TOX/2020/44 Annex B

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Additional data regarding UK specific mycotoxin biomonitoring data and mode of action toxicity mechanisms for single mycotoxins

Overview of mycotoxin families and their associated mycotoxins

As described in paragraph 3 of the cover page, a detailed overview of all mycotoxin families previously covered in the scope of TOX/2017/30[1] has been tabulated in the attached Annex.

The collated information incudes; their associated mycotoxins, the species of fungus that produces them, their mode of action, key toxicological endpoints, as well as their recommended health-based guidance values as set by authoritative bodies such as the European Food Safety Authority (EFSA), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Scientific Committee on Food (SCF), World Health Organisation (WHO), National Institute for Public Health and the Environment (RIVM), Federal Institute for Risk Assessment (BfR), Committee for Medicinal Products for Veterinary Use (CVMP), and French Food Safety Agency (AFSSA).

It is hoped that the gathered data will be able to assist the Committee in the grouping of mycotoxins based on their mode of action or toxic endpoints.

Secretariat
September 2020

[1] TOX/2017/30 scoping paper available on the COT website.

Table 1 provides an overview of the Aflatoxins family and its associated mycotoxins; aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2. These mycotoxins are produced by *Aspergillus flavus*, *nomius* and *parasiticus*. They do not have an associated health-based guidance value(s) since the mycotoxins in this family are genotoxic and carcinogenic. The mode of action and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative report	Mode of action	Endpoint	Key Study
AFB_1 **AFB_2** **AFG_1** **AFG_2**	SCF (1996)	Forms adducts – mechanism not further detailed.	Hepatotoxicity – leading to liver carcinogenicity.	Various, refer to report link.
	JECFA (1998)	Formation of DNA adducts on the N7 position of the guanine nucleotide cause by AFB_1-8,9-epoxide.		
	JECFA (2002) AFM_1	Since AFM_1 is a metabolite of AFB_1, it is presumed to induce liver cancer in rodents by a similar mechanism to its parent compound.	Hepatotoxicity – leading to liver carcinogenicity, a NOEL was determined at 0.1 mg total intake over 21 months (male Fischer rat study).	Cullen *et al*., (1987)
	JECFA (2007) JECFA (2016)	Formation of DNA adducts on the N7 position of the guanine nucleotide cause by AFB_1-*exo*-8,9-epoxide.	Hepatocarcinogenic.	Wogan *et al*., (1974)
	EFSA (2007) AFB_1		Hepatotoxicity – leading to liver carcinogenicity, a $BMDL_{10}$ of 0.17 µg/kg bw/day was utilised for Margin of Exposure calculations (male Fischer rat study).	

Abbreviations: AFB_1 = Aflatoxin B_1; AFB_2 = Aflatoxin B_2; AFG_1 = Aflatoxin G_1; AFG_2 = Aflatoxin G_2; HBGV = Health-based guidance value; NOEL = No observed effect level, BMDL10 = Benchmark dose level, 10%.

Table 2 provides an overview of Ochratoxin A, which is produced by the *Penicillium* spp. and *Aspergillus* spp. families. Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoint are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (ng/kg)	Endpoint	Key Study
OTA	SCF (1996)	Not detailed.	TDI; 0.2-16 ng/kg bw/day, provisionally supports the lower range (*i.e.* 0.2-5).	Potential genotoxicity on the lower range, whilst the highest is nephrotoxicity.	Refer to report, range is based on other estimates of TDIs by other authoritative bodies.
	SCF (1998)	Remains to be established whether DNA-adducts represents direct, covalent binding of OTA metabolites or represent secondary based changes due to indirect mechanisms.	TDI; 1.2-14 ng/kg bw/day but preferably below 5 ng/kg bw/day.		
	JECFA (2001) JECFA (2007)	Summarised as follows: genotoxicity from direct interaction of OTA or a reactive metabolite with DNA; generation of tumours secondary to chronic renal toxicity and compensatory cell proliferation or inhibition of phenylalanine-tRNA Phe synthetase and protein synthesis; disruption of cell-cell signalling pathways and process of cell division; alternation of intracellular calcium homeostasis and; mitochondrial dysfunction leading to oxidative stress and indirect induction of DNA damage.	PTWI; 100 ng/kg bw.	Nephrotoxicity; LOEL 8 µg/kg bw/day representing an early marker of renal toxicity in female pigs.	Cumulative studies by Krogh & Elling groups (1977-1988), where effects on enzymes and kidney function were not examined in the 2-year study; however, from these studies the LOEL of 8 µg/kg for effects on the kidneys was established by JECFA in their 2001 evaluation.
	EFSA (2006) EFSA (2010)	Genotoxic mode of action is still a matter of debate.	Initially a TDI of 18 ng/kg bw, however, considering the long half-life of OTA in humans a TWI of 120 ng/kg bw was considered to be more appropriate. The PTWI of 120 ng/kg by was retained in 2010 evaluation.	Nephrotoxicity; LOEL 8 µg/kg bw/day representing an early marker of renal toxicity in female pigs (total uncertainty factor applied was 450).	

Abbreviations: OTA = Ochratoxin A; DNA = Deoxyribonucleic acid; HBGV = Health-based guidance value; TDI = Tolerable daily intake; PTWI = Provisional tolerable daily intake; LOEL = Lowest observed effect level.

