

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

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

Introduction

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that will inform the Government's dietary recommendations for infants and young children. The SACN is examining the nutritional basis of the advice. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) was asked to review the risks of toxicity from chemicals in the diet of infants, most of which has been completed, and young children. The reviews will identify new evidence that has emerged since the Government's recommendations were formulated, and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years.

2. A scoping paper (TOX/2015/32) "COT contribution to SACN review of complementary and young child feeding; proposed scope of work for 1-5 year old children" was reviewed by the COT in 2015. This scoping paper is in response to the COT conclusion of: "Members requested that for mycotoxins, an exposure assessment should be undertaken for all those mycotoxins measured in the UK Total Diet Study (TDS) – Mycotoxin Analysis (samples had been analysed and the data were currently being processed), and from this a decision should be made as to what depth of review was required for each mycotoxin" (COT, 2015).

Structure of the scoping paper

3. This scoping paper provides estimated mycotoxin exposures for infants and young children in the UK aged 0 to 12 months and 1 to 5 years, respectively. There are currently no Government dietary recommendations for infants and young children which relate to mycotoxins.

4. Mycotoxins are produced as secondary metabolites by filamentous fungi and are toxic to vertebrates and other animal classes at low concentrations (Bennett and Klich, 2003). The mycotoxins considered in this scoping paper are aflatoxins, citrinin, cyclopiazonic acid, ergot alkaloids, fumonisins, moniliformin, ochratoxin A, patulin, sterigmatocystin, zearalenone and tricothecenes including deoxynivalenol and its acetylated derivatives, diacetoxyscirpenol, fusarenon-X, neosolaniol, nivalenol and T2 and HT2.

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5. Each mycotoxin is in a separate annex and comprises an introductory section, and overviews of an health based guidance value (HBGV) or Margin of Exposure (MOE) section, an exposure assessment, risk characterisation and conclusions sections. For mycotoxins, for which the European Food Safety Authority (EFSA) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have not produced an evaluation, an additional section covering absorption, distribution, metabolism and excretion (ADME) data and toxicity data has been included.

TDS survey

6. This scoping paper has considered exposures based on concentration data measured in the mycotoxins TDS by the Food and Environment Research Agency (FERA).

7. An overview of the TDS survey and the accompanying data are provided in a Food Survey Information Sheet (FSIS) (Annex Q). This also includes a brief risk assessment and conclusions. The FSIS covers exposures from all age groups, not just infants and young children aged 1 to 5. Advice provided by the COT from this paper will be reflected in the FSIS.

Risk assessments of the mycotoxins

Hazard characterisation

8. The toxicology sections and associated HBGVs or MOE approaches have all been summarised from evaluations from either EFSA or JECFA, where available. Fusarenon-X, neosolaniol and cyclopiazonic acid have not been considered by either EFSA or JECFA. Fuarenon-X and neosolaniol have been evaluated by The Netherlands National Institute for Public Health and the Environment (RIVM) and this has been used in the risk characterisations of this scoping paper. There was no evaluation for cyclopiazonic acid and so ADME and toxicity data are summarised from the literature.

9. Deoxynivalenol, diacetoxyscirpenol and moniliformin are currently being considered by EFSA. Aflatoxins, diacetoxyscirpenol, fumonisins and sterigmatocystin were considered by JECFA in November 2016. A technical report has been published (FAO/WHO, 2017) but monographs are currently not available.

Exposure assessment

10. Exposure assessments for the mycotoxins were carried out using occurrence data from the TDS – mycotoxin analysis (Stratton *et al.*, 2015) and consumption data from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (DH, 2013) and the National Diet and Nutrition Survey rolling programme (NDNS) (Bates *et al.*, 2014; Bates *et al.*, 2016). Exposures were assessed for infants aged 4 - <6, 6 - <9 and 9 - <12 months, and for young children aged 12 - <15, 15 – <18, 18 – <24 and 24 – <60

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months. Consumption data from DNSIYC was used for children aged 4 – 18 months and from NDNS for children aged 18 – 60 months.

11. Possible exposures were calculated from TDS – mycotoxin analysis data (Stratton *et al.*, 2015) for different groups of mycotoxins. Generally there was a high proportion of analytical data below the limit of detection (LOD) or quantification (LOQ) and thus, exposure assessments were expressed as a range of lower bound (LB) (where 0 is used as the analytical value) and upper bound (UB) (the limit of detection/quantification is used).

Questions on which the views of the Committee are sought

12. Members are invited to consider the following questions and specific questions in the annexes B, C, F, L, M, N and Q.

General

- i). Do Members agree with the HBGV/MOE approach for each of the mycotoxins?
- ii). Are there any mycotoxins for which Members would like a full review?
- iii). Would Members like breast milk data to be considered in the risk characterisation, for those mycotoxins for which it is available?
- iv). How would Members like to see the exposure tables presented?
- v). This scoping paper only considered the mycotoxins measured in the TDS. Are there other mycotoxins that Members think should be considered?

