	89	0.7	10.5	Yes
Fruit juice and herbal tea for infants and young children	–	–	–	–	2	0	ND	ND	–	0	NA

Food group / Food	AFB$_1$ Total no. of samples[a]	AFB$_1$ No. of samples detected[b]	AFB$_1$ Minimum (µg/kg)	AFB$_1$ Maximum (µg/kg)	FB$_1$ Total no. of samples[a]	FB$_1$ No. of samples detected[b]	FB$_1$ Minimum (µg/kg)	FB$_1$ Maximum (µg/kg)	% samples detected[c] AFB$_1$	% samples detected[c] FB$_1$	Co-occurrence?
Infant formula, liquid	6	0	ND	ND	–	–	–	–	0	–	NA
Infant formula, powder	36	1	ND	0.2	54	0	ND	ND	2.8	0	No
Ready-to-eat meals for infants and young children	140	24	ND	0.2	251	48	ND	151	17.1	19.1	Yes
Yoghurt, cheese and milk-based desserts for infants and young children	5	1	ND	0.1	4	0	ND	ND	20	0	No
Fruit and fruit products											
Apple	7	1	ND	0.2	1	0	ND	ND	14.3	0	No
Apricot	171	5	ND	2	5	0	ND	ND	2.9	0	No
Banana	1	0	ND	ND	1	0	ND	ND	0	0	No
Berries and other small fruits	24	2	ND	1	4	0	ND	ND	8.3	0	No
Blueberries	2	0	ND	ND	–	–	–	–	0	–	NA
Carob	1	0	ND	ND	1	0	ND	ND	0	0	No
Citrus fruits	3	0	ND	ND	–	–	–	–	0	–	NA
Currants, red, black, white	3	0	ND	ND	–	–	–	–	0	–	NA
Date	323	6	ND	69	–	–	–	–	1.9	–	NA
Dewberries, including boysenberry and loganberry	1	0	ND	ND	–	–	–	–	0	–	NA
Dried fruits	5 299	880	ND	258	38	0	ND	ND	16.6	0	No
Elderberries	1	0	ND	ND	–	–	–	–	0	–	NA
Fig	3 664	904	ND	1 139	16	8	ND	177	24.7	50	Yes
Fruit and fruit products, nes	188	52	ND	15	7	0	ND	ND	27.7	0	No
Gooseberries	1	1	0.2	0.2	–	–	–	–	100	–	NA
Grapes	5	0	ND	ND	4	0	ND	ND	0	0	No
Guava	2	0	ND	ND	–	–	–	–	0	–	NA
Kumquats	1	0	ND	ND	–	–	–	–	0	–	NA
Mango	8	0	ND	ND	1	0	ND	ND	0	0	No

Table 1 (continued)

	AFB_1				FB_1				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB_1	FB_1	Co-occurrence?
Mulberries	2	0	ND	ND	1	0	ND	ND	0	0	No
Nectarine	4	0	ND	ND	–	–	–	–	0	–	NA
Olive oil, refined	8	0	ND	ND	–	–	–	–	0	–	NA
Papaya	4	0	ND	ND	–	–	–	–	0	–	NA
Pear	1	0	ND	ND	–	–	–	–	0	–	NA
Persimmon, Japanese	1	0	ND	ND	–	–	–	–	0	–	NA
Pineapple	13	0	ND	ND	–	–	–	–	0	–	No
Plum (including dried)	60	2	ND	3	13	0	ND	ND	3.3	0	No
Rambutan	1	0	ND	ND	–	–	–	–	0	–	NA
Raspberries, red, black	1	0	ND	ND	–	–	–	–	0	–	NA
Rose hips	2	0	ND	ND	–	–	–	–	0	–	NA
Stone fruits	6	0	ND	ND	–	–	–	–	0	–	NA
Strawberry	3	0	ND	ND	–	–	–	–	0	–	NA
Fruit and vegetable juices											
Concentrated fruit juice	2	1	ND	0.2	6	2	ND	2	50	33.3	Yes
Fruit juice	12	1	ND	0.2	6	1	ND	10	8.3	16.7	Yes
Fruit or vegetable juice, nes	4	0	ND	ND	4	0	ND	ND	0	0	No
Herbs, spices and condiments											
Anise seed	18	0	ND	ND	–	–	–	–	0	–	NA
Basil	12	1	ND	1	–	–	–	–	8.3	–	NA
Bay leaves	10	0	ND	ND	–	–	–	–	0	–	NA
Camomile or chamomile	–	–	–	–	2	0	ND	ND	–	0	NA
Caraway seed	37	1	ND	2	–	–	–	–	2.7	–	NA
Cardamom seed	23	4	ND	8	–	–	–	–	17.4	–	NA

Food group / Food	AFB_1 Total no. of samples[a]	AFB_1 No. of samples detected[b]	AFB_1 Minimum (µg/kg)	AFB_1 Maximum (µg/kg)	FB_1 Total no. of samples[a]	FB_1 No. of samples detected[b]	FB_1 Minimum (µg/kg)	FB_1 Maximum (µg/kg)	% samples detected[c] AFB_1	% samples detected[c] FB_1	Co-occurrence?
Celery seed	1	0	ND	ND	–	–	–	–	0	–	NA
Chives	4	0	ND	ND	–	–	–	–	0	–	NA
Cinnamon bark	60	1	ND	1	–	–	–	–	1.7	–	NA
Cloves, buds	20	0	ND	ND	–	–	–	–	0	–	NA
Coriander, seed	33	2	ND	1	–	–	–	–	6.1	–	NA
Cumin seed	44	1	ND	0.2	–	–	–	–	2.3	–	NA
Dill	3	0	ND	ND	–	–	–	–	0	–	NA
Dill seed	6	1	ND	1	–	–	–	–	16.7	–	NA
Fennel, seed	23	0	ND	ND	–	–	–	–	0	–	NA
Fenugreek, seed	17	2	ND	1	–	–	–	–	11.8	–	NA
Ginger, root	333	147	ND	15	2	0	ND	ND	44.1	0	No
Herb, spice or condiment, nes											
Herbs	51	6	ND	3	2	0	ND	ND	11.8	0	No
Hops, dry	1	1	0.23	0.2	1	0	ND	ND	100	0	No
Juniper, berry	1	1	0.6	1	–	–	–	–	100	–	NA
Mace	27	2	ND	1	–	–	–	–	7.4	–	NA
Nutmeg	676	480	ND	107	–	–	–	–	71	–	NA
Parsley	5	0	ND	ND	4	0	ND	ND	0	0	NA
Pepper (black, white)	970	245	ND	21	2	2	356	397	25.3	100	Yes
Rosemary	2	0	ND	ND	–	–	–	–	0	–	NA
Sauce and condiments	204	115	ND	3	9	1	ND	113	56.4	11.1	Yes
Spices	3 762	1 970	ND	202	4	0	ND	ND	52.4	0	No
Thyme	13	0	ND	ND	2	0	ND	ND	0	0	No
Turmeric, root	15	2	ND	10	–	–	–	–	13.3	–	NA
Legumes and pulses											
Beans (dry)	2	0	ND	ND	–	–	–	–	0	–	NA

Table 1 (continued)

	AFB_1				FB_1				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB_1	FB_1	Co-occurrence?
Beans except broad bean and soybean (green pods and immature seeds)	146	8	ND	1	12	0	ND	ND	5.5	0	No
Beans, shelled (immature seeds)	10	0	ND	ND	1	0	ND	ND	0	0	No
Broad bean (dry)	4	0	ND	ND	–	–	–	–	0	–	NA
Broad bean, shelled (immature seeds)	4	0	ND	ND	–	–	–	–	0	–	NA
Chickpea (dry)	72	4	ND	1	6	0	ND	ND	5.6	0	No
Legume vegetables	25	8	ND	8	3	0	ND	ND	32	0	No
Legumes and pulses, nes	172	20	ND	27	27	0	ND	ND	11.6	0	No
Lentil (dry)	158	8	ND	0.2	15	0	ND	ND	5.1	0	No
Lentils	6	5	ND	0.2	4	0	ND	ND	83.3	0	No
Peas (dry)	9	1	ND	0.2	7	0	ND	ND	11.1	0	No
Peas, shelled (immature seeds)	10	1	ND	0.2	–	–	–	–	10	–	NA
Pulses	21	0	ND	ND	–	–	–	–	0	–	NA
Soybean (dry)	94	4	ND	0.2	92	6	ND	53	4.3	6.5	Yes
Soybean (immature seeds)	–	–	–	–	191	1	ND	27	–	0.5	NA
Soybean oil, refined	3	0	ND	ND	3	0	ND	ND	0	0	No
Meat and meat products (including edible offal)											
Cattle meat	31	0	ND	ND	–	–	–	–	0	–	NA
Cattle, liver	151	0	ND	ND	–	–	–	–	0	–	NA
Chicken meat	41	5	ND	2	–	0	ND	ND	12.2	0	No
Chicken, edible offal of	291	0	ND	ND	–	–	–	–	0	–	NA
Duck meat	2	0	ND	ND	–	–	–	–	0	–	NA
Duck, edible offal of	26	0	ND	ND	–	–	–	–	0	–	NA
Edible offals (mammalian)	13	0	ND	ND	–	–	–	–	0	–	NA

Food group / Food	AFB_1 Total no. of samples[a]	AFB_1 No. of samples detected[b]	AFB_1 Minimum (µg/kg)	AFB_1 Maximum (µg/kg)	FB_1 Total no. of samples[a]	FB_1 No. of samples detected[b]	FB_1 Minimum (µg/kg)	FB_1 Maximum (µg/kg)	% samples detected[c] AFB_1	% samples detected[c] FB_1	Co-occurrence?
Goose, edible offal of	2	0	ND	ND	–	–	–	–	0	–	NA
Meat and meat products, nes	240	2	ND	0.3	13	1	ND	120	0.8	7.7	Yes
Pig liver	256	1	ND	0.1	4	0	ND	ND	0.4	0	No
Pig meat	81	1	ND	0.1	12	0	ND	ND	1.2	0	No
Poultry meat	4	0	ND	ND	4	0	ND	ND	0	0	No
Sheep liver	78	0	ND	ND	–	–	–	–	0	–	NA
Sheep meat	8	1	ND	2	–	–	–	–	12.5	–	NA
Turkey meat	18	0	ND	ND	–	–	–	–	0	–	NA
Turkey, edible offal of	110	0	ND	ND	–	–	–	–	0	–	NA
Milk and dairy products											
Butter and other animal fat emulsions	2	0	ND	ND	–	–	–	–	0	–	NA
Cattle milk	1	0	ND	ND	8	0	ND	ND	0	0	No
Cheese and analogues	4	0	ND	ND	–	–	–	–	0	–	NA
Fermented milk products	69	33	ND	16	1	0	ND	ND	47.8	0	No
Milk and dairy products, nes	3	0	ND	ND	5	0	ND	ND	0	0	No
Milks	38	2	ND	0.3	9	0	ND	ND	5.3	0	No
Nonalcoholic beverages (excluding milk, fruit and vegetable juice, water and stimulants)											
Nonalcoholic beverage, nes	10	1	ND	0.2	79	1	ND	1 025	10	1.3	Yes
Soft drinks	3	3	0.2	0.2	12	0	ND	ND	100	0	No
Nuts and oilseeds											
Almonds	3 889	767	ND	322	2	0	ND	ND	19.7	0	No
Brazil nut	375	90	ND	898	1	0	ND	ND	24	0	No
Cashew nut	1 060	112	ND	2	3	0	ND	ND	10.6	0	No
Chestnuts	119	6	ND	2	3	0	ND	ND	5	0	No
Coconut	211	5	ND	1	7	0	ND	ND	2.4	0	No
Hazelnut	6 952	2 140	ND	638	–	–	–	–	30.8	–	NA

Table 1 (continued)

	AFB$_1$				FB$_1$				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB$_1$	FB$_1$	Co-occurrence?
Linseed	166	14	ND	1	1	0	ND	ND	8.4	0	No
Macadamia nut	174	14	ND	1	1	0	ND	ND	8	0	No
Nuts	600	154	ND	132	4	0	ND	ND	25.7	0	No
Nuts and oilseeds, nes	3	1	ND	0.2	–	–	–	–	33.3	–	NA
Oilseeds	1 700	211	ND	9 065	30	0	ND	ND	12.4	0	No
Peanut, shelled	7 464	2 882	ND	1 460	19	0	ND	ND	38.6	0	No
Pecan	167	20	ND	10	–	–	–	–	12	–	NA
Pine nut	66	8	ND	1	–	–	–	–	12.1	–	NA
Pistachio nut	12 105	2 276	ND	711	3	0	ND	ND	18.8	0	No
Poppy seed	217	8	ND	1	2	0	ND	ND	3.7	0	No
Rape seed	30	2	ND	0.2	2	0	ND	ND	6.7	0	No
Sesame seed	266	70	ND	10	36	0	ND	ND	26.3	0	No
Sunflower seed	471	42	ND	3	98	1	ND	41	8.9	1	Yes
Walnut	1 226	115	ND	12	5	0	ND	ND	9.4	0	No
Other foods											
Other foods (foods which cannot be included in any other group)	67	24	ND	147	52	4	ND	48	35.8	7.7	Yes
Products for special nutritional use											
Dietary supplements	47	12	ND	1	8	0	ND	ND	25.5	0	No
Dietetic food for diabetics (labelled as such)	2	0	ND	ND	–	–	–	–	0	–	NA
Food for sports people (labelled as such)	20	20	0.2	3	–	–	–	–	100	–	NA
Food for weight reduction	6	6	0.2	0.2	1	0	ND	ND	100	0	No
Medical food	20	2	ND	0.2	228	103	ND	2 809	10	45.2	Yes

Food group / Food	AFB_1 Total no. of samples[a]	AFB_1 No. of samples detected[b]	AFB_1 Minimum (µg/kg)	AFB_1 Maximum (µg/kg)	FB_1 Total no. of samples[a]	FB_1 No. of samples detected[b]	FB_1 Minimum (µg/kg)	FB_1 Maximum (µg/kg)	% samples detected[c] AFB_1	% samples detected[c] FB_1	Co-occurrence?
Products for special nutritional use, nes	12	10	ND	2	8	3	ND	441	83.3	37.5	Yes
Snacks and desserts											
Ices and desserts	432	122	ND	31	2	0	ND	ND	28.2	0	No
Snack food	587	162	ND	2	1 039	474	ND	4 824	27.6	45.6	Yes
Snack or dessert, nes	18	12	ND	2	2	2	102	157	66.7	100	Yes
Starchy roots and tubers											
Arrowroot	5	0	ND	ND	36	0	ND	ND	0	0	No
Carrot	2	0	ND	ND	2	0	ND	ND	0	0	No
Cassava	2	0	ND	ND	–	–	–	–	0	–	NA
Parsley, turnip-rooted	2	1	ND	1	–	–	–	–	50	–	NA
Potato	46	0	ND	ND	15	0	ND	ND	0	0	No
Starchy roots and tubers, nes	22	0	ND	ND	9	0	ND	ND	0	0	No
Sweet potato	3	2	ND	0.2	–	–	–	–	66.7	–	NA
Stimulant beverages, dried and diluted excluding cocoa products											
Coffee (beverage)	13	0	ND	ND	4	0	ND	ND	0	0	No
Coffee beans	60	0	ND	ND	–	–	–	–	0	–	NA
Coffee beans, roasted	57	1	ND	0.3	–	–	–	–	1.8	–	NA
Tea (infusion)	9	3	ND	1	32	0	ND	ND	33.3	0	No
Tea, green, black (black, fermented and dried)	26	15	ND	1	47	0	ND	ND	57.7	0	No
Teas and herbal teas (solid)	8	1	ND	0.2	16	1	ND	67	12.5	6.3	Yes
Sugar and confectionery (including cocoa products)											
Cocoa beans	159	7	ND	1	–	–	–	–	4.4	–	NA
Cocoa butter	1	0	ND	ND	–	–	–	–	0	–	NA
Cocoa mass	19	10	ND	1	–	–	–	–	52.6	–	NA