Table 3 provides an overview of Patulin which is produced by *Aspergillus* spp. and *Penicillium* spp families including; *A. clavatus*, *P. expansum*, *P. patulum*, *P. aspergillus*, *P. byssochlamys*, and *P. expansum.* Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
PAT	JECFA (1990) JECFA (1995)	Ability to bind to sulph-hydryl groups of various enzymes.	PTWI; 7 µg/kg bw	Combined reprotoxicity, long-term toxicity/carcinogenicity study. NOEL; 0.1 mg/kg bw/day in Wistar rats.	Becci *et al*., (1981)
	SCF (1996) SCF (2000)		PMTDI; 0.4 µg/kg bw	Combined reproductive toxicity, long-term toxicity/carcinogenicity study in Wistar rats. NOEL; 0.1 mg/kg bw (administered 3 times weekly; equivalent to 43 µg/kg bw/day), with an application of UF of 100.	

Abbreviations: PAT = Patulin; HBGV = Health-based guidance value; PTWI = Provisional tolerable daily intake; PMTDI = Provisional maximum tolerable daily intake; NOEL = No observed effect level.

Table 4 provides an overview of the Type A trichothecene family and its associated mycotoxins; T-2 and HT-2 toxins. These mycotoxins are produced by *Fusarium* spp. including; *F. sporotrichoides*, *F. poae*, *F. equiseti*, *F. acumninatum*, or *Cephalosporium, Verticimonosporum, Trichoderma, Trichothecium* and *Stachybotrys* which are other crop invasive species. The mode of action and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
T-2	SCF (2001) SCF (2002)	Inhibition of protein synthesis by biding to the ribosome, the inhibitory effect on RNA and DNA synthesis, and toxic effect on cell membranes.	Combined t-TDI; 0.06 µg/kg bw	Sub-acute (3 weeks) (leukopenia/reduced antibody production) LOEL; 0.029mg/kg bw/day in pigs. Applied UF of 500.	Rafai *et al.*, (1995)
HT-2	JECFA (2001)	T-2 toxin was considered a potent inhibitor of protein synthesis both *in vivo* and *in vitro* (interaction with the peptidyl transferase centre on the 60S ribosomal unit and inhibited transpeptidation of peptide-bond formation).	PMTDI; 0.06 µg/kg bw		
	EFSA (2017)	Based on similar toxic profile and potency and HT-2 is an immediate metabolite of T2, EFSA concluded that T2 and HT-2 can be included in a group ARfD.	ARfD; 0.3 µg T2 or HT2/kg bw	Acute (emesis); $BMDL_{10}$ of 2.97 µg/kg bw/day calculated for emetic effects in mink for both T2 and HT2 toxins. UF of 10 was applied.	Wu *et al.*, (2016)

Abbreviations: T-2 = T-2 toxin; HT-2 = HT-2 toxin; HBGV = Health-based guidance value; t-TDI = Temporary tolerable daily intake; PMTDI = Provisional maximum tolerable daily intake; ARfD = Acute reference dose; LOEL = Lowest observed effect level; $BMDL_{10}$ = Benchmark dose level, 10%; UF = Uncertainty factor; RNA = ribonucleic acid; DNA = deoxyribonucleic acid.

Table 5 provides an overview of the Type A trichothecene family and its associated mycotoxin; 4,15-diacetoxyscirpenol. This mycotoxin is produced by species from the *Fusarium* spp. family. Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports[2]	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
4,15-DAS	JECFA (2017)	Inhibits the initial step of protein synthesis. The target organelle is the 60S subunit of eukaryotic ribosomes within inhibition of peptidyl transferase.	PMTDI; 60 ng/kg bw per day for 4,15-DAS, T-2 and HT-2 toxins, alone or in combination.	4,15-DAS and T-2/HT-2 toxins 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.	Refer to JECFA report.
	EFSA (2018)	Binding to ribosomes, inducing a 'ribotoxic stress response' with activation of ribosome associated MAPKs and inhibition of protein synthesis. Further possesses, cytotoxic properties, no clear indication for ROS production are available. Increase in CCK levels considered the mechanism of DAS (and tricothecenes) induced anorexia. *In vitro* assays indicated cytotoxic properties on haemopoietic progenitors which could be due to stimulation of apoptosis or inhibition of protein synthesis.	ARfD; 3.2 µg/kg bw. TDI; 0.65 µg/kg bw.	Emesis. NOAEL 32 µg/kg bw (equivalent to 1.2 mg/m² (i.v. administration of phase I clinical trials of Anguidine). NOAEL of 65 µg/kg bw for haematotoxicity and myelotoxicity based on Phase I clinical trials of Anguidine (cytostatic anticancer drug). Reported health effects at doses from 3-5 mg/m² from Phase II clinical trials (equivalent to 81-135 µg/kg bw.	Murphy *et al.*, (1978)

Abbreviations: 4,15-DAS =4,15-diacetoxyscirpenol; HBGV = Health-based guidance value; PMTDI = Provisional maximum tolerable daily intake; ARfD = Acute reference dose; TDI = Temporary tolerable daily intake; NOAEL = No observed adverse effect level; i.v = intravenous. MAPK = Mitogen-activated protein kinase; ROS = Reactive oxygen species; CCK = Cholecystokinin.

[2] Note that the COT has reviewed the EFSA 2018 report in 2018. The COT agreed with the EFSA establishment of an ARfD for DAS. The discussion paper can be found on the COT website.