Secretariat

June 2017

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References

Bates B, Lennox A, Prentice A, Bates C, Page P, Nicholson S, Swan G (2014). National Diet and Nutrition Survey (NDNS): results from Years 1 to 4 (combined) of the rolling programme for 2008 and 2009 to 2011 and 2012. Available at: <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012>

Bates B, Cox L, Nicholson S, Page P, Prentice A, Steer T, Swan G (2016). National Diet and Nutrition Survey Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 – 2013/2014). Available at: <https://www.gov.uk/government/statistics/ndns-results-from-years-5-and-6-combined>

Bennett JW and Kilch M (2003). Mycotoxins. *Clinical Microbiology Reviews* 16(3): 497-516. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/12857779>

COT (2015). Minutes of the meeting held on Tuesday, 27th October 2015 in Aviation House, London. (paragraph 44). Available at: <https://cot.food.gov.uk/sites/default/files/cotfinalminutes-27oct15.pdf>

DH (Department of Health) (2013). Diet and Nutrition Survey of Infants and Young Children. Available at: <https://www.gov.uk/government/publications/diet-and-nutrition-survey-of-infants-and-young-children-2011>

FAO/WHO (2017). WHO Technical Report Series. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

Stratton J., Anderson S., Leon I., Hepworth P., Chapman S., Christy J., Jardine S., Philips D., Setter R., Clough J. and MacDonald S. (2015) Final report diet study (TDS) – mycotoxin analysis. FS102081. Unpublished report.

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

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

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

Introduction

13. This risk characterisation for aflatoxins makes use of an EFSA Opinion (EFSA, 2007¹).

14. The toxicology of the aflatoxins is reasonably well documented and currently the approach to the hazard and risk characterisation is unlikely to change. Therefore an ADME and Toxicology section has not been included for this group of mycotoxins. This paper provides a summary of the data used for the Margin of Exposure (MOE) approach for risk characterisation of AFB1 and total aflatoxins.

15. The MOE approach can be used for AFB1 and total AF data. This paper uses the MOE approach to compare the individual AFs to the point of departure. There isn't a Total AF value as this was not provided by FERA when the sample analysis was done in the mycotoxins TDS.

Background

16. Aflatoxins produced primarily by two species of *Aspergillus* fungus which are found in areas with hot, humid climates. *A. flavus* produces B aflatoxins and is ubiquitous generally favouring the leaves and flowers of plants. *A. parasiticus* produces both B and G aflatoxins, is more adapted to a soil environment and has more limited distribution. (EFSA, 2007)

17. Aflatoxins are 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. They are most commonly associated with groundnuts, tree nuts, dried fruit, spices, figs, crude vegetable oils, cocoa beans, maize, rice, cottonseed and copra. 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 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 contaminants. (EFSA, 2007).

¹ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/446>

18. Most of the available toxicological data relate to AFB1. However, 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). (FAO/WHO, 2017).

19. The health effects of aflatoxins have been reviewed by a number of expert groups. The International Agency for Research on Cancer (IARC) has 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. (IARC, 1993; IARC, 2002).

20. Aflatoxins have been reviewed by the Scientific Committee for Food (SCF) in 1996, EFSA in 2007 and JECFA in 1998, 2001 (AFM1) and 2017.

Margin of Exposure

21. EFSA considered the liver carcinogenicity of aflatoxins to be the pivotal effect for the risk assessment. 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 (FAO/WHO, 1998; IARC, 1993 and 2002).

22. The potential carcinogenicity in humans of the aflatoxins (either total or AFB1) 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. Exposure to aflatoxins appears to present an additional risk, which is enhanced by simultaneous exposure to hepatitis B virus, and possibly hepatitis C virus (FAO/WHO, 1998; IARC 1993 and 2002).

23. Because aflatoxins are both genotoxic and carcinogenic EFSA could not establish a no observed adverse effect level (NOAEL) as a point of departure for the risk assessment. Therefore, the Panel considered dose-response modelling of experimental data from animal experiments and data from epidemiological studies. The Panel noted that the available database for dose-response modelling would only be sufficient for AFB1. Therefore, and taking into account that AFG1 and AFB2 were also shown to be carcinogenic in rodents, albeit at lower potency than AFB1, in the risk characterisation, EFSA, as a conservative approach assumed that the carcinogenic potency of “total aflatoxins” would be similar to that of AFB1.