Table 1 (continued)

	AFB$_1$				FB$_1$				% samples detected[c]		
Food group / Food	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB$_1$	FB$_1$	Co-occurrence?
Cocoa powder	401	53	ND	1	1	0	ND	ND	13.2	0	No
Confectionery	667	223	ND	89	17	3	ND	52	33.4	17.6	Yes
Honey	25	2	ND	35	10	0	ND	ND	8	0	No
Sugar	98	83	ND	2	–	–	–	–	84.7	–	NA
Sugar and confectionery, nes	168	43	ND	2	11	0	ND	ND	25.6	0	No
Vegetables and vegetable products (including fungi)											
Asparagus	–	–	–	–	7	0	ND	ND	–	0	NA
Cabbage, head	1	0	ND	ND	–	–	–	–	0	–	NA
Corn salad	1	0	ND	ND	–	–	–	–	0	–	NA
Fruiting vegetables	29	0	ND	ND	24	0	ND	ND	0	0	No
Fungi, edible (not including mushrooms)	57	0	ND	ND	13	0	ND	ND	0	0	No
Garlic	48	1	ND	1	1	0	ND	ND	2.1	0	No
Indian mustard	16	4	ND	2	–	–	–	–	25	–	NA
Leafy vegetables	145	145	0.2	14	–	–	–	–	100	–	NA
Melons, except watermelon	2	0	ND	ND	–	–	–	–	0	–	NA
Mushrooms	8	0	ND	ND	–	–	–	–	0	–	NA
Onion, bulb	2	1	ND	0.2	–	–	–	–	50	–	NA
Peppers, chilli	135	63	ND	26	–	–	–	–	46.7	–	NA
Peppers, sweet (including pim(i)ento)	65	35	ND	7	–	–	–	–	53.8	–	NA
Pumpkins	7	0	ND	ND	–	–	–	–	0	–	NA
Sweet corn (kernels)	119	4	ND	1	27	0	ND	ND	3.4	0	No
Tomato	2	1	ND	0.2	14	0	ND	ND	50	0	No
Vegetables and vegetable products, nes	57	13	ND	166	5	0	ND	ND	22.8	0	No

Food group / Food	AFB_1				FB_1				% samples detected[c]		Co-occurrence?
	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	Total no. of samples[a]	No. of samples detected[b]	Minimum (µg/kg)	Maximum (µg/kg)	AFB_1	FB_1	
Watermelon	1	0	ND	ND	–	–	–	–	0	–	NA
All foods	84 647	18 170	ND	9 065	21 924	6 148	ND	35 400	22	28	–

AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; NA: cannot be determined as there are no occurrence data for one of the mycotoxins assessed; ND: not detected; nes: not elsewhere specified; no.: number

[a] Number of samples of a food that had a record for AFB_1 or for FB_1 in the GEMS/Food contaminants database.

[b] Number of samples of a food in which AFB_1 or FB_1 was detected.

[c] Percentages of positive samples.

While these results provide a general guide as to the types of foods where co-occurrence may occur, a more detailed analysis of individual food samples is required to show the degree of co-occurrence.

(b) Results by unique sample identification number for AFB_1 and FB_1

The majority of the data in the GEMS/Food contaminants database for aflatoxins and fumonisins have unique sample identification numbers. Those without sample identification numbers were excluded from this analysis. Therefore, the sample identification number was used to summarize the data from the GEMS/Food contaminants database based on the food names and food groupings as included by the data submitters. No further classifications or edits to food groupings or names were applied.

Data points for which a unique sample identification number was provided were evaluated to determine the samples that were analysed for both AFB_1 and FB_1. Where this was the case, an analysis was undertaken to determine if they co-occurred in the same sample, that is, where both AFB_1 and FB_1 had detectable concentrations above the LOD.

Overall, 5018 samples across all countries and foods were analysed for both AFB_1 and FB_1. Of these, 84 samples (1.7%) had co-occurrence of AFB_1 and FB_1. This low proportion could be a result of the countries that have submitted data and the foods for which data were available. For example, there were a small number of countries in the database for which foods with occurrence of aflatoxins and fumonisins are commonly associated (e.g. from Africa, the central areas in the Americas and Asia). These countries commonly have maize, for which contamination is common, as a dietary staple; however, the data from the four African countries were all for sorghum.

By country

There were 17 countries and one WHO region that had unique sample identification numbers for individual analytical samples and therefore could be evaluated for co-occurrence. These countries/regions are Australia, Austria, Belgium, Burkina Faso, Canada, Cyprus, Ethiopia, France, Germany, Hungary, Ireland, Mali, Poland, Romania, Slovenia, Spain, Sudan and the WHO European Region.

There were nine countries from which no co-occurrence was identified, as the samples had no detected (<LOD) concentrations of AFB_1 and/or FB_1. Appendix 1 includes a list of these countries, the number of samples tested and the foods that were analysed.

Eight countries and one region had both AFB_1 and FB_1 in at least one sample. Table 2 lists these countries, the foods with the co-occurrence and

Table 2

Countries/regions with AFB_1 and FB_1 co-occurrence in at least one sample from the GEMS/Food contaminants database based on individual samples

Country	Total no. of samples for all foods[a]	No. of detects[b]	% total samples with both AFB_1 and FB_1	Foods with AFB_1 and FB_1 co-occurrence: Food	No. of detects[b] / total no. samples	Range of positive concentrations (µg/kg)	Foods analysed with no co-occurrence (no. of samples)
Burkina Faso	367	10	2.7	Sorghum	10/367	AFB_1: 8.3–159 FB_1: 22–2 451	None
Canada	2 397	41	1.7	Bread and other cooked cereal products	23/742	AFB_1: 0.8–6.8 FB_1: 23–157	Arrowroot (5), barley (23), bran unprocessed (wheat, oat, corn) (69), buckwheat (28), cereals and cereal-based products, nes (flours, starches, chips, grains, cake mixes) (71), coconut (1), millet (13), oats (grain, oatmeal, flour) (190), pea flour (2), popcorn (10), rice (160), rye (grains, flour) (18), soy flour (1), wheat (grain, pasta, flour) (459)
				Cereal-based food for infants and young children	1/450	AFB_1: 1.1 FB_1: 35	
				Maize	17/155	AFB_1: 1.0-12.5 FB_1: 12–741	
Ethiopia	380	3	0.8	Sorghum	3/380	AFB_1: 8.6–101 FB_1: 62–259	None
Germany	109	7	6.4	Cereals and cereal-based products, nes	7/49	AFB_1: 0.2–1.9 FB_1: 139–1 104	Barley (1), bread and other cooked cereal products (9), cereal grains (4), confectionery (1), dietary supplements (6), dried fruits (1), figs (5), maize (3), medical foods (16), oilseeds (6), rape seed (1), wheat (7)
Ireland	116	4	3.4	Maize	4/29	AFB_1: 0.3–1.2 FB_1: 94–2 164	Maize, oats, rice, snack foods, barley (15), bread and cooked cereal products, nes (1), cereal grains (5), cereals and cereal-based products, nes (41), meals for infants (1), legumes and pulses (2), oats (7), rice (6), snack foods (4), wheat (11)
Mali	336	8	2.4	Sorghum	8/336	AFB_1: 7.7–272 FB_1: 20–424	None
Poland	258	3	1.2	Cereals and cereal-based products, nes	2/92	AFB_1: 0.9–1.3 FB_1: 558–1 140	Bread and other cooked cereal products (1), maize (78), ready-to-eat meal for infants (2), snack foods (14)

Table 2 (continued)

Country	Total no. of samples for all foods[a]	No. of detects[b]	% total samples with both AFB_1 and FB_1	Foods with AFB_1 and FB_1 co-occurrence: Food	No. of detects[b] / total no. samples	Range of positive concentrations (µg/kg)	Foods analysed with no co-occurrence (no. of samples)
				Cereal-based food for infants and young children	1/71	AFB_1: 0.02 FB_1: 57	
Slovenia	109	5	4.6	Cereals and cereal-based products, nes	5/55	AFB_1: 0.3–3.5 FB_1: 177–565	Cereal grains (7), cereal-based foods for infants and young children (4), maize (35), snack food (1), sweet corn on the cob (7)
WHO European Region	166	3	1.8	Cereals and cereal-based products, nes	2/30	AFB_1: 0.07–0.2 FB_1: 63–260	Barley (16), beans except broad and soy (1), berries (1), bread and other cooked cereal products (11), cereal grains (10), cereal-based food for infants and young children (6)
				Maize	1/1	AFB_1: 1.7 FB_1: 393	

AFB_1: aflatoxin B_1; FB_1: fumonisin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; nes: not elsewhere specified; no.: number; WHO: World Health Organization

[a] Number of samples of a food that had a record for AFB_1 or FB_1 in the GEMS/Food contaminants database.

[b] Number of samples with both AFB_1 and FB_1.

the degree of co-occurrence, and the foods for which no co-occurrence was demonstrated.

The degree of co-occurrence for individual countries varied due to variation in the total number of samples analysed and the different number and types of foods included. Because of this variation, which countries/regions have a high degree of co-occurrence compared with others cannot be determined. However, this dataset does indicate that there are a number of regions that do have co-occurrence, including Africa, Europe and North America.

Across all countries/regions with at least one sample with co-occurrence, the range of co-occurrence was between 0.8% and 6.4%.

Three countries and one region showed co-occurrence in cereals and cereal-based products not elsewhere specified (Germany, Poland, Slovenia and the WHO European Region). Two countries and one region showed co-occurrence in maize (Canada, Ireland and the WHO European Region). Three countries from Africa demonstrated co-occurrence in sorghum (Burkina Faso, Ethiopia and Mali). These countries were three out of the four listed in the GEMS/Food contaminants database with data from the FAO/WHO project on mycotoxins in sorghum. This project, implemented from 2012 to 2014 at the request of CCCF, provided mycotoxin occurrence data and information on farming and production practices. Sudan was the fourth country with data on sorghum in the database from this project, but this country did not demonstrate any co-occurrence (Appendix 1).

By food group

Another analysis with the data from the GEMS/Food contaminants database used unique sample identification numbers that evaluated co-occurrence of AFB_1 and FB_1 within the same foods. This analysis therefore did not consider the country from which the data originated. This evaluation was done in order to determine if some foods showed a higher degree of co-occurrence than others. These results are shown in Table 3.

The foods with demonstrated co-occurrence of AFB_1 and FB_1 were maize (5.5%), cereals and cereal-based products (4.2%), bread and other cooked cereal products (2.8%), sorghum (1.4%) and cereal-based foods for infants and young children (0.4%).

3.2.2 Evaluation of co-occurrence based on the literature

There is a large body of literature in the area of co-occurrence of aflatoxins and fumonisins. Papers were identified following a scoping review conducted by the JECFA Secretariat as supporting work for the Committee. Key papers were also identified by experts on the Committee. Additional papers were selected based

Table 3

Occurrence of AFB_1 and FB_1 in foods based on the concentration data in the GEMS/Food contaminants database using unique sample identification numbers

Food / Food group	Total no. of samples[a]	No. of samples with both AFB_1 and FB_1	% total samples with both AFB_1 and FB_1	Concentration (µg/kg) in samples with co-occurrence			
				Min. AFB_1	Max. AFB_1	Min. FB_1	Max. FB_1
Arrowroot	5	0	0	–	–	–	–
Barley	59	0	0	–	–	–	–
Beans except broad bean and soybean (green pods and immature seeds)	1	0	0	–	–	–	–
Beans-based meals	4	0	0	–	–	–	–
Berries and other small fruit	1	0	0	–	–	–	–
Bran, unprocessed, of cereal grain (except buckwheat, canihua, quinoa)	69	0	0	–	–	–	–
Bread and other cooked cereal products	832	23	2.8	0.8	6.8	14	517
Buckwheat	28	0	0	–	–	–	–
Cereal grains	87	0	0	–	–	–	–
Cereal-based food for infants and young children	539	2	0.4	0.02	1.1	35	57
Cereals and cereal-based products, nes	381	16	4.2	0.07	3.5	63	1 140
Chickpea (dry)	1	0	0	–	–	–	–
Coconut	2	0	0	–	–	–	–
Composite food, nes	4	0	0	–	–	–	–
Confectionery	1	0	0	–	–	–	–
Dietary supplements	6	0	0	–	–	–	–
Dried fruits	9	0	0	–	–	–	–
Fig	5	0	0	–	–	–	–
Food for infants or children, nes	34	0	0	–	–	–	–
Honey	10	0	0	–	–	–	–
Infant formula, powder	1	0	0	–	–	–	–
Legume vegetables	1	0	0	–	–	–	–
Legumes and pulses, nes	1	0	0	–	–	–	–
Lentil (dry)	3	0	0	–	–	–	–

Food / Food group	Total no. of samples[a]	No. of samples with both AFB_1 and FB_1	% total samples with both AFB_1 and FB_1	Concentration (µg/kg) in samples with co-occurrence			
				Min. AFB_1	Max. AFB_1	Min. FB_1	Max. FB_1
Maize	401	22	5.5	0.3	12.5	12	2 165
Medical food	16	0	0	–	–	–	–
Millet	13	0	0	–	–	–	–
Oats	197	0	0	–	–	–	–
Oilseeds	7	0	0	–	–	–	–
Peas (dry)	2	0	0	–	–	–	–
Pistachio nut	1	0	0	–	–	–	–
Popcorn	10	0	0	–	–	–	–
Rape seed	1	0	0	–	–	–	–
Ready-to-eat meals for infants and young children	15	0	0	–	–	–	–
Rice	181	0	0	–	–	–	–
Rye	19	0	0	–	–	–	–
Snack food	23	0	0	–	–	–	–
Sorghum	1 533	21	1.4	7.7	272	20	2 451
Soybean (dry)	2	0	0	–	–	–	–
Spices	1	0	0	–	–	–	–
Sugar and confectionery, nes	1	0	0	–	–	–	–
Sunflower seed	2	0	0	–	–	–	–
Sweet corn (corn on the cob)	8	0	0	–	–	–	–
Tap water	1	0	0	–	–	–	–
Walnut	3	0	0	–	–	–	–
Wheat	484	0	0	–	–	–	–
White bread	11	0	0	–	–	–	–
Wholemeal bread	2	0	0	–	–	–	–
All foods	5 018	84	1.7	0.02	272	12	2 451

AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; Max.: maximum; Min.: minimum; nes: not elsewhere specified

[a] Number of samples of a food that had a record for AFB_1 or FB_1 in the GEMS/Food contaminants database.

on the reference lists of reviewed papers. An internet search was conducted to find other scientific materials, such as agency reports or total diet study reports. Papers may have used the term "coexistence" as an alternative to "co-occurrence".

