TOX/2019/54

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

Review of potential risks from contaminants in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Mycotoxins

Background

1. As part of the review by the Scientific Advisory Committee on Nutrition (SACN) of Government recommendations on complementary and young child feeding, the Committee in Toxicology (COT) was asked to review the toxicity of chemicals in the diets of infants and young children aged 0-5 years.

2. Following on from a scoping paper presented to the Committee in 2017 summaries on a number of mycotoxins were presented at the meeting in July this year (TOX/2019/30). In agreement with their previous assessment the Members decided full reviews on ergot alkaloids (EAs), Sterigmatocystin (STC), zearalenone (ZEN) and nivalenol were unnecessary. However, the Members requested cancer studies on neonatal/prenatal rats to be provided for aflatoxins, if available, to allow for information regarding the sensitivity differences between infants and adults and additional information on IARCs classification of citrinin and further detail on the reproductive/maternal toxicity studies.

3. The Committee asked for the exposure ranges (LB, UB) to be provided, this will be included in the final text for the addendum.

4. Annex A provides the additional information requested by the Committee for aflatoxins and citrinin. The original papers presented to the Members at the July meeting are attached in Annex B (aflatoxin) and Annex C (citrinin) for information.

Questions to be asked to the Committee

1. Do the Committee consider the additional information sufficient to conclude on the potential risk from aflatoxin, including the sensitivity differences between infants and adults?

2. With the additional information available, do the Committee agree with IARCs classification of citrinin?

3. Do the Committee agree with their initial assessment that a full review of these mycotoxins will not be necessary and that they can be included in the Addendum to the Overarching Statement?

4. Are there any points The Committee would like to emphasis?

5. Do the Members have any other comments?

Secretariat

September 2019

Additional information on neonatal/prenatal exposure to aflatoxins

5. The following table and paragraphs provide summaries of the studies assessed by IARC (1993[1]) and EFSA (2007[2]) as well as publications retrieved from a literature search (from 1993, the IARC publication date, to August 2019).

Table 1. Summaries of rodent studies assessing prenatal/neonatal exposure to aflatoxin (B1).

Compound	Concentration and exposure route	Exposure and study duration	Results	Reference
Rats				
Aflatoxin B1	2 mg/kg of diet (0.1 mg/kg bw)[a]	Throughout pregnancy and lactation Male and female weaned offspring Male and female weaned offspring of untreated rats	>75% of animals in both treatment groups developed malignant liver neoplasms, which were the main cause of death Hyperplasic hepatic nodules, two types of differently stained areas of hyperplasia in the liver	Ward et al., 1975 (IARC)

[1] https://monographs.iarc.fr/wp-content/uploads/2018/06/mono56-14.pdf
https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100F-23.pdf
[2] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2007.446

		were exposed to same diet from 6-7 weeks of age Treatment until death or until all were killed at 74 weeks	Increase in colonic tumour incidence observed in rats exposed from conception and from rats exposed from 6-7 weeks of age (groups exposed in utero, postnatally and exclusively postnatally were not separated, sex unspecified)	
Aflatoxin	Female rats: 0.5 mg/kg bw i.p. injection Offspring: 0 or 0.5 mg/kg bw i.p. injection	Female rats from GD 17-18 or GD 18-20 Offspring of both sexes on days 2-5 or 14-17 after birth	Dams: Increased incidence of benign and malignant neoplasms in various organs (liver, GI tract, endocrine organs, mammary gland) Offspring exposed in utero (with or without treatment after birth): Significant increase in total number of malignant tumours, except for mammary gland and leukaemia.	Goerttler et al., 1980 (IARC)
Aflatoxin B1	0.7, 1.4, 3.5 and 7.0 mg/kg bw	GD 8 or 16	Average fetal weight decreased	Sharma and Sahai, 1987 (IARC)

	Subcutaneous injection		Malformations were not observed	
Aflatoxin B1	2 mg/kg bw i.p. injection	GD 8-10 or GD 15-17	Significant increase in liver triglyceride content in 1-2 month old offspring of dams exposed at GD 8-10 Decreased motor activity in 1 month old offspring exposed at GD 8-10 or 15-17; behaviour became normal again at the age of 2 or 3 months Persistent neuronal degeneration in the brains.	Chentanez et al., 1986 (IARC)
Aflatoxin B1	10, 20, 50 or 100 µg/kg bw per day Intramuscular injection	GD 12-19	In utero exposure severely compromised postnatal development in female offspring, including reduced birth weight and locomotor activity	Supriya et al., 2016 https://www.ncbi.nlm.nih.gov/pubmed/26956420
Aflatoxin B1	10, 20 or 50 µg/kg bw daily Dosing method not given	GD 12-19	Severely compromised postnatal development of male offspring, causing a delay in testes descent and reduction in steroidogenesis and spermatogenesis	Supriya and Reddy, 2015 Abstract only https://www.ncbi.nlm.nih.gov/pubmed/25911313