24. For the evaluation of human and experimental animal data the EFSA Scientific Committee has proposed the use of the benchmark dose (BMD) methodology to derive a reference point on the dose-response curve. The Scientific Committee was of the opinion that the use of the BMDL², calculated

² The BMDL is the 95th percentile lower confidence limit of the BMD.

for a benchmark response (BMR) of 10% (BMDL₁₀³), is an appropriate reference point for compounds that are both genotoxic and carcinogenic. Such a value is the lowest statistically significant increased incidence that can be measured in most studies, and would normally require little or no extrapolation outside the observed experimental data.

25. The animal species most sensitive to the liver carcinogenicity of AFB1 appears to be the rat. EFSA considered the study in male Fisher rats performed by Wogan et al. (1974) as the most adequate study for dose-response modelling.

26. Groups of male Fisher rats were fed diets containing 0, 1, 5, 15, 50, or 100 µg/kg diet of AFB1 (purity >95%) until clinical deterioration of animals was observed, at which time all survivors in that treatment group were killed. EFSA converted the dietary concentrations of AFB1 into daily intakes assuming that an average adult male rat consumed 40 g diet per kg body weight per day. EFSA also adjusted the daily intake to 104 weeks in order to compensate for the shorter study duration in some of the AFB1 groups. In the modelling of the results from the Wogan et al. (1974) study the highest dose was omitted because this dose resulted in a 100% tumour incidence.

27. The US EPA BMD software (BMDS) was used (US EPA, 2006) for modelling the liver carcinoma dose-response in male Fisher rats and to calculate BMD and BMDL values for an extra 10% risk compared to the background.

28. The calculated BMD₁₀ values ranged from 0.41 to 0.48 µg/kg b.w. per day and the BMDL₁₀ values from 0.17 to 0.34 µg/kg b.w. per day. In order to be prudent the Panel used the lowest BMDL₁₀ of 0.17 µg/kg b.w. per day in the risk assessment.

29. Also, in line with the terms of reference and the opinions of the EFSA Scientific Committee and of the JECFA on substances that are genotoxic and carcinogenic (EFSA, 2005; FAO/WHO, 2009), Margins of Exposure (MOEs) were calculated by dividing the BMDL values for AFB1 derived from animal (rat) carcinogenicity and human epidemiological data by the estimates of dietary exposure. The Panel derived MOEs from the lowest BMDL₁₀ value of 170 ng/kg bw/day derived from the animal data and the lowest BMDL₁₀ value of 870 ng/kg bw/day or the lowest BMDL₁ (1% extra cancer risk) value of 78 ng/kg bw/day derived from epidemiological data. The EFSA Scientific Committee proposed that a MOE of 10,000 or higher, based on a BMDL₁₀ from an animal study, would be of low concern from a public health point of view (EFSA, 2005). To date there have been no conclusions on the magnitude of an MOE based on human data that would be of low concern.

³ The BMDL₁₀ is the 95th percentile lower confidence limit of the BMD for a 10 % increase in the risk of tumours

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Exposure Assessment

30. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. The results from all of the food samples that were analysed for AFs were below the limit of quantification (LOQ). Therefore the calculated exposures provided in Table 1 are expressed as lower bound (LB) and upper bound (UB).

31. For aflatoxin exposures, for all age groups, the mean values were all below 0.005 µg/kg bw/day and the 97.5th percentile exposures were all below 0.019 µg/kg bw/day.

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Table 1. Estimated aflatoxin chronic exposures from the TDS in infants and young children aged 4 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

Aflatoxin	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)		12 to 15 month-olds		15 to 18 month-olds		18 to 24 month-olds		24 to 60 month-olds	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
B1	0.000	0.000-0.001	0.000-0.001	0.000-0.004	0.000-0.002	0.000-0.005	0.000-0.003	0.000-0.007	0.000-0.004	0.000-0.010	0.000-0.004	0.000-0.011	0.000-0.005	0.000-0.012
B2	0.000	0.000-0.001	0.000-0.001	0.000-0.003	0.000-0.001	0.000-0.004	0.000-0.002	0.000-0.006	0.000-0.003	0.000-0.008	0.000-0.004	0.000-0.008	0.000-0.004	0.000-0.009
G1	0.000	0.000-0.002	0.000-0.001	0.000-0.004	0.000-0.002	0.000-0.005	0.000-0.003	0.000-0.008	0.000-0.004	0.000-0.010	0.000-0.005	0.000-0.009	0.000-0.005	0.000-0.011
G2	0.000	0.000-0.002	0.000-0.001	0.000-0.004	0.000-0.002	0.000-0.006	0.000-0.003	0.000-0.009	0.000-0.004	0.000-0.015	0.000-0.005	0.000-0.019	0.000-0.005	0.000-0.017
M1	0.000-0.002	0.000-0.007	0.000-0.001	0.000-0.006	0.000-0.001	0.000-0.004	0.000-0.001	0.000-0.003	0.000-0.001	0.000-0.002	0.000-0.001	0.000-0.002	0.000-0.001	0.000-0.002

Risk characterisation

32. MOEs were calculated for all AF exposures (Table 2). The MOE approach is normally used for AFB1 and total AF exposures. Total AF results were not provided to the FSA as part of the TDS and due to the inconsistent reporting of the total AFs across the EU it is not certain whether total exposures could be calculated from the data available. Therefore in this paper the MOE approach has been used for individual AFs.