Table 6 provides an overview of the Type B trichothecene family and its associated mycotoxin; Deoxynivalenol (and its acetylated metabolites). This mycotoxin is produced by species from the *Fusarium* spp. family. Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
DON (15-Ac DON and 3-Ac DON naturally occurring metabolites)	SCF (1999) SCF (2002)	Inhibits the synthesis of DNA and RNA and protein synthesis at the ribosomal level. Toxin has a haemolytic effect on erythrocytes and lymphocytes.	t-TDI; 1 µg/kg bw	Chronic (growth retardation) NOAEL; 0.1 mg/kg bw day in mice and an uncertainty factor of 100.	Iverson *et al*., 1995
	JECFA (2001)		PMTDI; 1 µg/kg bw for DON and acetylated forms.		
	JECFA (2011)	Toxicological studies in mice, rats and pigs provided new insight in MoA of DON in causing reduced weight gain; due to the induction of suppressors of cytokine signalling and to effects on the pituitary GH axis.	ARfD; 8 µg/kg bw for DON and acetylated, however, limited data from human case reports indicate that dietary exposures of up to 50 µg/kg bw/day are not likely to induce emesis.	Acute (emesis); $BMDL_{10}$ of 0.21 mg/kg bw/day calculated for emetic effects in pigs for both DON and its acetylated derivatives.	Young *et al*., (1983); Pollman *et al*., (1985)
	EFSA (2017)	Binds to ribosomes, leading to inhibition of protein synthesis and thus RNA and DNA synthesis. This binding further induces ribotoxic stress and activates MAPKs to cause cellular apoptosis, inflammation and oxidative stress.	TDI; 1 µg/kg bw ARfD; 8 µg/kg bw for DON and acetylated forms.	Chronic (growth retardation) NOAEL; 0.1 mg/kg bw day in mice and an uncertainty factor of 100. Epidemiological data from mycotoxicoses NOAEL of 26 µg DON/kg bw per eating occasion for vomiting (default uncertainty factor of 3.16 for toxicokinetic differences in the human population was needed).	Iverson *et al*., (1995) with the support of Bondy *et al*., (2016) Luo *et al*., (1987)

Abbreviations: DON = Deoxynivalenol; 15-AcDON = 15-acetyldeoxynivalenol; 3-AcDON = 3-acetyldeoxynivalenol; HBGV = Health-based guidance value; -t-TDI = Temporary tolerable daily intake; TDI= Tolerable daily intake; PMTDI = Provisional maximum tolerable daily intake; ARfD = Acute reference dose; NOAEL = No observed adverse effect level; $BMDL_{10}$ = Benchmark dose level, 10%; RNA = ribonucleic acid; DNA = deoxyribonucleic acid; GH = growth hormone; MoA = Mode of action; MAPKs = Mitogen-activated protein kinase.

Table 7 provides an overview of the Type B trichothecene family and its associated mycotoxin; Nivalenol and Fusarenon-X. Nivalenol is produced by *Fusarium* spp species including; *F. crookwellense*, *F. poae*, *F. culmorum* and *F. graminearum*, whilst Fusarenon-X in addition can also be produced by *F. nivale and F. equiseti*. Their mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
NIV	SCF (2000)	The mechanism of nucleic inhibition is not known.	t-TDI; 0.7 µg/kg bw	Chronic (growth retardation) LOAEL; 0.7 mg/kg bw/day in mouse studies 1 and 2 years.	Ohtsubo *et al*., (1989); Ryu *et al*., (1988)
	RIVM (2002)	Inhibition of the initial step of protein synthesis.			
	VKM (2013)	Binds to the 60s ribosomal subunit and inhibit protein synthesis with the activity of the enzyme peptidyl transferase.			
	EFSA (2013)		TDI; 1.2 µg/kg bw	Immunological differences (decrease in WBC) $BMDL_{05}$; 0.35 mg NIV/kg bw/day for haematological disturbances in WBC in rats. Application of 300 as an uncertainty factor.	Takahashi *et al*., (2008)
FUS-X	RIVM (2002)	Yet to be fully elucidated, however, it is generally accepted and known to evoke a ribotoxic response; targeting the 60s subunit organelle of eukaryotic organelles. FUS-X binds to peptidyl transferase and causes inhibition of the initiation of protein synthesis.	Unable to establish a temporary TDI due to data insufficiencies.	Acutely toxic (oral); LD_{50} 4.4 mg kg/bw in rats and 4.5 mg/kg bw in mice. Fus-x ribotoxic, actively targets organs that contain actively proliferating cells (*e.g.* thymus, spleen, small intestine, testes and bone marrow).	Ueno *et al*., (1983); Ueno *et al*., (1984)
	COT (2019)		Comparison with the HBGV for DON (ARfD; 8 µg/kg bw) was considered appropriate since the oral emetic potency of Fus-X relative to DON is 1.04. Comparative toxicity data suggests that it is more toxic than	Acute (emesis); $BMDL_{10}$ of 0.21 mg/kg bw/day calculated for emetic effects in pigs for both DON and its acetylated derivatives. Epidemiological data from mycotoxicoses NOAEL of 26 µg	Young *et al*., (1983); Pollman *et al*., (1985) Luo *et al*., (1987)

			other type B trichothecenes (DON and acetylated forms and NIV) when administered orally, however, had lower emetic potency than Type A.	DON/kg bw per eating occasion for vomiting (default uncertainty factor of 3.16 for toxicokinetic differences in the human population was needed).	