The aim of the literature search was to find papers that included analysis of and results for AFB_1 and FB_1. Papers that included analysis of aflatoxins only or fumonisins only were excluded unless separate papers were found that reported the results for the two contaminants from the same set of samples. Papers that included analysis of any aflatoxins or fumonisins, in total or subtypes, were reviewed to determine if they included analysis of both AFB_1 and FB_1. Papers that presented no numerical results were excluded.

For the studies reported in the literature, it was not always possible to evaluate if the co-occurrence was in the same individual food sample. Detection in the same sample (as was assessed using data from the GEMS/Food contaminants database using unique sample identification numbers) differs from detection in the same type of food from the same study. In many cases the percentage of detections from all the samples of the food tested for aflatoxins is given and the percentage of samples with detected fumonisins is given separately, and often no data are provided for the percentage of individual samples that contained both mycotoxins. However, where both mycotoxins have over 50% of occurrence individually, it can be concluded that at least some of the individual samples would have co-occurrence.

(a) Co-occurrence of AFB_1 and FB_1

Some studies have analysed for both AFB_1 and FB_1 in foods but have found no co-occurrence. These studies are summarized in Appendix 1. Some studies analysed more than one food, with some but not all of these foods showing co-occurrence. The foods from those studies that showed no co-occurrence are noted in Appendix 1, whereas those that had co-occurrence are summarized in this section. Many of the studies that had no co-occurrence were from total diet studies. These included those from China, Hong Kong Special Administrative Region (SAR) (FEHD, 2013), France (Sirot, Fremy & Leblanc, 2013) and the Netherlands (Lopez et al., 2016). These are also summarized in Appendix 1.

Many studies have demonstrated the co-occurrence of AFB_1 and FB_1. In a review of published studies from 19 countries, co-occurrence of AFB_1 and FB_1 was demonstrated in all but two studies (in which no aflatoxins were detected). The review included studies of between 10 and 1655 maize samples, with co-occurrence between 6% and 100%.

Table 4 summarizes the findings of the published papers assessing occurrence of both AFB_1 and FB_1. There were studies from a number of different countries from Africa, Europe, Asia, North America and South America. The

Table 4
Summary of occurrence of AFB_1 and FB_1 in foods based on literature search[a]

WHO Region / Country	Food group	Food	Mycotoxin	No. of samples	% occurrence[b]	Mean? (µg/kg)[c]	Mean +ve (µg/kg)[d]	Mean total (µg/kg)[e]	Range (µg/kg)	Median (µg/kg)	Reference
African											
Burkina Faso	Cereals and cereal products	Maize	AFB_1	26	50	–	–	–	3.4–636	23.6	Warth et al. (2012)
			FB_1	26	81	–	–	–	22.5–1 343	269	
Cameroon	Cereals and cereal products	Maize	AFB_1	165	22	–	35	–	ND–345	–	Ediage, Hell & De Saeger (2014)
			FB_1	165	74% ($FB_1 + FB_2 + FB_3$)	–	1 329	–	ND–5 412	–	
Democratic Republic of Congo	Cereals and cereal products	Maize (mixed)	AFB_1	4	100	1.4	–	–	1–120	–	Manjula et al. (2009)
			FB_1	4	100	0.3	–	–	0.05–9.6	–	
		Maize (white)	AFB_1	4	100	0.55	–	–	0.4–10.8	–	
			FB_1	4	100	0.14	–	–	0.02–1.7	–	
Ethiopia	Cereals and cereal products	Sorghum	AFB_1	70	12.9	29.5	–	–	ND–62.5	30.4	Chala et al. (2014)
			FB_1	70	14.3	14.7	–	–	ND–30.1	12.9	
		Millet (finger millet)	AFB_1	34	6.1	1.1	–	–	ND–1.4	1.1	
			FB_1	34	45.5	15.5	–	–	ND–49.2	5.3	
Côte d'Ivoire	Cereals and cereal products	Maize	AFB_1	51	100	–	–	128.7	4.5–330	75.4	Kouadio et al. (2014)
			FB_1	51	100	–	–	226	22.6–1 464	175.9	
Malawi	Cereals and cereal products	Maize	AFB_1	90	38	–	91	–	ND–592	–	Matumba et al. (2014)
			FB_1	90	100	–	1 3	–	4 6 (max)	–	
Mozambique	Cereals and cereal products	Maize	AFB_1	13	46	–	–	–	16.3–363	69.9	Warth et al. (2012)
			FB_1	13	92	–	–	–	159–7 615	869	
Nigeria	Cereals and cereal products	Maize	AFB_1	47	67	394	–	–	0.4–6 738	74	Adetunji et al. (2014)
			FB_1	65	93	1 552	–	–	1.8–10 447	1 064	
		Rice	AFB_1	21	100	–	–	37.2 ± 14.0	4.1–309	–	Makun et al. (2011)
			FB_1	21	14.3	–	–	0.2 ± 0.2	0.4–4.4	–	

Table 4 (continued)

WHO Region / Country	Food group	Food	Mycotoxin	No. of samples	% occurrence[b]	Mean? (µg/kg)[c]	Mean +ve (µg/kg)[d]	Mean total (µg/kg)[e]	Range (µg/kg)	Median (µg/kg)	Reference
		Maize-based snack	AFB_1	8	63	–	–	–	ND–69.5	45	Kayode et al. (2013)
			FB_1	8	100	–	–	–	44–339	124	
	Mixed food	Maize and groundnut snack	AFB_1	2	100	–	–	–	9.3–12	11	Kayode et al. (2013)
			FB_1	2	100	–	–	–	124–130	127	
	Meat and meat products	Banda (hard-smoked meat)	AFB_1	30	10	–	244	–	ND–262	–	Oladejo & Adebayo-Tayo (2011)
			FB_1	30	90	–	1.2	–	ND–2.3	–	
South Africa	Cereals and cereal products	Maize	AFB_1	114	13	–	23	–	ND–133	–	Mngqawa et al. (2016)
			FB_1	114	68	–	658	–	ND–18 924	–	
		Maize	AFB_1	40	70	–	–	–	ND–741	–	Chilaka et al. (2012)
			FB_1	40	100	–	–	–	8–892	–	
United Republic of Tanzania	Cereals and cereal products	Maize	AFB_1	60	50	–	65	–	2–1 081	–	Kamala et al. (2015)
			FB_1	60	73	–	1 361	–	16–18 184	–	
		Maize porridge	AFB_1	101	48	–	5	–	ND–35	–	Geary et al. (2016)
			FB_1	100	100	–	298	–	13–1 850	–	
		Maize	AFB_1	120	12	–	–	–	5–90	38	Kimanya et al. (2008)
			FB_1	120	52	–	–	–	ND–6 125	206	
		Maize (flour-based food for infants)	AFB_1	67	51	–	–	–	0.9–56	3.5	Magoha et al. (2016)
			FB_1	67	30	–	–	–	52.7–974	140	
		Maize (flour-based food for infants)	AFB_1	41	24	–	–	–	0.5–364	1.3	Kimanya et al. (2014)
			FB_1	41	80	–	–	–	57–1 672	329	
Zimbabwe	Cereals and cereal products	Maize	AFB_1	95	1	–	11	–	ND–11	–	Hove et al. (2016a)
			FB_1	95	95	–	242	–	ND–1 106	–	
		Maize	AFB_1	388	21	–	–	–	ND–26.6	3.2	Murashiki et al. (2017)
			FB_1	388	100	–	–	–	10–607	292 and 360 (2 districts)	

WHO Region / Country	Food group	Food	Mycotoxin	No. of samples	% occurrence[b]	Mean? (µg/kg)[c]	Mean +ve (µg/kg)[d]	Mean total (µg/kg)[e]	Range (µg/kg)	Median (µg/kg)	Reference
Americas											
Argentina	Cereals and cereal products	Maize, freshly harvested	AFB_1	1 655	5.9	2.8	–	–	ND–339	–	Garrido et al. (2012)
			FB_1	1 655	96.7	3 572	–	–	ND–498 200	–	
		Maize, stored	AFB_1	1 591	10.4	2.4	–	–	ND–427	–	
			FB_1	1 591	98.6	3 676	–	–	ND–31 701	–	
Brazil	Cereals and cereal products	Corn	AFB_1	200	11	20.2	–	–	ND–1 393	–	Rocha et al. (2009)
			FB_1	200	98	1 752	–	–	ND–9 670	–	
		Corn	AFB_1	300	8.8	30.7	–	–	ND–56	–	Moreno et al. (2009)
			FB_1	300	98.5	2 083	–	–	ND–18 780	–	
Canada	Cereals and cereal products	Rice	AFB_1	199	50	–	0.36	0.18	–	–	Bansal et al. (2011)
			FB_1	199	8	–	5.1	0.4	–	–	
Ecuador	Cereals and cereal products	Rice (paddy)	AFB_1	43	7	–	20.6	–	ND–47.4	–	Ortiz et al. (2013)
			FB_1	43	23	–	159	–	ND–1 146	–	
Guatemala	Cereals and cereal products	Maize, shelled	AFB_1	572	36	–	–	63	ND–2 655	–	Torres et al. (2015)
			FB_1	640	98.9	–	–	1 800	ND–17 100	–	
		Incaparina (maize- and cottonseed flour–based infant food)	AFB_1	8	100	–	–	–	3–214	–	Trucksess et al. (2002)
			FB_1	8	100	–	–	–	200–1 700	–	
South-East Asian											
Sri Lanka	Herbs and spices	Black pepper	AFB_1	82	NS	–	9.1	–	ND–17.3	–	Yogendrarajah et al. (2014a)
			FB_1	11	5	–	123	–	ND–135	–	
European											
Spain	Stimulant beverages	Coffee, various types	AFB_1	169	22	–	0.9	0.2	ND–1.1	–	García-Moraleja et al. (2015)
			FB_1	169	4	–	15.5	0.6	ND–18.3	–	

Table 4 (continued)

WHO Region / Country	Food group	Food	Mycotoxin	No. of samples	% occurrence[b]	Mean? (µg/kg)[c]	Mean +ve (µg/kg)[d]	Mean total (µg/kg)[e]	Range (µg/kg)	Median (µg/kg)	Reference
Eastern Mediterranean											
Iran (Islamic Republic of)	Cereals and cereal products	Rice	AFB_1	65	21.5	–	3.9	–	ND–30.8	–	Nazari et al. (2014)
			FB_1	65	9.2	–	110.7	–	54.5–177	–	
Western Pacific											
China	Cereals and cereal products	Rice	AFB_1	29	100	–	–	–	0.1–1.4	–	Sun et al. (2011)
			FB_1	29	89.7	–	–	–	ND–500	–	
		Wheat flour	AFB_1	16	100	–	–	–	0.1–0.9	–	
			FB_1	16	81.3	–	–	–	ND–400	–	
		Corn	AFB_1	108	100	–	–	–	0.4–136.8	–	
			FB_1	108	92.6	–	–	–	ND–37 000	–	
	Cereals and cereal products	Maize	AFB_1	60	6.7	–	0.9	–	0.3–1.7	–	Wang et al. (2013)
			FB_1	60	23.3	–	5.7	–	2.2–17.4	–	
Viet Nam	Cereals and cereal products	Maize	AFB_1	97	56	–	137	–	ND–844	–	Phuong et al. (2015)
			FB_1	97	67	–	1 249	–	ND–10 799	–	

AFB_1: aflatoxin B_1; FB_1: fumonisin B_1; LOD: limit of detection; LOQ: limit of quantification; Max.: maximum; nes: not elsewhere specified; ND: not detected; NS: not specified

[a] Literature search collated from January 2011 to August 2016.

[b] Percentage of positive samples, i.e. above the LOD/LOQ.

[c] Calculation not further specified.

[d] Mean of positive samples.

[e] Mean of all samples.

majority of the studies were from the African Region and included a number of different foods, but mainly maize and maize products. Other foods included other cereals (including sorghum, millet, rice, wheat), coffee, black pepper and smoked meat. The proportion of samples with AFB_1 and FB_1 varied between 1% and 100%, depending on the mycotoxin assessed; the sensitivity of the analytical method (i.e. the level of the LOD or LOQ); the food and the number of samples analysed; and the country from which the samples were obtained. Many studies detected FB_1 in or very near to 100% of samples. These studies also found occurrence of AFB_1, indicating co-occurrence of the two contaminants within the same sample.

Studies reporting co-occurrence within the same food samples were limited. Murashiki et al. (2017) noted that 22% (n = 36/166) of maize samples from the Shamva district in Zimbabwe and 20% (n = 44/222) from the Makoni district contained AFB_1 and FB_1. A study of 60 samples of maize from China (Wang et al., 2013) found four with AFB_1 and 14 with FB_1, but only one that contained both AFB_1 and FB_1 (i.e. 1.7% co-occurrence). Another study of grains from three regions in China (Sun et al., 2011) found that 92.6% (n = 100/108) of corn samples were positive for both AFB_1 and FB_1, as were 89.7% (n = 26/29) of rice samples and 81.3% (n = 13/16) of wheat flour samples.

One other study that sampled a number of cereal-based foods (wheat, oats, barley, spelt, maize, rice and sorghum) from several Mediterranean countries (Serrano et al., 2012) described co-occurrence of AFB_1 and FB_1 in one of the pasta samples but did not provide enough detail to determine whether specific foods from specific countries were co-contaminated.

A small number of samples were found to have co-occurrence of aflatoxins and fumonisins in general in the same food samples. In one study of freshly harvested corn in Brazil, aflatoxins and fumonisins co-occurred in 7% (n = 200) of the samples tested (Rocha et al., 2009). A study of maize-based complementary foods consumed by Tanzanian children aged 18–24 months found that total aflatoxins and total fumonisins co-occurred in 29% of 41 samples examined (Kimanya et al., 2014).

In a study of yams in Nigeria, Somorin et al. (2012) noted that aflatoxins and fumonisins co-occurred in 31% of white yam flour (n = 81 samples) but not water yam flour (n = 19 samples). All of the sliced whole yams from one of the regions studied had co-occurrence (n = 9 samples).