33. The range of exposures across all age groups, from 4 to 60 months, for AFB1 is 0 – 0.012 µg/kg bw/day, which corresponds to MOEs of 14 or higher. The range of exposures for AFB2, AFG1, AFG2 and AFM1 is up to 0.009, 0.011, 0.019 and 0.007, respectively which corresponds to MOEs of greater than 19, 15, 8.9 and 24, respectively.

34. The exposures, and consequently MOEs, were not based on measured values, but on LB and UB values. All results were below the calculated LOQs which ranged from 0.13 µg/kg for AFB1 in dried fruit to 2.41 µg/kg for AFG1 in sugar confectionary.

35. Exposures to all of the AFs in infants and young children are somewhere between 0 and the UB exposure. Therefore the actual MOEs would be higher than those calculated in Table 2.

Conclusions

36. 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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Table 2. Estimated aflatoxin MOEs for infants and young children aged 4 to 60 months

Aflatoxin	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)		12 to 15 month-olds (n=670)		15 to 18 month-olds (n=605)		18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
B1		> 170	> 170	> 43	> 85	> 34	> 57	> 24	> 43	> 17	> 43	> 15	> 34	> 14
B2		> 170	> 43	> 57	> 170	> 43	> 85	> 28	> 57	> 21	> 43	> 21	> 43	> 19
G1		> 85	> 170	> 43	> 85	> 34	> 57	> 21	> 43	> 17	> 34	> 19	> 34	> 15
G2		> 85	> 170	> 43	> 85	> 28	> 57	> 19	> 43	> 11	> 34	> 8.9	> 34	> 10
M1	> 85	> 24	> 170	> 28	> 170	> 43	> 170	> 57	> 170	> 85	> 170	> 85	> 170	> 85

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Secretariat

June 2017

References

EFSA (2005). Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. *The EFSA Journal*. **282**: 1-31.

Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/282>

EFSA (2007). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *The EFSA Journal*. **446**: 1-127 Available at:

<https://www.efsa.europa.eu/en/efsajournal/pub/446>

FAO/WHO (1998). Who Food additives series 40. Safety evaluation of certain food additives and contaminants. Available at:

<http://www.inchem.org/documents/jecfa/jecmono/v040je01.htm>

FAO/WHO (2001). WHO Food Additives Series 47. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at:

<http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm>

FAO/WHO (2009). Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240. Available at:

<http://www.who.int/foodsafety/publications/chemical-food/en/>

FAO/WHO (2017). WHO Technical Report Series. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Available at:

<http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

IARC 1993. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 56. Available at:

<http://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf>

IARC 2002. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 82. Available at:

<https://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf>

SCF 1996. Reports of the Scientific Committee for Food (Thirty-fifth series). Available at:

https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_alfatoxins_scf_reports_35_en.pdf

Wogan,G.N., Paglialunga,S., and Newberne,P.M. (1974). Carcinogenic effects of low dietary levels of aflatoxin B1 in rats. *Food Cosmet. Toxicol.* **12**: 681-685. Available at:

<http://www.sciencedirect.com/science/article/pii/0015626474902399>

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

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Citrinin

Background

37. An EFSA (2012⁴) opinion has been used as the basis for this risk characterisation of citrinin.
38. Citrinin is produced by several species of the genera *Aspergillus*, *Penicillium*, and *Monascus*. Some of these fungi also produce ochratoxin A or patulin. Citrinin 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. (EFSA, 2012).
39. There are no specific toxicokinetic studies available for oral administration. In a study in pregnant female rats dosed ip with 35 mg/kg bw on the 12th day of gestation. The primary route of excretion is renal. ¹⁴C-citrinin-derived radioactivity elimination from plasma was biphasic. The half-lives for the rapid and slow phases of elimination were 1.95 and 39.7 hours, respectively. Approximately 74, 1.7 and 1.4 % of the radioactivity was detected in urine at 24, 48 and 72 hours, respectively. Plasma levels in pigs dosed with 0.02 mg/kg bw once or daily for a period of 8 weeks were 84 and 111 ng/ml at week 6 and day 57, respectively. (EFSA, 2012).
40. The acute lethal toxicity of citrinin ranged between 19 and 134 mg/kg bw, which was dependent upon species and route of administration. Studies with repeated dosing all confirmed the nephrotoxicity of citrinin and highlighted the differences in susceptibility between species. Only one subchronic study was available and this suggested that a dose of 20 mg/kg bw/day citrinin can be considered a NOAEL for rats. (EFSA, 2012).
41. Only one long-term feeding study had been identified in rats with one dose group, with a high dietary exposure to citrinin (initially about 70 mg/kg bw/day). The kidney was the principal target organ and showed progressive histopathological changes and incidences of adenomas. This study was limited to 80 weeks and therefore it cannot be excluded that carcinomas would have occurred if the study duration had been that of a carcinogenic study. The data from immunotoxicity studies were generally incomplete and did not allow a conclusive evaluation to be made. (EFSA, 2012).