Abbreviations: NIV = Nivalenol; FUS-X = Fusarenon=X; HBGV = Health-based guidance value; -t-TDI = Temporary tolerable daily intake; TDI= Tolerable daily intake; LOAEL = Lowest observed adverse effect level; WBC = White blood cells; $BMDL_{05}$ = Benchmark dose level, 5%; LD_{50} = Lethal dose, 50%; $BMDL_{10}$ = Benchmark dose, 10%.

Table 8 provides an overview of Zearalenone, its respective limits in cereal based food commodities; in accordance to Regulation (EC) 1881/2006 (and its amendments). Zearalenone is produced my species of the Fusarium spp. family including; *F. graminearum, F. culmorum, F. equiseti* and *F. verticillioides*. Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
ZEN	JECFA (1998)	Was shown to be a weak oestrogen in long-term studies in mice, rats, dogs, and monkeys.	ADI; 0-0.5 µg/kg bw (Note: this is for the metabolite α-zearalenone).	Level causing no hormonal effect in monkeys (ovariectomised female cynomolgus monkeys); 0.5 mg/kg bw/day.	Singh *et al*., (1984); CIC, (1985)
	JECFA (2000)	Have been shown to bind activate both α- and β oestrogen receptors.	PMTDI; 0.5 µg/kg bw	No hormonal effects in pigs (15 days study in pigs), most sensitive species. NOEL 40 µg/kg bw/day.	Edwards *et al*., (1987)
	SCF (2000)		t-TDI; 0.2 µg/kg bw	No hormonal effects in pigs (15 days study in pigs), most sensitive species. NOEL 40 µg/kg bw/day, safety factor of 200.	Bauer *et al*., (1987)
	EFSA (2011) EFSA (2016)	Oestrogenic activity; binds to both α- and β oestrogen receptor, with a higher affinity to the former sub-type.	Group TDI of ZEN and its modified forms; 0.25 µg/kg bw/day	EDC (pituitary adenomas) in male B6C3F1 mice. $BMDL_{10}$ of 6.39 mg/kg bw/day. NOEL 10.4 µg/kg bw/day.	NTP (1982); Dölll *et al*., (2003)

Abbreviations: ZEN = Zearalenone; HBGV = Health-based guidance value; ADI = Acceptable daily intake; PMTDI = Provisional maximum daily intake; t-TDI = Temporary tolerable daily intake; TDI = Tolerable daily intake; NOEL = No observed effect level; EDC = Endocrine; $BMDL_{10}$ = Benchmark dose level, 10%; CIC = Coulston International Corp; NTP = National Toxicology Program.

Table 9 provides an overview of the *Fumonisins* family and its associated mycotoxins; fumonisin B_1, fumonisin B_2, and their respective limits in cereal based food commodities; in accordance to Regulation (EC) 1881/2006 (and its amendments). These mycotoxins are produced by Fusarium spp species including; *F. verticillioides*, *F. proliferatum*, *F. fujikuroi*, *F. anthophilum*, *F. dlamini*, *F. napiforme* and *F. thapsinum*. Their mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key study
FB_1 **FB_2**	JECFA (2001)	Two proposed mechanisms; inhibition of ceramide synthase (key enzyme in the biosynthesis of synthesis sphingolipids and changes in the polyunsaturated fatty acid and phospholipid pools.	PMTDI; 2 µg/kg bw for FB_1, FB_2 and FB_3, alone or in combination.	Nephrotoxicity (for FB1); NOEL for renal toxicity in Fischer 344N rats as 0.2 mg/kg bw/day with a safety factor of 100.	Howard *et al*., (2001) for chronic rat study; Hard *et al*., (2001) for re-evaluation of renal tumours.
	JECFA (2012) JECFA (2017)	Disruption of lipid metabolism as a consequence of inhibition of ceramide synthases (sphingoid based *N*-acyltransferases), key enzymes in the *de novo* sphingolipid biosynthesis pathway.		Hepatotoxicity; $BMDL_{10}$ of 0.165 mg/kg bw/day calculated for megalocytic hepatocytes in mice (uncertainty factor 100).	Bondy et al., (2012)
	EFSA (2018)	Inhibition of ceramide synthase.	TDI: 1 µg/kg bw; group TDI with all subtypes (FB_{1-4}) based on assessment for structural similarity.	Hepatoxocixity; $BMDL_{10}$ of 0.1 mg/kg bw/day calculated for megalocytic hepatocytes in mice (uncertainty factor 100).	

Abbreviations: FB_1 = Fumonisin B_1; FB_2 = Fumonisin B_2; HBGV = Health-based guidance value; PMTDI = Provisional maximum tolerable intake; TDI = Tolerable daily intake, NOEL = No observed effect level; $BMDL_{10}$ = Benchmark dose level, 10%;

Table 10 provides an overview of Citrinin and its respective limits in food supplements based on rice fermented with red yeast (*Monascus purpureus*); in accordance to Regulation (EC) 1881/2006 (and its amendments). This mycotoxin is produced by species of *Aspergillus* spp., *Penicillium* spp. and *Monascus* spp. The mode of action is described. It does not have a recommended health-based guidance value, however a level of no concern based on nephrotoxicity was established based on the European Food Standards Authority (EFSA) 2012 review.