Studies evaluating co-occurrence have also been done using samples from experimental plots. While the products from these may not go into the main food supply, their results add to the evidence base in this area. One study of maize in Brazil found 100% occurrence of FB_1 in 110 samples (mean: 6790 µg/kg) and 55% occurrence of AFB_1 (mean: 309 µg/kg) (Camargos, Machinski & Soares, 2001). Another study evaluating the occurrence of aflatoxins and fumonisins

in rotational crops of corn and soybeans in the Mississippi Delta detected both contaminants in both commodities across the 4 years of the study: for corn, mean concentrations of aflatoxins were 2.4–54.8 μg/kg and of fumonisins were 200–3600 μg/kg; for soybean, mean concentrations of aflatoxins were 0.1–6.8 μg/kg and of fumonisins were 100–200 μg/kg (Abbas et al., 2012).

(b) Co-occurrence of aflatoxins and fumonisins with other mycotoxins

AFB_1 and FB_1 have been shown to co-occur with other aflatoxins and fumonisins. Of the maize samples found to have AFB_1 and FB_1 in Zimbabwe, FB_2 was also found in 31%, FB_3 in 3%, and AFG_1 and AFB_2 in 1% (Hove et al., 2016a). Maize in Viet Nam was also found to contain AFB_2, AFG_1 and FB_2 (Phuong et al., 2015).

Aflatoxins and fumonisins have also been shown to co-occur with other mycotoxins. Maize from two provinces in South Africa (Mngqawa et al., 2016) had detectable concentrations of AFB_1 and FB_1 as well as of 18 other mycotoxins, including other aflatoxins and fumonisins, ochratoxin, deoxynivalenol, HT-2 toxin and T-2 toxin, at an occurrence of 3–83%. In Viet Nam, Phuong et al. (2015) showed that maize (n = 97) contained aflatoxins (56%), fumonisins (67%) and zearalenone (28%). Wheat from the Syrian Arab Republic (n = 40) contained aflatoxins and fumonisins with deoxynivalenol, sterigmatocystin, ochratoxin A, zearalenone and zearalenol (Alkadri et al., 2014). Rice sold in Canada was shown to contain AFB_1, FB_1 and ochratoxin A (Bansal et al., 2011). High rates of contamination with a number of mycotoxins were found in paddy rice (n = 43; 23% deoxynivalenol, 23% FB_1, 7% AFB_1, 2% AFG_1 and 2% AFG_2) in Ecuador (Ortiz et al., 2013). A study in Nigeria showed that while AFB_1 and FB_1 co-occurred in samples of yams and yam flour, no deoxynivalenol, ochratoxin or zearalenone was detected in those samples (Somorin et al., 2012). Aflatoxins and fumonisins have been shown to co-occur with deoxynivalenol, ochratoxin A and zearalenone in maize in Burkina Faso, Mozambique and the United Republic of Tanzania (Warth et al., 2012; Kamala et al., 2015). A study of maize (n = 40) and beans (n = 30) in the Democratic Republic of Congo showed co-occurrence of aflatoxins and fumonisins as well as of ochratoxin A and zearalenone in both foods (Mulunda et al., 2013). A study of maize (n = 165) in Cameroon showed co-occurrence of AFB_1 and FB_1 as well as of zearalenone, acetyldeoxynivalenol, roquefortine C and beauvericin (Ediage et al., 2014).

3.3 Summary of the co-occurrence of aflatoxins and fumonisins in foods

- There is co-occurrence of individual and total aflatoxins and fumonisins in foods, particularly in maize and other cereal grains.
- Of the 5018 samples in the GEMS/Food contaminants database that had unique sample identification numbers, across all countries and foods, 84 (1.7%) had co-occurrence of AFB_1 and FB_1. This proportion was driven by the countries that submitted data and the range of foods available. Only a limited number of African countries, for example, supplied data, and these were only for sorghum.
- The foods with co-occurrence of AFB_1 and FB_1, based on evaluating data by unique sample identification number from the GEMS/Food contaminants database, were maize (5.5%); cereals and cereal-based products (4.2%); bread and other cooked cereal products (2.8%); sorghum (1.4%); and cereal-based foods for infants and young children (0.4%). It was not possible to identify countries that had higher degrees of co-occurrence than others.
- Many papers showed co-occurrence of AFB_1 and FB_1. Most of the studies were from African countries, and the main food assessed was maize. Other foods in which AFB_1 and FB_1 co-occurred were rice, wheat and coffee.
- Where studies from the literature have noted the degree of co-occurrence of aflatoxins and fumonisins from individual samples, up to approximately 90% of samples, particularly maize, have had this co-occurrence.
- AFB_1 and FB_1 occur in the same foods from the same surveys, even though this may not be in the exact same sample, as demonstrated from GEMS/Food data (analysis not based on unique sample identification numbers) and the literature.
- The degree of co-occurrence of aflatoxins and fumonisins can be influenced by factors such as variety of the commodity, region, time of sampling, storage and food preparation.
- Co-occurrence of aflatoxins and fumonisins as well as other mycotoxins in the same foods is common.

4. Co-exposure to aflatoxins and fumonisins in the diet

4.1 Assessing co-exposure

An evaluation was undertaken by the Committee of the co-exposure to aflatoxins and fumonisins from the diet.

Co-exposure to aflatoxins and fumonisins can occur when both mycotoxins are consumed from the diet as a whole, that is, they are present in different foods that are not necessarily contaminated with both mycotoxins. The whole diet was the basis for the co-exposure evaluation as it is the amount obtained from the diet (and not from individual foods) that is of interest from a toxicological point of view. (The amount that can be obtained from individual foods is of relevance in an acute exposure setting.) As with the co-occurrence, the co-exposure evaluation focused on AFB_1 and FB_1.

Populations consuming foods that have relatively high concentrations of either AFB_1 or FB_1 and/or are staple foods in the diet are those with the potential of greater risk of the adverse effects of co-exposure to these contaminants. At the current meeting, the Committee summarized concentrations of AFB_1 and FB_1 in foods and estimated dietary exposures at the national and international level for both AFB_1 and FB_1 separately.

To determine populations that may have an increased risk from co-exposure to aflatoxins and fumonisins in this evaluation, the following was undertaken:

- Reviewing which foods have high concentrations of AFB_1 and FB_1;
- Evaluating the amounts of key foods consumed at the international level from the GEMS/Food cluster diets;
- Determining the foods that were high contributors to dietary exposure to AFB_1 or FB_1 at both the national and international level;
- Comparing estimates of dietary exposure to AFB_1 and FB_1 at the international level to determine clusters that have a higher exposure to both mycotoxins; and
- Comparing estimates of dietary exposure to AFB_1 and FB_1 at the national level (both those calculated by the Committee and those from the literature) where estimates for both mycotoxins were available for the same country.

Biomarkers of exposure, including in blood and urine, can also be evaluated to determine co-exposure (see section 2.3.1).

4.2 Foods with high concentrations of AFB_1 and FB_1

Concentrations of AFB_1 in foods were assessed by the Committee (see "Aflatoxins (addendum)", Tables A1-1, A1-2, A1-3 and A1-4 for aflatoxin concentrations from the literature, and Tables A1-5 and A1-6 for aflatoxin concentrations from the GEMS/Food contaminants database). The foods with the highest concentrations were as follows:

- Cereals, including maize (up to 6738 µg/kg in the literature and 476 µg/kg in the GEMS/Food contaminants database, with the highest concentrations in African countries); rice (up to 1707 µg/kg in the literature and 24 µg/kg in the GEMS/Food contaminants database); sorghum (up to 25 µg/kg in the literature and 359 µg/kg in the GEMS/Food contaminants database); and wheat (up to 254 µg/kg in the literature);
- Tree nuts and oilseeds including groundnuts (peanuts and peanut products; up to 937 µg/kg in the literature and 754 µg/kg in the GEMS/Food contaminants database); pistachios (up to 123 µg/kg in the literature and 711 µg/kg in the GEMS/Food contaminants database); and other nuts including almonds, Brazil nuts and hazelnuts (up to between 480 and 898 µg/kg in the GEMS/Food contaminants database); and
- Spices including chillies (whole, up to 687 µg/kg from the literature) and chilli powder (114 µg/kg from the GEMS/Food contaminants database).

This current review by the Committee only includes data since 2007 (after the sixty-eighth JECFA meeting). These data comprise the most recent from over the past decade for a number of foods, including those foods commonly known to contain aflatoxins.

Foods with the highest FB_1 concentrations based on data from the GEMS/Food contaminants database (see "Fumonisins (addendum)", Tables 7 and 8) are cereals and cereal-based foods and products (lower bound [LB] to upper bound [UB] mean: 129–194 µg/kg; maximum 35 400 µg/kg), with maize and maize-based products having the highest concentrations (mean 310–392 µg/kg LB to UB; maximum 23 800 µg/kg).

4.3 Populations with high consumption of key food commodities

The GEMS/Food cluster diets were evaluated to determine what areas in the world have high consumption of key commodities contaminated with AFB_1 and FB_1 (as shown in Table 5).

Table 5

Consumption data from the GEMS/Food cluster diets for selected food commodities that have high concentrations of AFB_1 and/or FB_1

	Consumption data per GEMS/Food cluster diet (g/capita per day)[a]																
Food	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
Maize	0.6	0	0.6	0	1.2	**12.3**	0	0	1.4	0	0	0	0	0.002	0.03	0	0
Maize flour	22.7	35.6	**87.3**	34.9	**46.7**	**49.1**	14.2	12.9	19.7	12.6	4.2	52.3	**94.3**	8.1	28.0	**55.9**	28.1
Groundnuts, with shell	0.1	0.03	0.2	0.2	1.1	0.1	0.1	0.2	5.2	0.03	0	0.9	1.2	0.3	0.2	**7.8**	0.01
Groundnuts, shelled	0.4	1.0	**6.9**	1.5	1.5	1.8	2.4	2.1	**6.8**	**4.4**	1.3	0.9	**7.7**	0.4	1.4	**8.5**	0.5
Prepared groundnuts	0.1	0.2	0.04	1.1	0.1	0.02	0.8	0.1	0.1	0.4	0.1	0.5	0.02	0.1	0.5	0.003	0
Tree nuts	3.6	3.3	5.1	8.6	15.9	9.3	5.2	7.0	13.2	4.2	9.2	**28.3**	6.7	**157.2**	4.0	0.0	**347.3**
Rice, paddy	0	0	0	0.3	0	0	0	0	0	0	0	0.3	0	0	0.2	0	0
Rice, husked	1.2	1.3	31.1	4.6	0.2	2.2	2.4	1.6	0.4	1.1	0	4.8	**13.5**	3.5	1.8	0	8.8
Rice, milled/husked	0	0	0	0.1	0.3	0	2.7	4.6	0	**28.4**	11.2	0	0	0	2.2	0	0
Rice, milled	33.9	9.3	36.5	82.3	**144.4**	65.9	9.6	5.3	**261.9**	28.6	0	62.2	23.9	**211.1**	10.4	12.8	49.9
Rice, broken	0.2	1	5.2	0	**5.5**	**4.6**	1	0.9	0.1	0.1	1.6	0.6	**6.3**	7.3	0.2	2.4	1.4
Rice flour	0	0.2	0	0.5	0.2	0	1	0.4	0.7	0	0.2	0.1	0	0.1	0.2	0	0
Sorghum	0	0	0	0	0	0	0	0	0	1.2	0	**7.1**	0	0	0	0	0
Sorghum flour	3.9	0	11.6	14.2	9.9	2.6	0	0	1.3	0	0	0	**76.0**	1.8	0	19.8	0
Wheat	0	1.1	0	0	0.6	0	0	0	0	0	0	0	0	0	0	0	1
Wheat flour	**299.2**	**263.3**	27.9	**214.1**	133.5	**340.0**	182.6	187.2	103.8	180.4	164.0	118.6	43.7	85.8	**206.2**	19.4	92.9

AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1

[a] Bold typeface indicates clusters with the highest consumption for that food.

For maize, the clusters that have the highest consumption are G06 (12.3 g/capita per day), followed by G09 at 1.4 g/capita per day and G05 at 1.2 g/capita per day. Countries in cluster G06 are Armenia, Cuba, Egypt, Greece, the Islamic Republic of Iran, Lebanon and Turkey. Cluster G09 includes primarily Asian countries, and cluster G05 is a mixture of countries from central and southern areas in the Americas, Asia and Africa. The highest consumption for maize is in the form of maize flour for which cluster G13 has the highest consumption (94.3 g/capita per day) and cluster G03 the next highest (87.3 g/capita per day). These two clusters are made up almost entirely of African countries. When considering the total consumption of maize and maize flour, the highest consumption is for cluster G13 (94.3 g/capita per day), followed by G03 (87.9 g/capita per day), G06 (61.4 g/capita per day), G16 (55.9 g/capita per day), G12 (52.3 g/capita per day) and G05 (47.9 g/capita per day).

Consumption of "wheat" itself is very small: only G02, G17 and G05 (from highest to lowest) had consumption data, at 1.1 g/capita per day or less (Table 5). The main consumption of wheat is reported as "wheat flour". Cluster G06 had the highest wheat flour consumption of 340 g/capita per day. Other clusters with high consumption include G01 (299.2 g/capita per day), G02 (263.3 g/capita per day) and G04 (214.1 g/capita per day).

For rice, the cluster with the highest consumption (including husked, milled, broken and paddy rice as well as rice flour) was cluster G09 (263.1 g/capita per day), which is primarily Asian countries. The next highest consumption was for G14 (which includes some Pacific island countries) (222 g/capita per day), followed by G05 (150.6 g/capita per day).

Consumption of sorghum (including sorghum and sorghum flour) was highest for cluster G13 (76 g/capita per day). This cluster, made up primarily of African countries, also had the highest consumption of maize flour.

Clusters that have a higher consumption of groundnuts (peanuts: in shell, shelled and prepared) include G16 (16.3 g/capita per day), G09 (12.1 g/capita per day), G13 (8.9 g/capita per day) and G03 (7.1 g/capita per day). These clusters consist primarily of African countries, except for cluster G09, which is made up primarily of Asian countries.

Tree nuts in the cluster diets (cluster level 2, code ID 22) include Brazil nuts, cashew nuts, chestnuts, almonds, walnuts, pistachios, kola nuts, areca nuts and coconuts. The clusters with the highest consumption of tree nuts are G17 (347.3 g/capita per day) and G14 (157.2 g/capita per day). These two clusters include many island countries including some in the Pacific Ocean and off the coast of Africa.

Some clusters have high consumption of cereals and tree nuts or groundnuts. Clusters that have a combination of high consumption of maize (maize and maize flour) and peanuts are clusters G13 (102 g/capita per day), G03

(95 g/capita per day) and G16 (65 g/capita per day). Cluster G16 had relatively high maize consumption and highest peanut consumption. The countries in G16 are Gabon, Rwanda and Uganda.

4.4 Commodities that are high contributors to dietary exposure

At the current meeting the Committee evaluated estimates of dietary exposure to AFB_1 and FB_1 both at the national level (including those estimated by the Committee and from the literature) and at the international level (estimated by the Committee). Based on these evaluations, the foods that contributed the most to dietary exposures were reviewed and are summarized below.