⁴ EFSA, 2012 available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2605>

42. Data from *in vitro* and *in vivo* studies have provided evidence for reproductive toxicity and teratogenic and embryotoxic effects of citrinin. However in these *in vivo* studies, maternal toxicity, including nephrotoxicity, was observed at the doses at which these effects occurred, indicating that these effects may be secondary to maternal toxicity. (EFSA, 2012).

43. The available data indicate that citrinin is not mutagenic in conventional bacterial assays either with or without metabolic activation. Only one study reported mutagenicity in the Ames test when rat hepatocytes were used as the activating system. When mammalian cells were used *in vitro*, citrinin did not induce DNA single-strand breaks, oxidative DNA damage or SCEs but induced micronuclei, aneuploidy and chromosomal aberrations. *In vivo* citrinin induced chromosome abnormalities and hypodiploidy in the bone marrow of mice. (EFSA, 2012).

Previous evaluations

44. No previous evaluations were identified.

HBGV

45. Given the limitations and uncertainties in the current database on citrinin, EFSA concluded that the derivation of a health based guidance value was not appropriate. For compounds that may be genotoxic and carcinogenic, EFSA recommends the use of a MOE approach for risk characterisation. However, due to the lack of data on human dietary exposure, no MOE could be calculated. In order to give risk managers a tool to evaluate the risk of citrinin in food and feed, EFSA decided to characterise the risk of citrinin on the available data on nephrotoxicity and determined therefore a level of no concern for nephrotoxicity. Applying a default uncertainty factor of 100 to the NOAEL of 20 µg/kg b.w. per day, accounting for inter-species variation and for inter-individual variation, EFSA concluded that there would be no concern for nephrotoxicity in humans at an exposure level of 0.2 µg/kg b.w. per day. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity. (EFSA, 2012)

Exposure Assessment

46. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. The results from all of the food samples that were analysed for citrinin were below the limit of quantification (LOQ). Therefore the exposures provided in Tables 1a-c are lower bound (LB) and upper bound (UB).

47. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.009 and 0 – 0.025 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.016 and 0 – 0.041 µg/kg bw/day, respectively. Calculated

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mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.019 and 0 – 0.044 µg/kg bw/day, respectively.

Table 1a. Estimated citrinin chronic exposures from the TDS for infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.001	0.000- 0.007	0.000- 0.005	0.000- 0.019	0.000- 0.009	0.000- 0.025

Table 1b. Estimated citrinin chronic exposures from the TDS for young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.013	0.000- 0.032	0.000- 0.016	0.000- 0.041

Table 1c. Estimated citrinin chronic exposures from the TDS for young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.019	0.000- 0.037	0.000- 0.019	0.000- 0.044

Risk characterisation

48. Mean and 97.5th percentile exposures of infants aged 0 to 12 months and young children aged 1 to 5 years are all below 0.2 µg/kg bw/day, the exposure level established by EFSA (2012) at which there would be no concern for nephrotoxicity in humans. However due to lack of available data, a concern for genotoxicity and carcinogenicity cannot be excluded at this level.

Conclusions

Questions on which the views of the Committee are sought

49. Members are invited to consider the following questions

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

- i). Do Members agree with the approach taken by EFSA regarding use of a POD for nephrotoxicity due to lack of exposure data for an MOE approach for citrinin?
- ii). Do Members think an alternative approach should be used?

Secretariat

June 2017

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

References

EFSA (2012). Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA Journal*. **10(3)**: 2605. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2605>

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

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

Cyclopiazonic acid (CPA)

Background

50. There is currently no evaluation of CPA available by European or International agencies or committees. Brief background information has been provided based on 2 reviews. ADME and toxicity data are provided in the relevant section and includes brief summaries of the data available from published studies.

51. CPA is produced by several species of both *Aspergillus* and *Penicillium*. CPA may also be found alongside aflatoxins and OTA and may lead to under reporting of its presence. CPA is normally formed under storage conditions. CPA has been found in a range of food types including seeds, grains, cheeses, meat products, milk and eggs. (Burdock and Flamm, 2000; Chang, Ehrlich and Fujii, 2009).