Mycotoxin	Authoritative reports	Mode of action	HBGV (µg/kg)	Endpoint	Key Study
CIT	EFSA (2012)	The EFSA CONTAM Panel concluded that citrinin toxicity is exerted *via* multiple pathways such as DNA and RNA synthesis inhibition, inhibition of microtubule assembly and of tubulin polymerisation, alteration of mitochondrial functionality with consequent increase in ROS and activation of the signal transduction pathway and the caspase-cascade system that results in apoptotic cell death.	No HBGV was set, although, a level of no concern for nephrotoxicity of 0.2 µg/kg bw/day was established.	Nephrotoxicity; NOAEL 20 µg/kg bw/day in rats (sub-chronic; 90-day), UF of 100.	Lee *et al*., (2010)

Abbreviations: CIT = Citrinin; HBGV = Health-based guidance value; NOAEL = No observed adverse effect level; UF = Uncertainty factor; CONTAM = Contaminants in the Food Chain; RNA = Ribonucleic acid; DNA = Deoxyribonucleic acid; ROS = Reactive oxygen species.

Table 11 Provides an overview of the Ergot alkaloid family and its 12 associated mycotoxins and their respective limits in cereal based food commodities; in accordance to Regulation (EC) 1881/2006 (and its amendments). These mycotoxins are produced by species from the *Claviceps* spp. family including; *C. Purpurea*, *C. fusiformis*. Their recommended health-based guidance values (HBGV) are also provided based on various authoritative reviews.

Mycotoxin	**Authoritative reports**	**Mode of action**	**HBGV (µg/kg)**	**Endpoint**	**Key Study**
Ergots considered as a sum of all twelve mycotoxins): Ergocristine, Ergotamine, Ergocryptine (α and β forms), Ergometrine, Ergosine, Ergocornine and their respective -inine forms. Note: -inine forms are described to be biologically inactive on the neuroreceptor sites, however, interconversion can take place in alkaline or acidic conditions.	WHO (1990)	Not described.	None set. It was concluded that human exposure to low levels of ergolines appears to be widespread. Outbreak data in Ethiopia and India indicate that *C. purpurea* alkaloids (*i.e.* ergotamine group) produced more severe effects. Highlighted that only low levels of ergolines remain in prepared foods as cleaning and milling processes remove the sclerotia; additionally, heat processing denatures/destroys most alkaloids of the ergotamine group.	N/A	N/A
	CVMP (1999)	Not described.	In human medicine usual oral doses are 500 µg; 3 times a daily or up to (1.8 mg daily ~0.03 mg/kg bw).	N/A	N/A
	BfR (2004)	Report in German: Adverse effects associated with lysergic acid derivatives. These have structural similarities with norepinephrine, dopamine and serotonin. Also exhibits inhibition of prolactin secretion from the anterior pituitary gland.	Report in German: Advises for pregnant and breastfeeding women to avoid consumption of rye bread which contains ~2,000-3,000 µg/kg. Other adult age groups not expected to observe adverse/fatal effects till 5-10 g fresh intake of ergot.	N/A	N/A

	AFSSA (2009)	Report in French: Causes stimulation of smooth muscles by inhibiting α and β adrenergic receptors.	Report in French: Doesn't seem to include any hard limits. Just sets out as it is in the regulation†.	N/A	N/A
	EFSA (2012)	Ergot alkaloids act on a number of neurotransmitter receptors, particularly adrenergic, dopaminergic and serotonergic receptors.	Group ARfD; 1 µg/kg bw for the sum of ergot alkaloids. TDI; 0.6 µg/kg bw for the sum of ergot alkaloids.	Tail muscular atrophy (13-week rat feeding study of ergotamine). $BMDL_{10}$ 0.33 mg/kg bw/day, uncertainty factor of 3. Tail muscular atrophy (13-week rat feeding study of ergotamine). $BMDL_{10}$ 0.33 mg/kg bw/day, uncertainty factor of 600.	Spieijers *et al*., (1993)

Abbreviations: HBGV = Health-based guidance value; ARfD = Acute reference dose; TDI = Tolerable daily intake; $BMDL_{10}$ = Benchmark dose limit; 10%.

Table 12 provides an overview of Cyclopiazonic acid (CPA). It does not currently have a regulatory limit as set in Regulation (EC) 1881/2006 (and its amendments), nor does it have a recommended health-based guidance value (HBGV). CPA is produced by species from the *Aspergillus* spp. and *Penicillium* spp. families. The Committee of Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) have reviewed the compound in 2019 and concluded that it does not pose a health concern for infants aged 0 to 12 months and children aged 1 to 5 years.

Mycotoxin	Authoritative reports	Mode of action†	HBGV	Endpoint	Key Study
CPA	COT (2019)	Potent, specific, and reversible inhibitor of the sarcoplasmic and endoplasmic reticulum Ca^{2+}-activated ATPases.	No risk assessments or evaluations of CPA by European or other international authoritative bodies. COT MOEs ranged from 4,500-100,000.	NOAEL of 0.1 mg/kg bw/day (sub-acute; 90 days) study in dogs (unknown provenance).	Nuehring *et al*., (1985)

† Burdock, G. A. & Flamm, W. G. (2000) Review article: Safety assessment of the mycotoxin cyclopiazonic acid. International Journal of Toxicology 19, pp. 195-218.

Abbreviations: CPA = cyclopiazonic acid; ATP = Adenosine triphosphate; HBGV = Health-based guidance values; MOE = Margin of Exposure; NOAEL = No observed adverse effect level.