Foods that were the highest contributors to AFB_1 dietary exposure were similar across the national estimates of dietary exposure (from the literature and those estimated by the Committee) and the international estimates (estimated by the Committee). The foods were cereals and cereal-based products, namely maize, rice, wheat and sorghum, as well as peanuts and spices.

The main foods contributing to FB_1 dietary exposure at the international level were maize and maize products for 15 of the 17 clusters (63–96% of FB_1 exposure). Wheat was the highest contributor for one cluster (95%) and the second highest contributor for four clusters (16–24%). For the national estimates of dietary exposure, a number of cereal-based foods were key contributors, including cakes, bread, biscuits, cereal bars, pies, rice and maize. These foods reflect the foods that have high concentrations of FB_1, particularly maize.

4.5 International estimates of dietary exposure

International estimates of dietary exposure to AFB_1 and FB_1 were estimated by the Committee at the current meeting. These are compared in Table 6. Clusters that have higher dietary exposures for both mycotoxins were evaluated. This is difficult given the range of foods included in each assessment and the limits of reporting used to estimate UB and LB mean concentrations and the wide range of resulting dietary exposure estimates with different levels of exposure between the LB and UB. Therefore, using the LB dietary exposures, the highest three exposures to AFB_1 (over 7 ng/kg bw per day) and FB_1 (over 400 ng/kg bw per day) were first identified and then evaluated to determine if this was the case for both mycotoxins. Two clusters had one of the top three exposures to AFB_1 and one of the top three exposures to FB_1: cluster G05, which includes countries such as Guatemala and Mexico, and cluster G13, which includes African countries.

Table 6

Estimated mean per capita dietary exposures to AFB_1 and FB_1[a]

		Estimated dietary exposure per GEMS/Food cluster diet (ng/kg bw per day)[b]																
Mycotoxin	**Scenario[c]**	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
AFB_1	LB	3.0	2.6	6.5	5.1	**7.1**	5.8	1.3	0.3	4.4	1.2	1.2	4.4	**31.6**	**7.1**	1.5	4.1	3.1
	UB	7.3	6.7	8.6	9.1	**10.8**	11.4	1.7	1.3	7.3	3.9	3.5	7.2	**34.8**	**10.2**	7.0	5.6	5.1
FB_1	LB	130	180	340	200	**560**	190	120	61	2	58	**430**	300	**400**	47	74	220	170
	UB	480	530	550	560	**940**	610	500	550	310	300	**650**	650	**660**	410	1 200	400	390

AFB_1: aflatoxin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; FB_1: fumonisin B_1; LB: lower bound; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound

[a] Estimated by the Committee based on concentration data from the GEMS/Food contaminants database and consumption data from the GEMS/Food cluster diets.

[b] Bold typeface indicates the three clusters with the highest dietary exposure to each of AFB_1 and FB_1 at the lower bound.

[c] LB mean samples below LOQ (not detected) entered as zero values; UB mean samples below LOQ (not detected) entered as LOD/LOQ value.

4.6 National estimates of dietary exposure

The Committee at the current meeting reviewed national estimates of dietary exposure to aflatoxins and fumonisins that were calculated by the Committee and those from the literature.

National estimates of dietary exposure were limited to 12 countries that had estimates for both AFB_1 and FB_1. These included Belgium, Bulgaria, Burkina Faso, Cyprus, Czech Republic, Germany, Hungary, Ireland, Italy, Sweden, Thailand and the USA. The results from these assessments are shown together in Table 7. For all these countries, the results show the potential for exposure in the diet from both AFB_1 and FB_1.

When evaluating the estimates of dietary exposure based on the LB means, four of the 12 countries had populations with dietary exposures above 2 ng/bw per day for AFB_1 and above 100 ng/bw per day for FB_1. These were Burkina Faso (adult women: AFB_1 2.3 and FB_1 191 ng/kg bw per day), Cyprus (adolescents 10–17 years: AFB_1 9 and FB_1 367 ng/kg bw per day), Italy (toddlers 12–35 months: AFB_1 2.7 and FB_1 246 ng/kg bw per day; other children 3–9 years: AFB_1 2.8 and FB_1 206 ng/kg bw per day; and adolescents 10–17 years: AFB_1 1.7 and FB_1 132 ng/kg bw per day) and the USA (children <6 years: AFB_1 3.8 and FB_1 127 ng/kg bw per day).

The primary limitation to drawing conclusions from dietary exposure estimates calculated using consumption data in the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss) and concentration data in the GEMS/Food contaminants database is that these are limited to countries that have submitted data, which may not capture all key populations of interest.

Estimates of dietary exposure from the literature for the same country were difficult to compare because they were based on different age/sex groups, included different foods and used different methodologies. There were only three countries with estimates of dietary exposure from the literature for both AFB_1 and FB_1 (China, France, Spain) from the same study.

The French estimates from the literature were from the Second French Total Diet Study (Sirot, Fremy & Leblanc, 2013). Estimated dietary exposure to AFB_1 for adults was 0.002–0.22 ng/kg bw per day at the mean and 0.01–0.39 ng/kg bw per day at the 95th percentile, and for children was 0.001–0.39 ng/kg bw per day at the mean and 0.008–0.74 ng/kg bw per day at the 95th percentile. For FB_1, estimated dietary exposures for adults were 7.5–29.1 (LB–UB) ng/kg bw per day at the mean and 22.9–65.6 ng/kg bw per day at the 95th percentile, and for children were 15.4–44.6 ng/kg bw per day at the mean and 50.4–106 ng/kg bw per day at the 95th percentile.

Table 7
Estimated national dietary exposures to AFB_1 and FB_1 by country[a]

Country	Population group (age in years)	Estimated dietary exposure (ng/kg bw per day)[b]							
		AFB_1				FB_1			
		LB mean	UB mean	LB high	UB high	LB mean	UB mean	LB P90	UB P90
Belgium	Toddlers (1–2)	0.83	2.1	1.7	4.2	270	1 252	540	2 505
	Other children (3–9)	0.21	1	0.43	1.9	298	1 069	597	2 138
	Adolescents (10–17)	0.03	0.19	0.06	0.38	100	454	199	908
	Adults (18–64)	0.03	0.17	0.06	0.34	74	364	149	729
	Elderly adults (65–74)	0.04	0.12	0.07	0.24	53	266	106	533
	Very elderly adults (>75)	0.05	0.12	0.09	0.23	52	268	104	537
Bulgaria	Infants (<1)	0	1.4	0	2.8	187	2 709	374	5 419
	Toddlers (1–2)	0	3.6	0	7.3	272	1 577	544	3 155
	Other children (3–9)	0.03	3.6	0.06	7.3	247	1 261	493	2 522
Burkina Faso	Adult women	**2.3**	3.9	4.6	7.8	**191**	913	382	1 826
Cyprus	Adolescents (10–17)	**9**	9.5	18	19	**367**	691	735	1 382
Czech Republic	Other children (3–9)	1	1.4	1.9	2.8	185	515	369	1 030
	Adolescents (10–17)	0.7	1	1.3	2	154	403	308	805
	Adults (18–64)	0.5	0.6	1	1.3	75	337	151	675
Germany	Toddlers (1–2)	0.3	2.4	0.5	4.8	199	2 057	397	4 114
	Other children (3–9)	0.4	2.2	0.8	4.5	184	696	368	1 393
	Adolescents (10–17)	0.3	1.4	0.7	2.8	86	286	171	571
	Adults (18–64)	0.3	1.4	0.6	2.8	77	275	154	550
	Elderly adults (65–74)	0.3	1.3	0.6	2.7	67	250	134	500
	Very elderly adults (>75)	0.3	1.3	0.6	2.6	78	241	155	482
Hungary	Adults (18–64)	0.09	4	0.19	8.1	35	362	69	724
	Elderly adults (65–74)	0.07	3.9	0.14	7.7	39	320	78	640
	Very elderly adults (>75)	0.07	4.3	0.15	8.5	43	389	87	778
Ireland	Adults (18–64)	0.04	0.57	0.07	1.1	41	437	82	874
Italy	Infants (<1)	0.44	1.6	0.87	3.2	107	2 354	213	4 708
	Toddlers (1–2)	**2.7**	7.9	5.3	15.7	**246**	1 965	491	3 929
	Other children (3–9)	**2.8**	7.8	5.6	15.6	**206**	1 270	411	2 540
	Adolescents (10–17)	**1.7**	4.8	3.4	9.5	**132**	752	264	1 504
	Adults (18–64)	1.1	3.3	2.2	6.6	70	571	141	1 035
	Elderly adults (65–74)	1.0	3.1	2.0	6.2	49	473	98	947
	Very elderly adults (>75)	1.1	3.2	2.2	6.4	52	513	104	1 027
Sweden	Other children (3–9)	0.49	0.54	0.98	1.1	420	1 010	840	2 021
	Adolescents (10–17)	0.37	0.4	0.73	0.81	303	653	606	1 307
	Adults (18–64)	0.2	0.22	0.4	0.44	214	484	429	968
Thailand	General population	1.1	1.4	2.3	2.9	17	616	34	1 231

Table 7 (continued)

Country	Population group (age in years)	Estimated dietary exposure (ng/kg bw per day)[b]							
		AFB_1				FB_1			
		LB mean	UB mean	LB high	UB high	LB mean	UB mean	LB P90	UB P90
USA	Children (<6)	**3.8**	10	7.5	20.4	**127**	530	254	1 060
	Women of childbearing age	1	2.9	2	5.9	36	141	71	283
	General population	1.4	3.9	2.7	7.8	46	192	92	383

bw: body weight; CIFOCOss: FAO/WHO Chronic Individual Food Consumption Database – Summary statistics; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound; P90: 90th percentile; UB: upper bound

[a] Estimated by the Committee based on concentration data from the GEMS/Food contaminants database and the CIFOCOss national consumption database.

[b] Bold typeface indicates population groups with LB mean exposures to AFB_1 of over 2 ng/kg bw per day and to FB_1 of over 100 ng/kg bw per day.

Estimates from Spain were based on dietary exposures to coffee only (García-Moraleja et al., 2015), and therefore do not include exposure from cereal-based foods. As a result, dietary exposures to AFB_1 are low: 0.003 ng/kg bw per day at the mean and 0.013 ng/kg bw per day at the 95th percentile for adults. FB_1 dietary exposures are also low at 0.14 ng/kg bw per day or less for mean and high exposures in adults and adolescents.

From China (Sun et al., 2011), based on occurrence data in maize, rice, wheat flour, oils and peanuts, mean dietary exposure to AFB_1 was reported to be in the range of 6.7–45 ng/kg bw per day on a 60 kg body weight basis. For FB_1, estimated mean dietary exposures were 1540–7667 ng/kg bw per day.

4.7 Summary of the evaluation of co-exposure to aflatoxins and fumonisins from the diet

- Foods with the highest concentrations of AFB_1 are groundnuts, cereals (namely, sorghum, maize, rice and wheat), tree nuts and some spices. Foods with the highest concentrations of FB_1 are maize and maize products.
- Based on consumption data from the GEMS/Food cluster diets, the highest consumption of maize and maize flour is from clusters G13, G03, G06, G16 and G12 (highest to lowest) (Table 5). These clusters include primarily African countries, but also some from the central areas in the Americas (Guatemala, Mexico). The highest consumption of groundnuts (with shell, shelled and prepared) is found for clusters G16, G09, G13 and G03 (highest to lowest). These clusters include African countries except cluster G09, which is mainly

Asian countries. High consumption of both maize and groundnuts is seen in clusters G13, G03 and G16 (highest to lowest).

- At the national and international levels, the main foods contributing to dietary exposure to AFB_1 are cereals and cereal-based products (namely, maize, rice, wheat and sorghum), peanuts and spices. The main foods contributing to FB_1 exposure were maize and maize-based products.
- From the international estimates of dietary exposure, the two clusters with the highest dietary exposures to both AFB_1 and FB_1 were G05 (including Guatemala and Mexico) and G13 (which is made up of African countries). LB mean AFB_1 exposures were over 7 ng/kg bw per day, and LB mean FB_1 exposures were over 400 ng/kg bw per day for these clusters.
- National estimates of dietary exposure were limited to 12 countries that had estimates for both AFB_1 and FB_1. Of these, four countries had LB mean exposures to AFB_1 over 2 ng/kg bw and to FB_1 over 100 ng/kg bw per day. These were Burkina Faso (adult women), Cyprus (adolescents), Italy (toddlers, children and adolescents) and the USA (children <6 years of age). From the literature, where the same studies included exposures to both AFB_1 and FB_1, there were estimates from three countries (France, Spain and China). Only the estimated exposures from China were high, with LB mean AFB_1 exposure of 6.7 ng/kg bw per day and with LB mean FB_1 exposures over 1500 ng/kg bw per day.

4.8 Co-exposure to aflatoxins and fumonisins for infants

Information on the occurrence of aflatoxins and fumonisins in breast milk is of high importance as WHO recommends exclusive breastfeeding up to 6 months of age, with continued breastfeeding along with appropriate complementary foods up to 2 years of age or beyond, because of the optimal nutrition and immunological benefits of breast milk (WHO, 2016). The Committee evaluated co-exposures to these two mycotoxins in human breast milk and in infant formula. Co-exposure from solid foods for infants was also evaluated.

The evaluation for infants also included AFM_1, as this metabolite is found in milk (human and animal) as a result of the exposure of the mother to aflatoxins through the diet.

4.8.1 Breast milk

A review of the literature and the GEMS/Food contaminants database was undertaken to evaluate concentrations of aflatoxins and fumonisins in human breast milk.

As there were no entries in the GEMS/Food contaminants database for human breast milk, an evaluation of co-occurrence of aflatoxins and fumonisins could not be undertaken from this data source.

A recently published systematic review of 63 studies with over 7000 participants from 31 countries assessed mycotoxins and their metabolites in breast milk. The most common aflatoxin evaluated was AFM_1, although some studies also evaluated AFB_1, with up to 4.8 µg/kg (range 0.05–372 µg/kg) detected in 18% of 113 samples in Sierra Leone (Cherkani-Hassani et al., 2016).

Studies on human breast milk by Magoha et al. (2014a,b) in the United Republic of Tanzania showed detectable concentrations of FB_1: 58 out of 131 samples (44%) contained FB_1 in the range of 6.5–472 µg/kg, and 100% of 143 samples of breast milk contained AFM_1 at a median concentration of 0.07 µg/kg (range 0.01–0.55 µg/kg). The authors estimated dietary exposures to FB_1 based on a consumption of 500 mL of breast milk per day, at a median of 3000 ng/kg bw per day (range 780–65 000 ng/kg bw per day). Dietary exposures to AFM_1 were estimated for 143 children as between 1.1 and 66.8 ng/kg bw per day. This indicates the potential for co-exposure to aflatoxins and fumonisins for breastfed infants; however, the Committee considered the method used to quantify the FB_1 in breast milk to be inadequate for this matrix.

4.8.2 Infant formula

An evaluation of the occurrence of aflatoxins and fumonisins in infant formula was undertaken by the Committee.