ADME and Toxicity

ADME

52. In a study by Byrem *et al.*, (1999) a single bolus injection of 20 mg CPA (in 2 ml 1 N NaOH) was injected into each of 4 pigs. Blood samples for CPA analysis were withdrawn at intervals between 2 minutes and 96 hours. Plasma was retained for analysis. In addition 3 pigs (97 ± 7 kg) were provided with a diet containing 10 mg CPA/kg feed (calculated as 0.3 mg CPA/kg bw/day) for 6 days *ad libitum*. The daily feed intake was $2.95 \text{ kg} \pm 0.23 \text{ kg}$ and plasma samples were taken on day 3, 4, 5 and 6 and skeletal muscle samples were taken within 10 minutes of exsanguination on day 6. The plasma kinetics for CPA were best described using a 3-compartment model, a rapid distribution and a large volume of distribution (49 L) in pigs given a 20 mg i.v. bolus. Cyclopiazonic acid was eliminated with a half-life of 24 h. Steady-state plasma CPA levels were reached within 6 days in pigs consuming a diet containing 10 mg/kgm CPA (0.3 mg CPA/kg bw/day). The measured concentrations of CPA in plasma were 410 ± 44 ng/mL and in skeletal muscle were 469 ± 86 ng/g. (Byrem *et al.*, 1999).

53. [^{14}C] CPA was administered to Sprague-Dawley rats intragastrically (5 mg/kg, 0.6 $\mu\text{Ci/kg}$) and parenterally (1 mg/kg, or 0.12 $\mu\text{Ci/kg}$) (Norred, 1990) or orally (5 mg/kg, 0.6 $\mu\text{Ci/kg}$) (Norred *et al.*, 1985). The biological half-life of [^{14}C] was 33 – 43 hours, depending on route of administration. Radioactivity

was not excreted into expired CO₂ indicating that extensive metabolic degradation of CPA did not happen. CPA was readily absorbed from the GI tract into the bloodstream (maximum levels of 10 % reached at 6 hours). The liver, heart, kidney and lung were relatively highly labelled however, approximately 45 – 50 % of the CPA dose was distributed to the muscles within the first 12 hours after dosing. (Norred 1990, Norred *et al.*, 1985). CPA or its metabolites were excreted in both urine and faeces, which is the major route. Chickens were dosed with 0, 0.5, 5.0 or 10 mg/kg bw CPA by crop intubation. The highest levels of CPA were found in meat 3 hours after dosing and were dose-dependent. In birds given low, or mid doses the muscle CPA content decreased rapidly. After 24 hours none and 25 % (compared to 3 hour levels) was detected, respectively. Birds given 10 mg/kg eliminated CPA from muscle at a much slower rate, with an approximate half-life of 60 hours. (Norred, 1990).

Acute toxicity

54. In a study by Purchase (1971) intraperitoneal injection of CPA to male Wistar-derived rats (8 - 25 mg/kg) produced hyperesthesia and convulsions followed by death in about 2 hours. Rats receiving 2.5 and 4.5 mg/kg died 1 – 3 days after dosing and rats receiving lower than 2.5 mg/kg (lowest dose 0.8 mg/kg) recovered and survived until day 10. An LD₅₀ of 2.3 mg/kg was calculated. Oral administration (30, 36.7, 45 and 55 mg/kg in males and 30, 36.7, 45, 55, 67.5 and 82.6 mg/kg in females) resulted in the death of 12/30 female rats within 36 hours of dosing and 3/20 and 8/20 male rats at 48 hours and between 4 and 6 days after dosing, respectively. LD₅₀'s of 36 mg/kg and 63 mg/kg were calculated for males and females, respectively. The lesions produced included degenerative changes and necrosis in the liver, spleen, exocrine and endocrine pancreas, kidney, salivary glands, myocardium skeletal muscle, bile ducts and other ducts. CPA produces focal necrosis in most organs at high doses and affects ducts or organs (such as the islets of Langerhans) originating from ducts at lower doses. (Purchase, 1971)

55. In a study by Nishif, Cole and Dorner (1985) the effects of single doses of 0, 5, 7.5, 10, 11 and 12.5 mg/kg bw in group of 5 – 26 mice on spontaneous motor activity were assessed. CPA caused a significant and dose-related reduction in the spontaneous motor activity (hypokinesia) at doses ≥ 5 mg/kg ip. A slight reduction in motor activity was already noticeable 5 minutes after CPA injection, and maximum hypokinesia was reached in 30-60 minutes and returned to normal after about 2 hours or more depending upon the dose. This hypokinesia was associated with slow respiration and ptosis. This sedated condition resembled sleep, but the mice moved about intermittently with ptosis and retained a positive righting reflex. Cataleptic and hypothermic effects of CPA were monitored at 30 – 60 minute intervals in a group of 10 mice dosed with 10 mg/kg bw CPA. Peak cataleptic effects occurred after 60 minutes. Peak CPA-induced hypothermia occurred 30 minutes after injection and although body temperature increased it was still not back to normal after 7 hours. The mice tested for catalepsy and hypothermia, and survivors of the higher doses of CPA given in the LD₅₀

determination were kept for 1 week to monitor changes in body weight, gross neurological effects and death rate.