			Behavioural changes, such as cliff avoidance, negative geotaxis, impairment of exploratory and locomotory activities	
Aflatoxin B1	0.1, 0.3 or 1.0 ppm (7.1, 21 or 67 µg/kg bw per day (gestation period)[b] Diet	GD 6 to day 21 delivery on weaning	Reversal effect on hypocampal neurogenesis targeting typ-3 progenitor cells	Tanaka et al., 2015 https://www.ncbi.nlm.nih.gov/pubmed/26260870
Aflatoxin B1	0.5, 1, 2, 3 or 4 mg/kg bw i.p. injection Suckling rats: 3 mg/kg bw Direct injection, single dose	GD 8.5	50 to 60% depletion of fetal liver GST towards CDNB Suckling rats: Liver GST significantly induced	Fatami et al., 2006 Abstract only https://www.ncbi.nlm.nih.gov/pubmed/16501953
[3H]Aflatoxin B1	Specific activity 18 mCi/mM) 8 µCi [^{3}H]AFB1 containing 40	Neonatal and young rats killed 2, 6, 12 and 24 hours after exposure	AFB1 was epoxidized more rapidly by the adult liver and lungs 2 hours after administration, compared to neonatals	Chelcheleh and Allameh 1995 https://www.ncbi.nlm.nih.gov/pubmed/7596201

	pg AFB1/100 g bw i.p. injection		Differences more pronounced in hepatic than pulmonary tissues	
Aflatoxin B1	15 µM (4.7 mg) In vitro	Cultured rat embryos	Neuronal tube defects	Geissler and Faustman, 1988 (IARC)
Mice				
Aflatoxin B1	16 or 32 mg/kg bw i.p. injection	GD 6-13	Maternal death, decreased bw, increased liver weight Reduced fetal weight, external and skeletal malformations in high dose group	Tanimura et al., 1982 (IARC)
Aflatoxin B1	4 mg/kg bw Oral intubation	GD 8 and 9	Fetal abnormalities after exposure on day 8 No abnormalities after exposure on day 9	Arora et al., 1981 (IARC)
Aflatoxin B1	15, 45 or 90 mg/kg i.p. injection or oral	GD 6-13 Exposure for 2-day periods	i.p.: Retardation of fetal development at 45 and 90 mg/kg Malformations of the diaphragm at 45 mg/kg Malformations of the diaphragm and kidneys at 90 mg/kg	Roll et al., 1990 (IARC)

			Oral: Diaphragmatic malformations at 45 mg/kg	
Aflatoxin B1	5 or 6 mg/kg (0.1 or 0.12 mg/kg bw)[c] for mutagenesis or mutation analysis, respectively i.p. injection 5 mg/kg (0.1 mg/kg bw)[c] for adduct analysis and mutagenesis Oral gavage	GD 14 Pregnant C57BL/6J females Carrying F1 gestation day 14 embryos of the B6C3F1 genotype	Adducts in GD 14 embryos were 20-fold more potent inducers of mutagenesis than adducts in parallel dosed adults Correlated with Ki67 staining of the liver, reflecting the proliferation potential of the liver tissue	Chawanthayatham et al., 2015 https://www.ncbi.nlm.nih.gov/pubmed/25070670
Aflatoxin B1	6 mg/kg (0.12 mg/kg bw)[c] i.p. injection	Day 4 after birth	10-fold increase in Spi(-)mutant fraction in liver DNA (over control) after 3 weeks After 10 weeks a further increase was observed	Wattanawaraporn et al., 2012 https://www.ncbi.nlm.nih.gov/pubmed/22539618

			Using a genetic loci, data show a strong preference for induction of GC to TA mutations after single exposure	
Aflatoxin B1	6 mg/kg (0.12 mg/kg bw)[c] i.p. injection	Newborn female and male	Similar amounts of DNA damage and mutation that may initiate neoplastic process Mutation frequencies in liver were increased by 20-30 fold 24 hours after dosing, AFB(1)-FAPY adduct was present in liver DNA	Woo et al., 2011 https://www.ncbi.nlm.nih.gov/pubmed/21507988
Aflatoxin B1	Neonatal (transgenic): total 6 mg/kg bw, 2 mg/kg bw per treatment i.p. injection Adult: Total 6 and 60 mg/kg bw, 2 and 20	Neonatal exposure at the age of 4, 7, and 10 days Three doses, every third day exposure Adult exposure at 120, 123 and 126 days of age	Neonatal: Liver tumours cII liver MF was 22-fold higher than control Frequency of GC to TA transversion was about 82-fold than the control and 31-fold higher than adults treated with 60 mg/kg bw Adult: No tumours	Chen et al., 2010 https://www.ncbi.nlm.nih.gov/pubmed/19642212

	mg/kg bw per treatment i.p. injection	Same exposure regime Animals were sacrificed 6 weeks after treatment	No significant increase in liver MF	