Table 13 provides an overview of Moniliformin (MON). It does not currently have a regulatory limit as set in Regulation (EC) 1881/2006 (and its amendments), nor does it have a recommended health-based guidance value. This mycotoxin can be produced by species from the *Fusarium* spp. and *Penicillium melanoconidium*. Its mode of action, recommended health-based guidance value (HBGV) and key toxicological endpoints are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports[3]	Mode of action	HBGV	Endpoint	Key Study
MON	EFSA (2018)	Mode of action is unclear. MON and pyruvate show structural similarity and the primary mode of action seems to be the inhibition of thiamine pyrophosphate-dependent enzymes, which compromises the tricarboxylic acid cycle.	Unable to establish a HBGV due to the limitations in the available data.	Acute (cardiotoxicity): NOAEL 6 mg/kg bw sub-acute study in rats. Chronic (haematoxicity): 28-day study in barrow pigs. $BMDL_{05}$ 0.20 mg/kg bw from the dose-response data on the decrease in haematocrit and haemoglobin = POD for MOE.	Johnsson *et al*., (2013) Jonsson *et al*., (2015)

Abbreviations: MON = Moniliformin; HBGV = Health-based guidance value; NOAEL = No observed adverse effect level; $BMDL_{05}$ = Benchmark dose level, 5%; POD = Point of Departure; MOE = Margin of Exposure.

[3] Note that the COT has reviewed the EFSA 2018 report in 2019, the COT agreed with the MOE approach taken by EFSA for assessing the human health risk of MON. The discussion paper can be found on the COT website.

Table 14 provides an overview of Neosolaniol (metabolite of T-2 toxin; Type A trichothecene) the mode of action is as described for the parent metabolite in *Table* 4. Its recommended health-based guidance values and key toxicological endpoint are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports[4]	HBGV (µg/kg)	Endpoint	Key Study
NEO	SCF (2002)	PMTDI for T2- and HT-2 (only); 0.06 µg/kg bw	Subacute (3 weeks) (leukopenia/reduced antibody production) LOEL; 0.029mg/kg bw/day in pigs. Applied UF of 500.	Rafai *et al.*, 1995
	JECFA (2001)	PMTDI for T2- and HT-2 (only); 60 ng/kg bw/day, alone or in combination.	LOEL of 0.029 mg/kg bw per day for changes in white and red blood cell counts identified in the 3-week dietary study in pigs.	
	EFSA (2011)	Group TDI; 1µg/kg bw (T2; x1, HT-2; x1, NEO; x0.3)	Immunological differences (reduction in antibody response to a specific antigen in pigs). LOAEL; 29 µg/kg bw/day NOAEL = $BMDL_{05}$ 10 µg/kg bw/day derived for T2 (uncertainty factor of 100).	
	EFSA (2017)	Group ARfD; 0.3 µg/kg bw	Acute (emetic effects in mink): $BMDL_{10}$-$BMDU_{10}$ of 2.97-49.8 µg/kg bw T2 or HT-2.	Wu *et al.*, (2016)
		Group TDI; 0.02 µg/kg bw (T2; x1, HT-2; x1, NEO; x0.3)	$BMDL_{10}$; 3.33 T2 µg/kg bw/day for reduction in the number of peripheral leucocytes in sub-chronic study in rats, uncertainty factor 200.	Rafai *et al.*, (1995) (total leucocyte count); Rahman *et al.*, (2014) (total leucocyte, thrombocyte, haem counts and body weight effects).

Abbreviations: NEO = Neosolaniol; HBGV = Health-based guidance value; PMTDI = Provisional maximum tolerable daily intake; TDI = Tolerable daily intake; ARfD = Acute reference dose; LOEL = Lowest observed effect level; LOAEL = lowest observed adverse effect level; NOAEL = No observed adverse effect level; $BMDL_{05}$ = Benchmark dose level, 5%; $BMDL_{10}$ = Benchmark dose level, 10%; $BMDU_{10}$ = Benchmark dose upper, 10%.

[4] Note that the COT has reviewed the above authoritative reports in 2018. In this, the exposure assessment; exceed EFSA TDI from 145 – 315% for infants and young children, however, unlikely that they are regularly exposed to these levels. Therefore, unlikely that dietary exposure levels of T2, HT-2 and NEO would be of any toxicological concern. Further details of the review can be found on the COT website.

Table 15 provides an overview of Sterigmatocystin (STC). It does not currently have a recommended health-based guidance value. It is produced by species from the *Aspergillus* spp. family including; *A. flavus*, *A. parasiticus*, *A. versicolor* and *A. Nidulans*. *A. versicolor* is the most common producer. STC also shares the same biosynthetic pathway with aflatoxins (STC is a pre-cursor; in aflatoxin samples it is possible to have traces of STC). Its mode of action and key toxicological endpoint are described based on reviews by various authoritative bodies.

Mycotoxin	Authoritative reports[5]	Mode of action	Endpoint	Key Study
STC	EFSA (2013)	Upon metabolic activation, forms N7-guanyl DNA adducts which are likely to be responsible for the mutagenic effects. Induces cytotoxicity, inhibition of cell cycle and mitosis, as well as increase formation of reactive oxygen species and lipid peroxidation *in vivo.*	$BMDL_{10}$; 0.16 m/kg bw/day based on the incidence of haemangiosarcomas in rats (based on limited tumorgenicity data).	Maekawa *et al*., (1979)
	JECFA (2016)	*Exo*-sterigmatocystin-1,2-oxide is the metabolite reacts with DNA and thus forms adducts.		