The GEMS/Food contaminants database contains entries for infant formulas from six countries/regions (Canada, Germany, Hungary, Slovakia, Spain and the WHO European Region) that have been analysed for AFB_1 (n = 161) and FB_1 (n = 61). As AFB_1 was detected in only one sample of infant formula powder (at 0.2 µg/kg) and FB_1 was not detected in either infant formula powder or follow-on formula powder (see Table 1), there was no co-occurrence of AFB_1 and FB_1 in infant formula.

The GEMS/Food contaminants database contains entries for infant formulas that have been analysed for AFM_1 (n = 876). The samples were from 14 countries/regions (Australia, Cyprus, France, Germany, Hungary, Ireland, Luxembourg, Malta, Poland, Portugal, Slovakia, Slovenia, Spain and the WHO European Region). There were only 30 samples with detected levels of AFM_1 (3.4%) from seven of the countries/regions (Luxembourg, Malta, Poland,

Portugal, Slovakia, Spain, the WHO European Region). As FB_1 was not detected in any infant formula, the GEMS/Food contaminants database does not show any evidence of co-occurrence of AFM_1 and FB_1; however, only a limited number of samples were analysed for FB_1.

No papers were found in the literature identifying FB_1 detections in infant formula, and therefore there was no indication of co-exposure to aflatoxins and fumonisins from infant formula.

4.8.3 Infant foods

In addition to breast milk and/or infant formula, infants under 12 months of age also consume solid foods once they are weaned, and may be exposed to both AFB_1 and FB_1 from infant foods (e.g. infant cereals, infant meals) and from the general food supply. Exposure to AFM_1 would also continue from breast milk and infant formula or dairy milk and dairy foods, if they have been introduced into the diet.

Analysis of the GEMS/Food contaminants database has shown AFB_1 and FB_1 to be in cereal-based foods for infants, ready-to-eat meals for infants and other not further specified foods for infants (Table 1). These data come from a number of countries, primarily in Europe but also from Canada. When assessing co-occurrence of AFB_1 and FB_1 in the same food sample based on the unique sample identification number, co-occurrence has been found in cereal-based foods for infants from Canada and Poland, but only in a very small proportion of samples (<1%). Again the data in the GEMS/Food contaminants database are from a limited number of countries and include mostly processed infant foods and no infant foods from African countries.

Published studies that included information on infant foods are summarized in Table 4. One study of 67 samples of maize flour–based foods for young children in the United Republic of Tanzania (Magoha et al., 2016) found that 51% of samples contained AFB_1 (0.9–56 µg/kg food) and 30% contained FB_1 (52.7–974 µg/kg food). A study of Incaparina (a corn and cottonseed flour–based supplementary infant food that is consumed by ~80% of infants) in Guatemala showed 100% occurrence of both AFB_1 (3–214 µg/kg) and FB_1 (200–1700 µg/kg) in eight samples (Trucksess et al., 2002). These studies indicate the potential for co-exposure to aflatoxins and fumonisins from noncommercial, traditionally and locally produced infant foods.

A study of co-exposure to mycotoxins, including aflatoxins and fumonisins, in maize-based complementary foods was undertaken in children aged 18–24 months in the United Republic of Tanzania (Kimanya et al., 2014). Two 24-hour recalls were conducted to collect food consumption data from 41 children. Samples of the maize used for their food were also analysed. Maize flour

samples had detectable concentrations of total aflatoxins of 0.11–386 μg/kg, of AFB_1 of 0.5–364 μg/kg, of total fumonisins of 63–2284 μg/kg and of FB_1 of 57–1672 μg/kg. Daily consumption of maize flour was 16–254 g per child. Estimated dietary exposures to aflatoxins (for the 13 children consuming aflatoxin-contaminated samples) were 1–786 ng/kg bw per day and to total fumonisins (for the 34 children consuming fumonisin-contaminated samples) were 190–26 300 ng/kg bw per day. Co-exposure to total aflatoxins and total fumonisins was found in 29% of the 41 children in the study.

In another study of co-exposure to aflatoxins and fumonisins in Tanzanian children consuming maize-based complementary foods (Magoha et al., 2016), 24-hour dietary recall was conducted to assess the amount of flour consumed by infants aged 3 (n = 121) and 5 months (n = 118). Samples of maize flour consumed by the families were collected for analysis. Of these samples, 58% contained total aflatoxins (0.33–69 μg/kg) and 31% detectable fumonisins (total) (48–1225 μg/kg). Estimated dietary exposures to total aflatoxins ranged between 0.14 and 120 ng/kg bw per day (median: 3.9 ng/kg bw per day), and to total fumonisins between 5 and 880 ng/kg bw per day (median: 140 ng/kg bw per day).

Therefore, there can be co-exposure to aflatoxins and fumonisins in the diet of infants from infant foods.

4.8.4 Summary of the evaluation of co-exposure to aflatoxins and fumonisins for infants

An evaluation was undertaken by the Committee to determine the potential for co-exposure to aflatoxins and fumonisins for infants from breast milk, infant formula or infant foods. The GEMS/Food contaminants database and the literature were searched for information relating to co-occurrence and co-exposure. While aflatoxins have been detected in breast milk, only one study found FB_1 in breast milk (Magoha et al., 2014a). However, the quality of the study was questioned by the Committee.

There were no data available showing co-occurrence of aflatoxins and fumonisins in infant formula. Some data indicated co-occurrence of aflatoxins and fumonisins in infant foods, primarily those that are cereal based, indicating that infant foods can be a source of co-exposure in infants.

5. Dose–response analysis and estimation of toxic/carcinogenic risk

No studies designed to reveal dietary dose–response relationships for co-exposure to pure FB_1 and AFB_1 have been conducted. The only available in vivo long-term

carcinogenicity co-exposure study was conducted in rainbow trout (Carlson et al., 2001) using multiple dietary doses of pure FB_1 but only a single non-dietary exposure to pure AFB_1. The Committee noted that although this study showed that dietary FB_1 could promote liver cancer in AFB_1-initiated animals, this outcome would be very difficult to model because the AFB_1 exposure was not oral/dietary and was at a single dose level.

6. Comments

6.1 Biochemical aspects

The biochemical aspects of aflatoxins and fumonisins were evaluated separately at the current meeting (see "Aflatoxins (addendum)" and "Fumonisins (addendum)").

The results from a rat study showed that co-exposure (single oral gavage) to pure AFB_1 (0.125 mg) and pure FB_1 (25 mg) resulted in a decrease in urinary excretion of AFB_1 (as measured by $UAFM_1$) and UFB_1 compared with the excretion when either mycotoxin was administered alone. In contrast, an increase in the serum AFB_1–alb adduct, derived from the metabolic activation pathway that forms the reactive AFB_1-8,9-epoxide intermediates, was observed. The mechanism for this pharmacokinetic interaction was not clear, yet it was suggested that the CYP metabolism of AFB_1 might be affected (Mitchell et al., 2014). The Committee acknowledged that if the co-exposure alters the AFB_1 metabolism and leads to a change in production of the reactive AFB_1-*exo*-8,9-epoxide intermediate, co-exposure could in principle alter the risk of hepatocarcinogenicity of AFB_1. However, there is currently no evidence that the interaction observed at the high concentrations used in the study also occurs at doses relevant to human exposure.

Another mechanism of interaction, as noted by the Committee at previous meetings, is based on the fact that AFB_1 is a genotoxic initiator of tumour formation in liver and that FB_1 is a potential cancer promoter in liver (Carlson et al., 2001; Gelderblom et al., 2002) (Annex 1, references *77* and *205*). The mutagenicity and carcinogenicity of AFB_1 have been described in detail in the monograph "Aflatoxins (addendum)". As for FB_1, recent human data have provided further support that daily exposure to high levels of fumonisins is likely to result in inhibition of ceramide synthase in humans, as is observed in animals (see "Fumonisins (addendum)"). Decreased ceramide biosynthesis and increased sphingosine kinase activity have been associated with the development and progression of many human tumours (Espaillat et al., 2015; Reimann et al.,

2015; Suh & Saba, 2015). Ceramide synthase 2 knock-out mice show elevated levels of sphinganine, apoptosis and proliferation in the liver, upregulation of cell cycle–related genes, and spontaneously developed liver tumours (Pewzner-Jung et al., 2010a,b). Many of the changes and effects seen in the liver of the ceramide synthase 2 knock-out mice are reminiscent of changes and effects reported in studies where ceramide synthases are inhibited by FB_1. These changes have been interpreted as the regenerative hyperplasia process that could promote the tumorigenic potential of DNA damage initiated by AFB_1.

6.2 Toxicological studies

The Committee at the seventy-fourth meeting reviewed the combined effects of fumonisins and other mycotoxins. These studies had limitations and showed inconclusive and sometimes contradictory results. The Committee at the seventy-fourth meeting concluded that because the fumonisins known to date do not share a similar mode of action with any other mycotoxin, it was unlikely that simple additive effects with other mycotoxins would occur (Annex 1, reference *205*).

In the present evaluation, the newly available in vivo and in vitro studies of combined effects were evaluated for evidence suggestive of interactions.

No long-term study on the effect of aflatoxin–fumonisin co-exposure has been done since the Committee's last evaluation.

In one mouse study, pure AFB_1 (80 µg/kg bw per day) and pure FB_1 (100 µg/kg bw per day) were given by oral gavage, either alone or in combination, for 14 days. Among the observed effects, some showed less than additivity (i.e. the end-point was affected to the same extent for AFB_1 only, FB_1 only and co-exposure groups), such as the increase in relative spleen weight, whereas others showed additivity, such as activities of enzymes indicative of oxidative stress (Abbès et al., 2016).

In a rat feeding study, F344 rats were exposed to pure AFB_1 (equivalent to 15 µg/kg bw per day for 14 days) and pure FB_1 (equivalent to 25 mg/kg bw per day for 21 days), alone or sequentially (the rats were treated with AFB_1 and then FB_1, with a recovery period of 21 days in between). The results showed that effects on some end-points such as body weight appeared to be less than additive, whereas others such as the effects on some liver enzymes appeared to be additive. Importantly, the lesions indicating liver damage, such as average number of apoptotic cells and the number and area of GST placental form positive ($GST\text{-}P^+$) foci, were found to be synergistic (Qian et al., 2016). These results support the hypothesis that fumonisins may be a promoter for aflatoxin-initiated hepatocarcinogenesis and confirmed the previous findings by Gelderblom et al. (2002), which, along

with the study in trout (Carlson et al., 2001), were the basis for the Committee to acknowledge the concern for the increased hepatocarcinogenicity under the condition of co-exposure to aflatoxins and fumonisins.

In another rat feeding study, Wistar rats were exposed for 90 days to FB_1 (100 mg/kg in diet from culture material extract) and pure AFB_1 (40 µg/kg diet), either alone or in combination. Spleen mononuclear cells were isolated and analysed immediately for a series of toxicological end-points, most of which suggested a less-than-additive effect (Theumer et al., 2010).

Chickens were fed diets containing aflatoxins (equivalent to 0.125 mg/kg bw, prepared with *A. flavus* culture material) or fumonisins (equivalent to 3.125 mg/kg bw, prepared with *F. verticillioides* culture material), alone or combined, for 56 days. Changes in plasma triglycerides, very low density lipoprotein levels and percentage of total liver lipids were observed in the co-exposure group (Siloto et al., 2013). The nature of the interactions was not defined by the authors, but they appeared to be additive or less than additive.

The Committee noted that the above studies were conducted using only one dose of aflatoxins and fumonisins; thus, the nature of the interaction could not be assessed. The errors and pitfalls of this approach have also been critiqued by Chou (2010).

Since 2011, there have been only a few in vitro studies published addressing the AFB_1 and FB_1 co-exposure interaction. In rat spleen mononuclear cells, AFB_1 and FB_1 each induced production of reactive oxygen species, with the combined effect being less than additive. However, other effects, such as on superoxide anion radical generation, protein oxidation, lipid peroxidation and DNA oxidation, were mostly suggestive of additivity in the combined treatment (Mary et al., 2012). In a rat liver hepatoma cell line, FB_1 alone or in combination with AFB_1 increased CYP1A transcription and activity, as well as upregulated the aryl hydrocarbon receptor in a dose-dependent manner. The effects were greatest in the cells treated with the FB_1–AFB_1 mixture, and in some cases the effects were suggestive of being more than additive (Mary et al., 2015). Because in vitro cytotoxicity of FB_1 and the metabolism of AFB_1 are highly dependent on the cell type, the Committee noted that the in vitro results need to be interpreted carefully.

With limited knowledge of the in vitro–in vivo extrapolation of many of the tested toxicological end-points and the above-mentioned limitations in the in vivo studies, the Committee concluded that the available toxicological studies do not provide adequate information on aflatoxin–fumonisin interactions to facilitate an understanding of the role of co-exposure as a contributing factor in human diseases.

6.3 Observations in humans

Urinary multi-biomarker analytical methods have recently been developed and increasingly used to estimate human exposure to mycotoxins. These methods are typically capable of simultaneously measuring the concentration of more than five urinary mycotoxin biomarkers, including UFB_1 and $UAFM_1$. In spite of a number of studies reporting the measurement of multiple urinary mycotoxin biomarkers concurrently, many provided the frequency of positive samples for each mycotoxin separately; thus, the status of co-occurrence of UFB_1 and $UAFM_1$ in the samples is unknown.

Very few multi-biomarker analytical studies provided the urinary mycotoxin biomarker co-occurrence information. In a study conducted in women in Guatemala, of a total of 602 urine samples, 287 (48%) were positive for UFB_1, 90 (15%) were positive for AFM_1 and 66 of the 90 AFM_1-positive samples were also positive for FB_1 (73%). This result was in concordance with the fact that the contamination of maize with fumonisins and aflatoxins is common in Guatemala (Torres et al., 2015). None of the other studies analysed samples from a population that was known to consume foods with frequent co-contamination of aflatoxin and fumonisin. With the low frequency of positive samples, no UFB_1 and $UAFM_1$ co-occurrence was found from those studies.

One study in the United Republic of Tanzania measured UFB_1 and plasma AF–alb in children. UFB_1 and AF–alb were detectable in 96% and 84% of the children, respectively, and 82% of the children had co-exposure. There was a significant positive correlation between levels of UFB_1 and AF–alb (Shirima et al., 2013).

Although evidence in laboratory animals has suggested an additive or synergistic effect of fumonisin and aflatoxin co-exposure in the development of preneoplastic lesions (Gelderblom et al., 2002; Qian et al., 2016) or hepatocellular carcinoma (Carlson et al., 2001), currently no data are available on such effects in humans.

Two prospective epidemiological studies were conducted in the United Republic of Tanzania to investigate the role of fumonisins, aflatoxins and their co-exposure in childhood growth. In one study, a significant negative association was observed between UFB_1, but not AF–alb, and length growth for 166 children followed up until 6–14 months of age. However, no interpretable results were found regarding the effect of fumonisin–aflatoxin co-exposure (Shirima et al., 2015). In the other study, exposure to fumonisins or aflatoxins, alone or in combination, was not significantly associated with underweight or stunting in 143 infants less than 6 months of age (Magoha et al., 2016).