[a] converted using a factor of 0.05 for chronic studies in (adult) rats, given by EFSA[3] (based on the initial assessment by the WHO in 1987)
[b] as given by the authors
[c] converted using an average bw of 0.02 kg for mice, given by JECFA (2016)[4]

Abbreviations: bw (body weight), GD (gestation day), i.p. (intraperitoneal), GI (gastrointestinal), GST (glutathione S-transferase), CDNB (1-chloro-2,4-dinitrobenzene), AFB1 (aflatoxin B1), Spi(-) (sensitive to P2 inhibition), GC (guanine-cytosine), TA (thymine-adenine), AFB(1)-FAPY (), clI gene (encodes a protein that activates transcriptional promoters in λ that are essential for lysogenization), MF (mutant frequency)

[3] http://www.efsa.europa.eu/sites/default/files/consultation/110707a.pdf
[4] https://apps.who.int/iris/bitstream/handle/10665/246173/9789241511155-eng.pdf%3bjsessionid=9E345DBB4E70BA9096BF57CD4165EB8D?sequence=1

6. In humans, aflatoxins are known to cross the placental barrier. There is some evidence suggesting the concentrations in cord blood are higher than maternal blood and that aflatoxins (e.g., B1, G1 and Q1) may accumulate in fetuses exposed in utero (Denning et al., 1990; Lamplugh et al., 1988). The metabolic activation of aflatoxin B1 in human adult and fetal liver to mutagenic metabolites was examined by Kitada et al. (1990). Mutation in the umu gene in a plasmid containing a strain of salmonella typhimurium was induced in both adult and fetal systems. Antibody inhibition studies indicate the involvement of cytochrome P450 3A4 in the adult system and P450 HFLa in the fetal system.

7. A number of studies have considered polymorphism in aflatoxin metabolism or DNA repair enzymes in relation either to the formation of DNA or protein adducts or in relation to risk of hepatocellular carcinoma (HCC). A paper by Wild et al. (1993) measured serum aflatoxin-albumin (AF-alb) in Gambian children in relation to glutathione S-transferase M1 (GSTM1) genotype and in Gambian adults in relation to GSTM1, glutathione S-transferase theta-1 (GSTT1), glutathione S-transferase Pi 1 (GSTP1) and epoxide hydrolase polymorphism and found no major differences in adduct levels by genotype. Another study in Gambian children (Turner et al., 2003) and one in Ghanaian adults (Jiang et al. 2005) indicate dietary exposure to AFB1 could result in impairment of cellular immunity that could decrease host resistance.

8. Studies by Gong et al. (2002; 2004) reported an increase in AF-alb adduct level with age, up to three years, and was significantly related to weaning status of the 1 to 3 year age group: weaned children had approximately two-fold higher mean aflatoxin–albumin adduct levels than those receiving a mixture of breast milk and solid foods.

9. Infection with hepatitis B (HBV) may increase aflatoxin metabolism. In HBV-infected children there was a higher level of AF-alb adducts than in non-infected children, an observation consistent with altered aflatoxin metabolism (Allen et al., 1992; Turner et al., 2000). However, similar studies in adults did not show such differences (Groopman et al., 1992; Wild et al., 2000).

Additional information on citrinin

IARC classification

10. IARC[5] evaluated a number of experimental studies and found citrinin to result in embryotoxicity in rodents, after injection of maternally toxic doses. Both positive and negative results were reported in the Bacillus subtilis rec assay; no mutagenicity, induction of recombination or unscheduled DNA synthesis was reported in bacterial or mammalian cell assays in vitro. Chromosomal aberrations, but no sister chromatid exchange, were induced by citrinin in Chinese hamster cells.

11. In vivo, citrinin produced renal tumours in one study that IARC considered adequate for carcinogenicity testing. Male rats in said study were exposed to citrinin orally via the diet at a concentration of 0.1% for 80 weeks (equivalent to 0.1 mg/kg bw[6]) (Arai and Hibino, 1983). A second study observed an increase in the incidence of renal tumours after exposure of rats to N-nitrosodimethylamine or N-(3,5-dichlorophenyl)succinimide in the diet in combination with citrinin (Shinohara et al., 1976).