Abbreviations: STC = Sterigmatocystin; HBGV = Health-based guidance value; $BMDL_{10}$ = Benchmark dose level.

[5] Note that the COT has reviewed the above authoritative reports in 2019. In this, the mean and 97.5th percentile margin of exposure values for UK infants and young children, based on the BMDL10 of 0.16 mg/g bw per day, are all > 10,000. Therefore, the exposures are unlikely to be of toxicological concern. Further details of the review can be found on the COT website.

References

Bauer, J., Heinritzi, K., Gareis, M. & Gedek, B. (1987) Veränderungen am Genitaltrakt des weiblichen Schweines nach Verfütterung praxisrelevanter Zearalenonmengen. Tierärztl. Prax. 15, 33-36.

Becci, P. J., Hess, F. G., Johnson, W. D., Gallo, M. A., Babish, J. G., Dailey, R. E. & Parent, R. A. (1981). Long-term carcinogenicity and toxicity studies of patulin in the rat. Journal of Applied Toxicology 1, pp. 256-261.

Bondy, G. S., Coady, L., Curran, I., Caldwell, D., Armstrong, C., Aziz, S.A., Nunnikhoven, A., Gannon, A. M., Liston, V., Shenton, J. & Mehta, R., (2016) Effects of chronic deoxynivalenol exposure on p53 heterozygous and p53 homozygous mice. Food and Chemical Toxicology 96, pp. 24–34.

Bondy, G., Mehta, R., Caldwell, D., Coady, L., Armstrong, C., Savard, M., Miller, J. D., Chomyshyn, E., Bronson, R., Zitomer, N. & Riley, R. T. (2012) Effects of long term exposure to the mycotoxin fumonisin B1 in p53 heterozygous and p53 homozygous transgenic mice. Food and Chemical Toxicology 50, pp. 3604-3613.

CIC. (1985) Maturation index in castrate cynomolgus monkeys receiving zeranol. Unpublished report from Coulston International Corp., Alamogordo, NM, USA. Submitted to WHO by International Minerals & Chemical Ltd., London, England.

Cullen, J. M., Reubner, B. H., Hsieh, L. S., Hyde, D. M. & Hsieh, L. S. (1987) Carcinogenecity of dietary aflatoxin M1 in male Fischer rats compared to aflatoxin B1. Cancer Research 47, pp. 1913-1917.

Döll, S., Dänicke, S. & Schnurrbusch, U. (2003a). The effect of increasing concentrations of *Fusarium* toxins in the diets for piglets on histological parameters of the uterus. Mycotoxin Research, 19, pp. 73-76.

Döll, S., Dänicke, S., Ueberschär, K. H., Valenta, H., Schnurrbusch, U., Ganter, M., Klobasa, F. & Flachowsky, G. (2003b) Effects of graded levels of *Fusarium* toxin contaminated maize in diets for female weaned piglets. Archiv für Tierernahrung, 57, 311-334.

Edwards, S., Cantley, T.C., Rottinghaus, G.E., Osweiler, G.D. & Day, B.N. (1987) The effects of zearalenone on reproduction in swine. I. The relationship between ingested zearalenone dose and anoestrus in non-pregnant, sexually mature gilts. Theriogenology 28, pp. 43-49.

Hard, G.C., Howard, P.C., Kovatch, R.M. & Bucci, T.J. (2001) Rat kidney pathology induced by chronic exposure to fumonisin B1 includes rare varients of renal tubule tumour. Toxicologic Pathology 29, pp. 379-386.

Howard, P.C., Eppley, R.M., Stack, M.E., Warbritton, A., Voss, K.A., Lorentzen, R.J., Kovach, R.M. & Bucci, T.J., (2001) Fumonisin B1, carcinogenicity in a two-year feeding study using F344 rats and B6C3F1. Environmental Health Perspectives 109 Supplement 2, pp. 277-282.

Howard, P.C., Warbritton, A., Voss, K.A., Lorentzen, R.J., Thurman, J.D., Kovach, R.M. & Bucci, T.J. (2001) Compensatory regeneration as a mechanism for renal tubule carcinogenesis of fumonisin B1 in the F344/N/Nctr BR rat. Environmental Health Perspectives 109 Supplement 2, pp. 309-314.

Iverson, F., Armstrong, C., Nera, E., Truelove, J., Fernie, S., Scott, P., Stapley, R., Hayward, S. & Gunner, S. (1995) Chronic feeding study of deoxynivalenol in B6C3F1 male and female mice. Teratogenesis, Carcinogenesis, and Mutagenesis 15, pp. 283-306.

Jonsson, M., Atosuo, J., Jestoi, M. N., Nathanail, A. V., Kokkonen, U. M., Anttila, M., Koivisto, P., Lilius, E. M. & Peltonen, K. (2015) Repeated dose 28-day oral toxicity study of moniliformin in rats. Toxicology Letters 233, pp. 38–44.

Jonsson, M., Jestoi, M. N., Nathanail, A. V., Kokkonen, U. M., Anttila, M., Koivisto, P., Karhunen, P. & Peltonen, K. (2013) Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food and Chemical Toxicology 53, pp. 27–32.

Lee, C. H., Lee, C. L. & Pan, T. M. (2010) A 90-d toxicity study of monascus-fermented products including high citrinin level. Journal of Food Science, 75, T91-97.