6.4 Co-occurrence of fumonisins and aflatoxins in foods

An evaluation of the co-occurrence of aflatoxins and fumonisins in foods was undertaken by the Committee. The degree of co-occurrence of aflatoxins and fumonisins can be influenced by many factors, including variety of the commodity, region, time of sampling, storage, food preparation and processing.

There is information available regarding the co-occurrence of a range of mycotoxins in foods that is broader than the aflatoxin and fumonisin groups. There is also information available for the range of different aflatoxins and fumonisins in foods either individually or as totals. Although this evaluation noted these aspects, the focus of the evaluation was co-occurrence of AFB_1 and FB_1.

Data used to evaluate the co-occurrence of aflatoxins and fumonisins in foods were obtained from two sources: the data in the GEMS/Food contaminants database and the literature from studies that analysed both mycotoxins. The evaluation aimed to determine the types of foods in which both mycotoxins occur, the degree of co-occurrence and the countries in which the co-occurrence exists.

Co-occurrence of aflatoxins and fumonisins in animal feed was not considered for this review because, as noted previously by the Committee at its seventy-fourth meeting, fumonisins do not transfer in significant amounts from feed to animal products.

The co-occurrence evaluation based on data from the GEMS/Food contaminants database was first based on all samples for each food and then by sample number for individual analytical samples. Co-occurrence was defined as where detected concentrations (>LOD or >LOQ, depending on the data available) were found for both AFB_1 and FB_1. For the analysis including all samples, there were over 84 000 data points for AFB_1 and over 19 000 for FB_1 in food samples from a variety of countries. The majority of detections of both AFB_1 and FB_1 were in the cereals and cereal-based products group. This included foods such as barley, maize, millet, rice, rye, sorghum and mixed cereal products. Although this provides a general guide as to the types of foods where co-occurrence may occur, a more detailed analysis of individual foods by sample was undertaken to determine the degree of co-occurrence within individual samples. Just over 5000 samples, across all countries and foods, in the GEMS/Food contaminants database had unique sample identification numbers. For 1.7% of these, co-occurrence of AFB_1 and FB_1 was reported. For individual samples, co-occurrence was found for maize (5.5%), cereals and cereal-based products (4.2%), bread and other cooked cereal products (2.8%), sorghum (1.4%) and cereal-based foods for infants and young children (0.4%). Of the 18 countries and one WHO region for which data were submitted by sample number, samples from nine countries

had no co-occurrence. For the others, the degree of co-occurrence varied as a result of the number of samples and types of foods included, and therefore it was not possible to identify countries that had higher degrees of co-occurrence than others. There were also a limited number of WHO regions with co-occurrence data represented in the GEMS/Food contaminants database, with data from only four African countries for sorghum only.

Based on an evaluation of the literature, co-occurrence of AFB_1 and FB_1 was identified in samples of foods within the same study; however, it was mostly not possible to determine the degree of co-occurrence within the same samples. In contrast to the data in the GEMS/Food contaminants database, most of the studies in the literature were from African countries, and the main food groups assessed were maize and maize-based products. Other foods where co-occurrence was observed were other cereals (including sorghum, millet, rice, wheat), coffee, smoked meat and black pepper. The proportion of samples with AFB_1 and FB_1 detection varied widely (1–100%).

6.5 Co-exposure to aflatoxins and fumonisins in the diet

An evaluation of the co-exposure to AFB_1 and FB_1 was undertaken at the overall diet level to determine populations that are likely to be consuming diets that result in exposure to both mycotoxins, irrespective of co-occurrence in foods. These populations are more likely to be at a higher level of risk of potential adverse effects associated with co-exposure. To do this, an evaluation was done to highlight foods with higher concentrations of AFB_1 and FB_1 and populations that may have a higher consumption of these foods. The foods that are the main contributors to dietary exposures to AFB_1 and FB_1 were also determined, as foods that may not have high concentrations may contribute to dietary exposure if they are staple foods in a diet. Finally, a comparison of dietary exposures to AFB_1 and FB_1 was made at the national and international levels to determine populations with high exposure to both mycotoxins. Much of the information for this evaluation was from the separate assessments for aflatoxins and fumonisins conducted by the Committee at the current meeting (see "Aflatoxins (addendum)" and "Fumonisins (addendum)").

Foods with the highest concentrations of AFB_1 are groundnuts, cereals (namely, sorghum, maize, rice and wheat), tree nuts and some spices. Foods with the highest concentrations of FB_1 are maize and maize products.

Based on consumption data from the GEMS/Food cluster diets, the highest consumption of maize and maize flour is from clusters G13, G03, G06, G16 and G12 (highest to lowest). These clusters include primarily African countries, but also some from central areas in the Americas (e.g. Guatemala, Mexico). The highest consumption of groundnuts (with shell, shelled and prepared) is found

for clusters G16, G09, G13 and G03 (highest to lowest). These clusters include African countries except cluster G09, which is mainly Asian countries. High consumption of both maize and groundnuts is seen in clusters G13, G03 and G16 (highest to lowest).

At the national and international levels, the main foods contributing to dietary exposure to AFB_1 are cereals and cereal-based products (namely, maize, rice, wheat and sorghum), peanuts and spices. The main foods contributing to FB_1 exposure were maize and maize-based products.

From the international estimates of dietary exposure, the two clusters with the highest dietary exposures to both AFB_1 and FB_1 were G05 (including Guatemala and Mexico) and G13 (which is made up of African countries) (Table 8). LB mean AFB_1 exposures were over 7 ng/kg bw per day, and LB mean FB_1 exposures were over 400 ng/kg bw per day for these clusters.

National estimates of dietary exposure were limited to 12 countries that had estimates for both AFB_1 and FB_1. Of these 12 countries, four countries had LB mean exposures to AFB_1 over 2 ng/kg bw per day and LB mean exposures to FB_1 over 100 ng/kg bw per day. These were Burkina Faso (adult women), Cyprus (adolescents), Italy (children and adolescents) and the USA (children less than 6 years of age). From the literature where the same study included exposures for both AFB_1 and FB_1, there were estimates from three countries (France, Spain and China). Only the estimated exposures from China were high, with LB mean AFB_1 exposure of 6.7 ng/kg bw per day and FB_1 exposures over 1500 ng/kg bw per day (Sun et al., 2011).

The Committee also reviewed the co-exposure for infants based on reports in the literature of detection of both aflatoxins and fumonisins in human breast milk. Contamination with AFM_1 was also included in this part of the evaluation, given that this is the aflatoxin metabolite found in milk as a result of dietary exposure to aflatoxins in the mother.

Only one study has been identified that shows detectable concentrations of FB_1 in human breast milk. This study (Magoha et al., 2014a), conducted in the United Republic of Tanzania, showed detectable concentrations of FB_1 in 44% of 131 samples, with a concentration range of 6.5–472 µg/kg. The authors estimated dietary exposures to FB_1, based on a consumption of 500 mL of breast milk per day, at a median of 3000 ng/kg bw per day (range 780–65 000 ng/kg bw per day). AFM_1 was detected in all 143 breast milk samples from the United Republic of Tanzania, with a median concentration of 0.07 µg/kg (range 0.01–0.55 µg/kg) (Magoha et al., 2014b). This indicates the potential for co-exposure to aflatoxins and fumonisins for breastfed infants; however, the Committee considered the method used in the Magoha et al. (2014a) study to quantify the FB_1 in breast milk to be inadequate for this matrix.

Table 8

Estimated mean per capita dietary exposures to AFB_1 and FB_1 estimated by the Committee

		Estimated dietary exposure for GEMS/Food clusters (ng/kg bw per day)[a,b]																
Mycotoxin	**Scenario**	**G01**	**G02**	**G03**	**G04**	**G05**	**G06**	**G07**	**G08**	**G09**	**G10**	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**
AFB_1	LB	3.0	2.6	6.5	5.1	**7.1**	5.8	1.3	0.3	4.4	1.2	1.2	4.4	**31.6**	**7.1**	1.5	4.1	3.1
	UB	7.3	6.7	8.6	9.1	**10.8**	11.4	1.7	1.3	7.3	3.9	3.5	7.2	**34.8**	**10.2**	7.0	5.6	5.1
FB_1	LB	131	176	341	197	**556**	190	124	61	2	58	**430**	296	**402**	47	74	224	166
	UB	481	534	547	564	**935**	605	500	551	307	303	**649**	648	**664**	408	1 150	397	390

bw: body weight; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; LB: lower bound, where not detected concentrations were assigned a concentration of zero; LOD: limit of detection; LOQ: limit of quantification; UB: upper bound, where not detected concentrations were assigned a concentration equal to the LOD/LOQ.

[a] Based on concentration data from the GEMS/Food contaminants database and consumption data from the GEMS/Food cluster diets.

[b] Bold typeface indicates the three clusters with the highest dietary exposure to each of AFB_1 and FB_1 at the LB.

There was no co-occurrence of AFM_1 and FB_1 or AFB_1 and FB_1 in infant formula from data in the GEMS/Food contaminants database, because there were no detections of FB_1 in infant formula. There were no papers identified in the literature reporting FB_1 levels in infant formula. Therefore, there is no indication that there would be co-exposure to aflatoxins and fumonisins from infant formula.

Infant foods, primarily those that are cereal based, are consumed by infants under 12 months of age; at the same time, infants may also be consuming breast milk and/or infant formula. AFB_1 and FB_1 have been detected in foods for infants, including cereal-based foods and meals for infants. This is the case from the GEMS/Food contaminants database and the literature. This includes staple maize-based foods for infants from Africa, where consumption of these foods resulted in estimated dietary exposures to total aflatoxins of 1–786 ng/kg bw per day, to total fumonisins of 190–26 300 ng/kg bw per day (Kimanya et al., 2014), to total aflatoxins of 0.14–120 ng/kg bw per day and to total fumonisins of 5–880 ng/kg bw per day (Magoha et al., 2016). Therefore, there can be co-exposure to aflatoxins and fumonisins in the diet of infants from infant foods.

7. Evaluation

Fumonisins and aflatoxins are both frequent contaminants in cereals and cereal-based foods. Aflatoxins are common contaminants in groundnuts and tree nuts. Co-exposure to both mycotoxins is likely in areas where these foods are regularly consumed.

From the international estimates of dietary exposure, two GEMS/Food clusters (G05 and G13) have high dietary exposure to both AFB_1 and FB_1. The countries (Guatemala and the United Republic of Tanzania) where co-exposure has been confirmed using urinary or plasma exposure biomarkers of FB_1 and AFB_1 belong to these two clusters.

Although evidence in laboratory animals from the previous and the present evaluations has suggested an additive or synergistic effect of fumonisin and aflatoxin co-exposure in the development of preneoplastic lesions or hepatocellular carcinoma, currently no data are available on such effects in humans.

Two prospective epidemiological studies do not support the hypothesis of an interaction between aflatoxins and fumonisins in childhood stunting.

The Committee concluded that there are few data available to support co-exposure as a contributing factor in human disease. However, the interaction between AFB_1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation (particularly

at exposures above the provisional maximum tolerable daily intake), remains a concern. This is due to the fact that the incidences of chronic liver disease and stunting are high in the areas of the world where the exposures to both mycotoxins are high and the co-exposure has been confirmed with biomarkers.

7.1 Recommendations

There is a need to reduce human exposure to aflatoxins and fumonisins, alone or in combination, in particular in developing countries.

With regard to human studies, the emphasis should be on biomarker-based approaches. Biomarker-based studies in high-risk areas should include attempts to characterize the health issues common in individuals within communities where exposure is high, which can be compared with similar communities where exposure is low.

Experimental animal feeding studies should also use biomarker-based approaches and should be designed with multiple dose levels that reflect the levels of contamination seen in areas at high risk for co-exposure.

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APPENDIX 1

Countries/regions and foods with no co-occurrence of AFB_1 and FB_1 from both the GEMS/Food contaminants database and the literature

Data from the GEMS/Food contaminants database were evaluated for co-occurrence of AFB_1 and FB_1 in the same sample. There were nine countries that showed no co-occurrence. These countries, the foods analysed and number of samples are shown in Table A1.

Table A1
Countries with no co-occurrence of AFB_1 and FB_1 from the GEMS/Food contaminants database based on individual sample number

Country	Total no. of samples	Foods analysed (no. of samples)
Australia	21	Baked beans (4) Infant cereal mixed grain (4) Breakfast cereals single or mixed grains (8) Meat pie (4) Tap water (1)
Austria	144	Bread and other cooked cereal products (51) Cereal grains (54) Cereals and cereal-based products, nes (33) Maize (2) Rice (4)
Belgium	79	Food for infants and young children (27) Maize (40) Ready-to-eat meals for infants and young children (12)
Cyprus	25	Bread and other cooked cereal products (15) Barley (4) Maize (5) Pistachio nuts (1)
France	36	Bread and other cooked cereal products (7) Cereal grains (7) Cereals and cereal-based products, nes (5) Maize (10) Snack foods (4) White bread (1) Wholemeal bread (2)
Hungary	1	Sugar and confectionery (no further food details provided) (1)
Romania	2	Maize (2)
Spain	22	Bread and other cooked cereal products (5) Cereal-based foods for infants and young children (4) Dried fruit (4) Infant formula powder (1) Maize (1) Rice (7)
Sudan	450	Sorghum (450)

AFB_1: aflatoxin B_1; FB_1: fumonisin B_1; GEMS/Food: Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme; nes: not elsewhere specified

Studies from the literature on the co-occurrence of AFB_1 and FB_1 in foods found no co-occurrence. Some studies analysed more than one food. Some of those foods showed co-occurrence and some did not. Therefore, the foods from those studies that showed no co-occurrence are noted here. Many of these studies showed occurrence of either AFB_1 or FB_1 (usually FB_1), but not both. These studies are summarized in Table A2. These studies were from a number of countries and were mainly on cereals and cereal-based foods, including wheat, maize, rice, barley, malt, cereal-based beverages (traditional local beverages, beer), dried fruit, cassava, yam, peanuts and peanut products, and herbs and spices.