56. The LD₅₀ value was determined using groups of 9-12 mice given 11, 12.5, 13 or 14 mg CPA/kg. The lowest dose of CPA causing significant weight loss in mice was 7.5 mg/kg (1 day). The ip LD₅₀ of CPA was found to be 13 + 0.05 mg/kg. The tremors induced by near-lethal doses of CPA were associated with voluntary or forced movements (action tremors), they worsened during the days following treatment, but they were weak compared with the exhausting and continuous tremors of the whole body caused by 20 mg tremorine/kg (used for comparison). When death occurred only 24-259 min after administration of CPA (11-14mg/kg), it was preceded by dyspnoea cyanosis, opisthotonus and clonic leg movements and tonic extension of hind legs (convulsions). When death was delayed (2-6 days after CPA administration), it was preceded by prostration, ptosis, hypothermia, tremor and cessation of food and water intake resulting in cachexia, convulsions were not seen in this group of mice 10 mg/kg ip CPA did not affect the rate of convulsion or death caused by either maximal electroshock or metrazol administration but it did delay the onset of metrazol-induced seizures.

57. In rabbits 10 mg CPA/kg bw initially produced tachycardia, tachypnoea and sedation with an activated electroencephalogram. Of three rabbits given 10 mg CPA/kg 1 died and in this rabbit slow delta waves were seen just before and during a brief period with clonic leg movements. In this animal death was accompanied by tonic extension of the hind legs, respiratory arrest and cardiac fibrillation. (Nishie, Cole and Dorner, 1985).

58. In a study by Porter *et al.* (1988) chickens dosed orally with CPA at 0.5, 5.0, and 10 mg/kg bw showed significant ($P \leq 0.05$) increases in brain dopamine and serotonin concentrations 96 hours after dosing. The increases coincide with significant decreases in homovanillic acid (HVA) and subtle increases of dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5HIAA) concentrations. The brain weights of the treated birds were statistically insignificant from their respective controls, although increases in brain weight-body weight ratio within treatments and with time correlated with CPA toxicity. No significant changes were observed in dopamine, DOPAC, HVA, serotonin, and 5HIAA concentrations among the treatments at 3, 24, and/or 48 hours after dosing. (Porter *et al.*, 1988).

59. Venkatesh *et al.* (2005) randomly distributed 28-day old broiler chicks into three groups of 12 birds. Groups were fed diets containing no treatment (control), 10 ppm CPA or 1ppm T2 toxin to determine the mechanism of cell death in spleen and thymus at 6, 12, 24, and 36 hours post-treatment. The CPA and T2 toxin treated groups showed significant ($P < 0.01$) induction of apoptosis in the spleen and thymus, respectively, with peak induction at 24 h post-treatment. These findings were confirmed ultrastructurally and with semi-thin sections of the spleen and thymus stained with toluidine blue.

Subacute/Repeat dose

60. Wistar-derived rats were given weekly doses of 0, 12 or 21 mg CPA/kg body weight in 1 N-sodium bicarbonate, using an intragastric dose volume of 2.5 ml/kg bw, and subgroups of 8 (4 for controls) with equal numbers of males and females were killed 1 week after doses 2, 5, 9 and 14 had been administered. Males on the highest dose level showed mild transient growth retardation in the first 4 weeks. Four rats died (with no gross pathological changes) suddenly during week 4. Three were from the higher dose group and 1 was from the lower dose group. No abnormal signs were observed in the surviving males or in any of the females throughout the 15 weeks of the experiment. Mild cellular degenerative changes were induced by CPA in the myocardium and in several other organs (kidneys, liver (but only in the high dose group at week 15), spleen, salivary glands, pancreas, male genital system and adrenal gland) where ballooning of nuclei, especially in ductal epithelia, was also characteristic. Generally, the changes noted were already evident after 2 doses of CPA and progressed slightly up to week 5. After this time the severity of some changes, was reduced. Some nuclear changes, however, especially in ductular epithelium such as that of the salivary glands, became more evident. The changes were only weakly related to dose level, sex and the number of doses given. (van Rensburg, 1984)