Luo, X. Y., Li, Y. W., Wen, S. F. & Hu, X. (1987) Food poisoning caused by scabby wheat and the detection of Fusarium mycotoxins. Journal of Hygiene Research 16, pp. 33–37.

Maekawa, A., Kajiwara, T., Odashima, S. & Kurata, H. (1979) Hepatic changes in male ACI/N rats on low dietary levels of sterigmatocystin. Gann, 70, pp. 777-781.

Murphy, W. K., Burgess, M. A., Valdivieso, M., Livingston, R. B., Bodey, G. P. & Freireich, E. J. (1978.) Phase I clinical evaluation of anguidine. Cancer Treatment Reports 62, pp. 1497–1502.

NTP. (1982) National Toxicology Program Carcinogenesis Bioassay of Zearalenone in F 344/N Rats and F6C3F1 Mice (Technical Report Series No. 235), Research Triangle Park, North Carolina, Department of Health and Human Services. Available at: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr235.pdf

Nuehring, L. P., Rowland, G. N., Harrison, L. R., Cole, R. J., Dorner, J. W. (1985) Cyclopiazonic acid mycotoxicosis in the dog. American Journal of Veterinary Research, 46, pp. 1670- 1676.

Ohtsubo, K., Ryu, LC., Nakamura, K., Izumiyama, N., Tanaka, T., Yamamura, H., Kobayashi, T. & Ueno Y. (1989) Chronic toxicity of nivalenol in female mice: a 2-year feeding study with Fusarium nivale Fn 2b moulded rice. Food and Chemical Toxicology 27, pp. 591-598.

Pollmann, D. S., Koch, B. A., Seitz, L. M., Mohr, H. E. & Kennedy, G. A. (1985) Deoxynivalenol-contaminated wheat in swine diets. Journal of Animal Science 60, pp. 239–247.

Rafai, P., Tuboly, S., Bata, A., Tilly, P., Vanyi, A., Papp, Z., Jakab, L. & Tury, E. (1995) Effect of various levels of T-2 toxin in the immune system of growing pigs. The Veterinary Record 136, pp. 511–514.

Rafai, P., Tuboly, S., Bata, A., Tilly, P., Vanyi, A., Papp, Z., Jakab, L. & Tury, E. (1995) Effect of various levels of T-2 toxin in the immune system of growing pigs. The Veterinary Record 136, pp. 136, 511-514.

Rahman, S., Sharma, A. K., Singh, N. D., Telang, A. G., Azmi, S. & Prawez, S. (2014) Clinico-haematological changes in T-2 toxicosis in Wistar rats. Indian Journal of Veterinary Pathology, 38, 22–28.

Ryu J.C., Ohtsubo, K., Izurniyarna, N., Nakamura, K., Tanaka, T., Yamarnura, H. & Ueno Y. (1988) The acute and chronic toxicities of nivalenol in mice. Fundamental and Applied Toxicology 11, pp. 38-47.

Singh, A.R. & Griffin, T.B. (1984) To determine oral dose of zeranol for no-hormonal effect in non-human primates. Part IV. Supplement. Unpublished report No. 820111 from Coulston International Corp., Alamogordo, NM, USA. Submitted to WHO by International Minerals & Chemical Ltd., London, England.

Singh, A.R., Griffin, T.B., & Coulston, F. (1984a). To determine oral dose of zeranol for no-hormonal effect in non-human primates. Part I. Studies on ovariectomized cynomolgus monkeys. Unpublished report No. 82011 from Coulston International Corp., Alamogordo, NM, USA. Submitted to WHO by International Minerals & Chemical Ltd., London, England.

Singh, A.R., Griffin, T.B., & Coulston, F. (1984b). To determine oral dose of zeranol for no-hormonal effect in non-human primates. Part II. Studies on ovariectomized cynomolgus monkeys. Unpublished report No. 82011 from Coulston International

Corp., Alamogordo, NM, USA. Submitted to WHO by International Minerals & Chemical Ltd., London, England.

Singh, A.R., Griffin, T.B., & Coulston, F. (1984c). To determine oral dose of zeranol for no-hormonal effect in non-human primates. Part III. Studies on ovariectomized cynomolgus monkeys. Unpublished report No. 82011 from Coulston International Corp., Alamogordo, NM, USA. Submitted to WHO by International Minerals & Chemical Ltd., London, England.

Speijers, G. J. A., Wester, P. N., van Leeuwen, F. X. R., de la Fonteyne-Blankestijn, L., Post, W., van Egmond, H. P., Sizoo, E. A. & Janssen, G. B. (1993) Sub-chronic toxicity experiment with rats fed a diet containing ergotamine tartrate. Report no. 618312002. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Wogan, G. N., Paglialunga, S. & Newberne, P. M. (1974) Carcinogenic effects of low dietary levels of aflatoxin B1 in rats. Food and Cosmetics Toxicology 12, pp. 681-685.

Wu, W., Zhou, H., Bursian, S. J., Link, J. E. & Pestka, J. J. (2016) Emetic responses to T-2 toxin, HT-2 toxin and emetine correspond to plasma elevations of peptide YY3-36 and 5-hydroxytryptamine. Archives of Toxicology 90, pp. 997–1007.

Young, L. G., McGirr, L., Valli, V. E., Lumsden, J. H. & Lun, A. (1983) Vomitoxin in corn fed to young pigs. Journal of Animal Science 57, pp. 655–664.