12. No renal tumours were reported in a 48 week rat study (0.02 or 0.05% citrinin, equivalent to 0.02 and 0.05 mg/kg bw[6]) (Shinohara et al., 1976), a 70 week study in mice (100 or 200 mg/kg of diet citrinin, equivalent to 20 or 40 mg/kg bw[7]) (Kanisawa, 1984) and only 1/35 medaka (Japanese rice fish) exposed to 150 mg/kg citrinin developed a liver cell nodule at 24 weeks (150 or 300 mg/kg citrinin for 12, 22 or 24 weeks, 800 mg diet per approximately 500 fish) (Hatanaka et al., 1982).

13. IARC concluded there was limited evidence for the carcinogenicity of citrinin in experimental animals, inadequate data of activity in short term tests and were furthermore unable to evaluate the carcinogenicity in humans due to a lack of case reports and/or epidemiological studies.

Reproductive/maternal toxicity

14. A study by Hood et al., (1976) exposing female rats to a single dose of 35 mg citrinin/kg bw on days 3 to 15 of gestation did not find any skeletal malformations of the fetus. However, enlarged kidneys and internal hydrocephalus and cleft palates were found and as 30 to 50% of the pregnant dams died, maternal toxicity might have influenced the study outcome. In another study in rats by Singh et al. (2006, 2007a/b, 2008), citrinin was administered to pregnant rats in the feed at a concentration of approximately 1 mg/kg bw during days 6 to 20 post coitum. Mild maternal toxicity was reported

[5] https://monographs.iarc.fr/wp-content/uploads/2018/06/mono40.pdf
[6] Assuming an average consumption for rats of 20 g per day as given by JECFA[4] and a conversion factor from diet to kg bw of 0.05, as given for a rat study by EFSA[3]
[7] Assuming a conversion factor of 0.2, as given for subchronic mice studies by EFSA[3]

in the form of degenerative liver changes, renal lesions and glomerular congestion. Fetal reabsorption was increased and 6.8% of the examined fetuses showed severe malformations, approximately 10% were retarded and fetal kidneys showed tubular degeneration, medullar tubular necrosis and interstitial fibrosation. EFSA noted that the extent of offspring exposure could not be determined in this study.

15. EFSA concluded in their assessment, that in vitro and in vivo studies provide clear evidence for reproductive toxicity, teratogenic and embryotoxic effects of citrinin. However, the given dose induced maternal toxicity, including nephrotoxicity and that the effects might be secondary to maternal toxicity.

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Aflatoxins: B1, B2, G1, G2 and M1 (AFB1, AFB2, AFG1, AFG2 and AFM1)

16. Aflatoxins are primarily produced by two species of Aspergillus fungus and can be found in foods as a result of fungal contamination both pre- and postharvest, with the rate and degree of contamination dependent on temperature, humidity, soil and storage conditions. Aflatoxins are most commonly associated with groundnuts, tree nuts, dried fruit, spices, figs, crude vegetable oils, cocoa beans, maize, rice, cottonseed and copra.

17. Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1) in humans and animals. It may be present in milk from animals fed on AFB1 contaminated feed and also in human breast milk. For the UK, exposure to aflatoxins is generally considered to occur mainly from imported materials. It is currently uncertain whether future changes in climate in the EU would lead to increased aflatoxin contamination.

18. Most of the available toxicological data relate to AFB1. Studies have consistently shown AFB1 to be both genotoxic and carcinogenic in experimental animals. Sufficient experimental evidence is also available for the carcinogenicity of naturally occurring mixtures of aflatoxins, and of AFG1 and AFM1, whereas there is only limited evidence for AFB2 and inadequate evidence for AFG2. The relative potency of aflatoxin congeners is available from bacterial mutagenicity and hepatocarcinogenic effects in the rainbow trout and rats, in the order of AFB1 > (AFG1, AFM1) >> (AFB2, AFG2).

19. The potential carcinogenicity of aflatoxins (either total or AFB1) in humans has been examined in a large number of epidemiology studies, generally carried out in Africa and Asia, where substantial quantities of aflatoxins occur in basic foodstuffs. The International Agency for Research on Cancer (IARC) concluded that naturally occurring aflatoxins are carcinogenic to humans (group 1), with a role in aetiology of liver cancer, notably among subjects who are carriers of hepatitis B virus (HBV) surface antigens.