Table A2
Summary of studies from the literature that showed no co-occurrence of AFB_1 and FB_1

Country	Food	Number of samples	Reference
Belgium	White pepper	7	Yogendrarajah et al. (2014a)
	Black pepper	20	
	Dry chilli	35	Yogendrarajah et al. (2014b)
Benin	Cassava	200	Gnonlonfin et al. (2008)
	Yam	200	
Brazil	Peanuts	25	Gonçalez et al. (2008)
Cameroon	Peanuts	90	Ediage, Hell & De Saeger (2014)
	Cassava (flakes and chips)	165	
China	Plant oil	39	Sun et al. (2011)
	Peanuts	17	
	Maize	25	Feng et al. (2011)
Czech Republic	Barley	28	Bolechová et al. (2015)
	Malt	24	
Ecuador	Polished rice	46	Ortiz et al. (2013)
	Oat flakes	42	
	White wheat noodles	43	
	Yellow wheat noodles	37	
Japan	Buckwheat dried noodle	30	Sugita-Konishi et al. (2006)
	Corn, frozen or canned	50	
	Corn, raw	10	
	Corn flakes	20	
	Corn grits	10	
	Popcorn	10	
Jordan	Wheat, barley, rice, beans, chickpeas, fababean, green coffee, peanut, walnut, pistachio, hazelnut, sunflower seeds, sesame seeds	108	Salem & Ahmad (2010)
Republic of Korea	Rice, polished	47	Kim et al. (2013)
	Barley	43	
	Corn, raw	84	

Country	Food	Number of samples	Reference
Nigeria	Kunu-zaki (beverage) from maize	NS	Ezekiel et al. (2015)
	Pito (beverage) from sorghum	NS	
	Groundnut-based snacks	10	Kayode et al. (2013)
Portugal	Wheat	15	Fernandes et al. (2015)
	Maize	4	
	Rice	2	
Serbia	Corn	35	Matic et al. (2010)
	Soy products	15	
Slovenia	Wheat and wheat products	80	Kirinčič et al. (2015)
	Maize and maize products	69	
	Oat and oat products	24	
	Buckwheat and buckwheat products	24	
	Rice and rice products	17	
	Rye and rye products	17	
	Barley and barley products	11	
	Millet and millet products	6	
	Triticale	2	
	Other cereals and cereal products (baked goods, biscuits, snack foods, breakfast cereal)	40	
Spain	Gluten-free foods (pasta, bread, pastries)	18	Cano-Sancho et al. (2012)
Sri Lanka	White pepper	11	Yogendrarajah et al. (2014a)
	Dry chilli	86	Yogendrarajah et al. (2014b)
Syrian Arab Republic	Wheat	40	Alkadri et al. (2014)
Tunisia and Spain	Dates	75	Azaiez et al. (2015)
	Dried vine fruits	71	
	Figs	28	
	Apricots	27	
	Plums	27	
Tunisia	Sorghum	60	Oueslati et al. (2014)
Various	Herbs and spices (black pepper, basil, oregano, nutmeg, paprika and thyme)	300	Reinholds et al. (2017)
Various European countries	Beer	33	Bertuzzi et al. (2011)
Various (China, India, Japan, Malaysia, Myanmar, Pakistan, Thailand, Viet Nam)	Rice	199	Lim et al. (2015)

NS: not specified

A number of total diet studies worldwide have investigated the presence of mycotoxins. Many of them have shown no co-occurrence of AFB_1 and FB_1. This was the case for the Netherlands total diet study in 2013 (Lopez et al., 2016),

where 88 foods were sampled and analysed and no food group had detectable concentrations of AFB_1 (only peanuts had occurrence in two composite samples, but this was below the LOQ) and FB_1 (only detected in breakfast cereals, but this was also below the LOQ). In both the first (Leblanc et al., 2005) and second (Sirot, Fremy & Leblanc, 2013) French total diet studies, no co-occurrence of AFB_1 and FB_1 was found. In the first study, 78 composite samples of foods were analysed, including (but not limited to) biscuits, cereal products, desserts, nuts, oilseeds and vegetables. No AFB_1 – only FB_1 – was detected in all foods. Similarly, there were no detections of aflatoxins in the foods analysed, including bread and bread products, breakfast cereals, biscuits and bars, in the second study.

Of the 60 composite samples analysed across a range of foods (including cereal-based, nuts, legumes, seeds, vegetables, confectionery, meat, fats and oils, etc.) in the first total diet study in Hong Kong Special Administrative Region (FEHD, 2013), none contained both aflatoxins and fumonisins. A total diet study in India, which analysed AFB_1 and FB_1 in sorghum flour, groundnut oil, buffalo milk, butter milk and dried chilli, found AFB_1 only in groundnut oil (all samples), sorghum (17/22 samples) and dried chilli (18/22 samples); FB_1 was not detected in these foods (Polasa & Rao, 2013).

Total diet studies are conducted by analysing foods "as consumed", so they are prepared and/or cooked before analysis. Many of the raw commodities or unprocessed versions of the foods from which foods are based or derived have also been analysed in other studies in their raw state and reported in the main section of this report. The degree of co-occurrence in the raw and/or unprocessed versions is higher. Therefore, the processing, preparation and cooking of foods may influence the results. However, more total diet studies from a broader number of countries, including those from Africa and Asia, would be needed to confirm this assumption.

ANNEX 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).

2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).

3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).

4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).

5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).

6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).

7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).

8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).

9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).

10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.

16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.

17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.

19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.

20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.

21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.

22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.

26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants (Fifteenth report

of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.

28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.

43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.

46. Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.

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ANNEX 2

Abbreviations used in the monographs

3-AcDON	3-acetyldeoxynivalenol
3-MCPD	3-(mono)chloro-1,2-propanediol; α-(mono)-chlorohydrin
4,15-DAS	4,15-diacetoxyscirpenol
ADH	alcohol dehydrogenase
AF	aflatoxin
AFB_1	aflatoxin B_1
AFB_2	aflatoxin B_2
AFG_1	aflatoxin G_1
AFG_2	aflatoxin G_2
AFM_1	aflatoxin M_1
AFT	total aflatoxins
AIN	American Institute of Nutrition
ALDH	aldehyde dehydrogenase
ALP	alkaline phosphatase
ALT	alanine transaminase
AOAC	Association of Official Analytical Chemists
AOACI	Association of Official Analytical Chemists International
AOCS	American Oil Chemists' Society
APCI	atmospheric pressure chemical ionization
ARI	aflatoxin risk index
AST	aspartate transaminase
ATM	ataxia telangiectasia mutated
ATP	adenosine triphosphate
AUC	area under the concentration–time curve
AVF	averufin
AVN	averantin
a_w	water activity
BEA	beauvericin
BER	base-excision repair
BFU-E	erythroid burst forming unit
BMD	benchmark dose
BMD_{10}	benchmark dose for a 10% response
BMDL	lower 95% confidence limit on the benchmark dose
$BMDL_{10}$	lower 95% confidence limit on the benchmark dose for a 10% response

BMDS	Benchmark Dose Software (USEPA)
BMI	body mass index
BMR	benchmark response
BUN	blood urea nitrogen
bw	body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CerS	ceramide synthase
CerS2	ceramide synthase 2
CFU-GM	colony forming unit–granulocyte and macrophage
CFU-MK	colony forming unit–megakaryocyte
ChE	cholinesterase
CHO	Chinese hamster ovary
CI	confidence interval
CIFOCOss	FAO/WHO Chronic Individual Food Consumption Database – summary statistics
C_{max}	maximum concentration
CONTAM	[EFSA] Panel on Contaminants in the Food Chain
COX-1	cyclooxygenase-1
CpG	5′–C–phosphate–G–3′
CYP	cytochrome P450
ART	direct analysis in real time
DAS	diacetoxyscirpenol
DHPMA	2,3-dihydroxypropyl mercapturic acid
diHOPrVal	*N*-(1,2-dihydroxypropyl)valine
DMSO	dimethyl sulfoxide
DON	deoxynivalenol
DONALD	DOrtmund Nutritional and Anthropometric Longitudinally Designed Study
d-SPE	dispersive solid-phase extraction
EC	European Commission
EC_{50}	half maximal effective concentration
EER	estimated energy requirement
EFSA	European Food Safety Authority
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
EPEC	enteropathogenic *Escherichia coli*
ESI	electrospray ionization
EU	European Union
F	female

FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FAPAS	Food Analysis Performance Assessment Scheme
FB_1	fumonisin B_1
FEDIOL	European Union Vegetable Oil and Proteinmeal Industry Union
FFQ	food frequency questionnaire
FGF12	fibroblast growth factor 12
FID	flame ionization detector
FPIA	fluorescence polarization immunoassay
FusX	fusarenon X
GC	gas chromatography
GC-ECD	gas chromatography–electron capture detector
GC-FID	gas chromatography–flame ionization detection
GC-MS	gas chromatography–mass spectrometry
GD	guideline
GDH	glutamate dehydrogenase
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GGT	gamma-glutamyl transpeptidase; γ-glutamyltransferase
GLP	good laboratory practice
GPC	gel permeation chromatography
GSH	glutathione
GST	glutathione *S*-transferase
GSTM1	glutathione *S*-transferase M1
GSTP1	glutathione *S*-transferase P1
GST-P+	glutathione *S*-transferase placental form positive
GSTT1	glutathione *S*-transferase T1
GTP	green tea polyphenols
HACCP	hazard analysis and critical control point
HAZ	height-for-age *z*-score
HBsAg	hepatitis B virus surface antigen
HBsAg–	negative hepatitis B surface antigen
HBsAg+	positive hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HepG2	human hepatoma cells
HLA	human leukocyte antigen
HO-1	haem oxygenase-1
HPLC	high-performance liquid chromatography

HPLC-FD	high-performance liquid chromatography coupled with fluorescence detection
HPLC-MS	high-performance liquid chromatography coupled with mass spectrometry
HPLC-MS/MS	high-performance liquid chromatography coupled with tandem mass spectrometry
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HPTLC	high-performance thin-layer chromatography
HPTS	high-pressure thermal sterilization
HR-LC–Orbitrap–MS	high-resolution liquid chromatography Orbitrap mass spectrometry
HRMS	high-resolution mass spectrometry
HR–Orbitrap–MS	high-resolution Orbitrap mass spectrometry
HSCAS	hydrated sodium calcium aluminosilicate
HSP70	heat shock protein 70
IAC	immunoaffinity column
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
IFN-γ	interferon-gamma
Ig	immunoglobulin
IGF	insulin-like growth factor
IGFBP3	insulin-like growth factor–binding protein-3
IgM	immunoglobulin M
IITA	International Institute of Tropical Agriculture
IL	interleukin
i.p.	intraperitoneal
IUNA	Irish Universities Nutrition Alliance Survey
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
k_{cat}	turnover number
k_{el}	elimination rate constant
k_{val}	second-order rate constant
K_m	affinity (Michaelis) constant
LAZ	length-for-age *z*-score
LB	lower bound
LC	liquid chromatography

LC-EIA	liquid chromatography coupled with enzyme-linked immunoassay
LC-ESI-MS/MS	liquid chromatography electrospray ionization tandem mass spectrometry
LC-HRMS	liquid chromatography coupled with high-resolution mass spectrometry
LC-MS	liquid chromatography coupled with mass spectrometry
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
LC-MS/MS-IT	liquid chromatography coupled with tandem mass spectrometry with ion trap
LC-TOF	liquid chromatography coupled with time-of-flight mass spectrometry
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LLE	liquid–liquid extraction
LOAEL	lowest-observed-adverse-effect level
LOD	level of detection; limit of detection
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LPS	lipopolysaccharides
M	male
m/m	mass/mass
m/z	mass-to-charge ratio
MAS	monoacetoxyscirpenol
max.	maximum
MB	middle bound
MCT	medium-chain triglyceride
min	minute
min.	minimum
MIP	molecularly imprinted polymers
miRNA	microribonucleic acid
ML	maximum level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MW	molecular weight
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NEO	neosolaniol
nes, Nes	not elsewhere specified
NIR	near-infrared (spectroscopy)

NIV	nivalenol
NK	natural killer
NMBzA	*N*-methyl-*N*-benzylnitrosamine
NMR	nuclear magnetic resonance
no. / No.	number
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NOR	norsolorinic acid
NR	not reported
NS	not significant
NSP	nanosilicate platelets
NT	not tested
NTD	neural tube defect
NTP	National Toxicology Program
NZW	New Zealand White
OECD	Organisation for Economic Co-operation and Development
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OEHHA	Office of Environmental Health Hazard Assessment
OMSTC	*O*-methylsterigmatocystin
OR	odds ratio
OTA	ochratoxin A
P	probability
PAH	polycyclic aromatic hydrocarbon
PAPAR	poly (ADP)-ribose
PARP	poly (ADP-ribose) polymerase
PBA	phenylboronic acid
PFC	plaque forming cell [assay]
PMTDI	provisional maximum tolerable daily intake
PPARα	peroxisome proliferator–activated receptor-α
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PTEC	porcine tracheal epithelial cells
qPCR	quantitative polymerase chain reaction
QTL	quantitative trait locus
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
RAP	resistance-associated protein
RBC	red blood cell
RIVM	Dutch National Institute for Public Health and the

	Environment
RNA	ribonucleic acid
RNAi	RNA interference
rpm	revolutions per minute
RTE	ready-to-eat
S9	9000 × *g* supernatant fraction from liver homogenate
S15	15 000 × *g* supernatant fraction from liver homogenate
Sa/So	sphinganine/sphingosine [ratio]
SAR	Special Administrative Region
SCE	sister chromatid exchange
SCFA	short-chain fatty acids
SCOOP	Scientific Co-operation on Questions relating to Food
SCP	scirpentriol
SD	standard deviation
SES	socioeconomic status
SGPT	serum glutamic–pyruvic transaminase
sLPS	*Salmonella typhimurium* lipopolysaccharide
SPE	solid-phase extraction
SRBC	sheep red blood cells
STC	sterigmatocystin
T	trichothecane
$t_{½}$	half-life
$t_{½\ \mathrm{initial}}$	initial plasma elimination half-life
T_3	triiodothyronine
TAS	triacetoxyscirpenol
TCA	tricarballylic acid
[^{3}H]TdR	tritiated thymidine
TIBC	total iron-binding capacity
tk	thymidine kinase
TLC	thin-layer chromatography
TLC 2D	two-dimensional thin-layer chromatography
TLR	toll-like receptor
T_{max}	time to reach the maximum concentration
TNF-α	tumour necrosis factor-α
TOFMS	time of flight–mass spectrometry
TSH	thyroid-stimulating hormone
$UAFM_1$	urinary aflatoxin M_1
UFB_1	urinary fumonisin B_1
UFLC	ultra-fast-pressure liquid chromatography
UHPLC	ultra-high-performance liquid chromatography

UHPLC/Q-TOF-MS	ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry
UHT	ultra-heat-treated
UNU	United Nations University
UPLC	ultra-performance liquid chromatography
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
UVA	ultraviolet A (radiation from about 320 to 400 nm in wavelength)
VAL	versiconal
V_{max}	maximum velocity
w/v	weight per volume
w/w	weight per weight
WAZ	weight-for-age *z*-score
WBC	white blood cell
WFP	World Food Programme
WHO	World Health Organization
WHZ	weight-for-height *z*-score
XRCC	X-ray repair cross-complementing

ANNEX 3

Joint FAO/WHO Expert Committee on Food Additives[1]

Rome, 8–17 November 2016

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This volume contains monographs prepared at the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 8 to 17 November 2016.

The detailed monographs in this volume summarize the technical, analytical, dietary exposure and toxicological data on a number of contaminants: aflatoxins; 4,15-diacetoxyscirpenol; fumonisins; glycidyl esters; 3-MCPD esters and 3-MCPD; and sterigmatocystin, as well as on co-exposure of fumonisins with aflatoxins.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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