20. EFSA did not consider it appropriate to establish a health-based guidance value (HBGV) since aflatoxins are both genotoxic and carcinogenic and therefore applied the margin of exposure (MOE) approach in their risk assessment. However, EFSA noted, that the available data would only be sufficient for AFB1, yet AFG1 and AFB2 were also shown to be carcinogenic in rodents, albeit at lower potency than AFB1. Therefore, as a conservative

approach EFSA assumed the carcinogenic potency of “total aflatoxin” to be similar to AFB1. EFSA proposed a MOE of 10,000 or higher would be of low health concern, if based on a $BMDL_{10}$ from an animal carcinogenicity study. To date there have been no conclusions on the magnitude of an MOE based on human data that would be of low concern.

21. Following EFSAs approach, the MOEs for aflatoxins were calculated using UK exposures and a $BMDL_{10}$ of 0.17 µg/kg bw per day, based on liver carcinogenicity in male rats exposed to 1 to 100 µg/kg diet of AFB1 (Wogan et al., 1974). Total aflatoxin was not available as part of the TDS and due to inconsistencies in the reporting across the EU it is not certain whether total exposure could be calculated from the data available.

22. For all children aged 4 to 60 months, the mean and 97.5th percentile MOEs for AFB1 are ≥ 14, the mean and 97.5th percentile MOEs for AFB2, AFG1, AFG2 and AFM1 are ≥19, 15, 8.9 and 24, respectively.

23. The exposures, and respective MOEs, were not based on measured values, but on lower bound (LB) and upper bound (UB) values, all results were below the calculated limit of quantification (LOQs). Therefore, the actual MOEs would be higher than those calculated.

24. Given that aflatoxins are genotoxic and carcinogenic their presence is always undesirable it is not possible to exclude a safety concern.

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Citrinin

25. Citrinin is produced by several species of the genera Aspergillus, Penicillium, and Monascus. and is normally formed under harvest and storage conditions. It occurs predominantly in grains but also in other plant products such as beans, fruit and herbs and spices. It is also found in red mould rice (RMR), used as a food colourant and preservative in Asian foods. Specific toxicokinetic studies with oral administration are not available. Experimental data indicate the occurrence of citrinin residues in edible tissues and eggs following oral exposure of animals with contaminated feed.

26. The acute lethal toxicity of citrinin ranged from 19 to 134 mg/kg bw depending on species and route of administration. Repeat dosing studies confirmed the nephrotoxicity of citrinin and again highlighted the differences in susceptibility between species. One available subchronic study in rats reported a no observed adverse effect level (NOAEL) of 20 mg/kg bw per day. One available long-term feeding study in rats exposed to high dietary citrinin (initially about 70 mg/kg bw per day) identified the kidney as principal target organ and reported progressive histopathological changes and incidences of adenomas. However, the study was limited to 80 weeks thus no conclusions on potential carcinogenicity can be drawn. Other in vivo studies showed the induction of chromosome abnormalities and hypodiploidy in mice bone marrow. Conventional bacterial and mammalian in vitro assays indicate that citrinin is not mutagenic, mutagenicity was only reported in one study using rat hepatocytes as the activation system in the Ames test. IARC concluded that citrinin is not classifiable as to its carcinogenicity to humans (Group 3).

27. Data from immunotoxicity studies were generally incomplete and did not allow for conclusions to be drawn. Data from in vitro and in vivo studies reported reproductive toxicity and teratogenic and embryotoxic effects of citrinin. However, the in vivo studies also reported maternal toxicity, including nephrotoxicity, indicating that the reproductive, teratogenic and embryotoxic effects may be secondary to maternal toxicity.

28. EFSA concluded that the derivation of a HBGV would not be appropriate, given the available data on genotoxicity and the limitations and uncertainties in the current database. This was not expanded upon by EFSA which furthermore concluded, that due to the lack of human dietary exposure data a MOE approach would not be appropriate. Instead, EFSA decided to characterise the

risk of citrinin and determine a level of no concern for nephrotoxicity in humans of 0.2 µg/kg bw per day based on a NOAEL of 20 µg/kg bw per day and application of an uncertainty factor (UF) of 100 for interspecies and interindividual variation. A concern for genotoxicity and carcinogenicity cannot be excluded at the level of no concern for nephrotoxicity.

29. Mean and 97.5th percentile exposures of infants aged 0 to 12 months and young children aged 1 to 5 years are all below the exposure level of 0.2 µg/kg bw per day considered of no concern for nephrotoxicity in humans by EFSA. Therefore, the exposures reported in the TDS are not of toxicological concern for nephrotoxicity. Due to lack and limitations of the available data, a concern for genotoxicity and carcinogenicity cannot be excluded.

30. Occurrence data from all food samples analysed for citrinin were below the LOQ and the exposures calculated are based on the LB and UB values.