61. Groups of 12 male SD rats received oral doses of CPA on 4 consecutive days at 0, 0.2, 2.0, 4.0, or 8.0 mg/kg/day. Only the 2 highest dose groups showed clinical signs of toxicity. Rats in the highest dose group exhibited abnormal behaviour, diarrhoea, rough coats, sunken eyes and other signs of toxicity after several days of dosing. Most of these rats were moribund before the last scheduled dose was administered. "Liver and spleen were more severely affected than other organs in the two highest dose groups. Livers contained diffuse pycnotic nuclei and, in some high-dose rats, focal areas of coagulative necrosis. In the high dose group aspartate and alanine aminotransferase activities were elevated, cytochrome P-450 concentration was decreased, and glutathione S-transferase activity was unchanged. Spleens were haemorrhagic and white pulp contained necrotic lymphocytes. White cell counts were decreased in a dose-related manner in the two highest dose groups. The gastrointestinal tract of high-dose rats contained pycnotic nuclei, and sites of necrosis were observed in the stomach, but these lesions were limited to several animals, and were generally mild. Pathologic changes in conjunction with decreased feed and water intake probably contributed to the general deterioration of high-dose rats that resulted in death". (Morrissey, 1985).

62. In a study by Gentles *et al.* (1999) the individual and combined effects of OTA and CPA were evaluated in Petersen x Hubbard broiler chickens from 1 day to 3 weeks of age. The experimental design was a 2 x 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed. CPA had a significant effect on body weight gain by the end of week 1 (11 % decrease compare to controls). For OTA and combination there was no significant change. Body weight gain was reduced ($P < 0.05$) by OA, CPA, and OA-CPA in combination at the end of 3 weeks. OTA significantly increased the relative weight of the kidney and serum uric acid and triglycerides and declines in total protein, albumin, and cholesterol were

observed. Increased relative weights of the pro-ventriculus and increased activity of creatine kinase were the effects of CPA. OA-CPA toxicity was characterized by increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid. Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen. (Gentles *et al.*, 1999)

63. Cullen *et al.* (1988) dissolved CPA in corn oil which was administered by gavage to broiler chicks daily, from the day of hatching for 23 days. Chicks were assigned to 4 groups (0, 1, 2, or 4 mg CPA/kg bw in corn oil). Each group was composed of 10 male and 10 female chicks. Surviving chicks were euthanatized and necropsied on day 24. In the group dosed with 4 mg/kg bw CPA 5/20 chicks died before day 23. Histologic examination revealed that the most common lesions consisted of necrosis and hemorrhage or hyperplasia of the mucosa of the proventriculus (up to 3 times normal depth) and hepatocellular vacuolation. Skeletal muscle degeneration, characterized by myofiber swelling or fragmentation accompanied by an infiltrate of macrophages and heterophils, was detected in the group given 4 mg/kg. This degeneration was associated with an increase of plasma creatine kinase activity. Focal hepatocellular and splenic necrosis also developed in the groups given 4 mg/kg. (Cullen *et al.*, 1988).

64. Dorner *et al.* (1983) artificially contaminated chicken rations with purified CPA at concentrations of 10, 50, and 100 µg/g and fed *ad libitum* to eight groups of chickens for 7 weeks. Chickens receiving feed with 100 µg/g of CPA had high mortality, decreased weight gain, and poor feed conversion when compared with birds receiving other doses. Postmortem examination showed that chickens fed the two greatest doses of CPA had proventricular lesions characterized by mucosal erosion and hyperemia (100 µg/g) and by thick mucosa and dilated proventricular lumens (50 µg/g). Birds given 100 µg/g of CPA in feed also had numerous yellow foci in their livers and spleens. Microscopic examination of tissues of birds that received 100 µg/g of CPA revealed ulcerative proventriculitis, mucosal necrosis in the gizzard, and hepatic and splenic necrosis and inflammation. Birds given 50 µg/g of CPA had microscopic lesions in the proventriculus (characterised by chronic mucosal inflammation and hyperplasia of the proventricular mucosal epithelium), liver, spleen and heart, however these lesions were generally more focal and less tissue destructive. Birds given 10 µg/g of CPA and control birds had no significant treatment-related lesions. (Dorner *et al.*, 1983).

65. In a study by Kamalavenkatesh *et al.* (2005) forty, newly hatched, unsexed broiler chicks were fed diets containing 10 ppm CPA and 1 ppm T2 toxin (T2) either individually or in combination for 28 days to study the immunopathological effects. Thymic atrophy and petechiae were observed in all toxin-fed birds. Necrotic foci were observed in the spleen of CPA fed birds. Lymphoid organs revealed lymphocytolysis and lymphoid depletion in all toxin fed birds. Changes in lymphocyte subsets in the thymus and spleen were assessed by measuring numbers of CD4⁺ and CD8⁺ lymphocytes. Thymic and

splenic CD4⁺ and CD8⁺ lymphocytes decreased significantly ($p < 0.01$) in toxin fed birds when compared to the control. Thymic CD8⁺ lymphocytes of T2 and CPA-T2 showed significant ($p < 0.01$) decrease from that of CPA and control groups. Splenic CD4⁺ and CD8⁺ lymphocytes showed significant ($p < 0.01$) decrease in CPA and CPA-T2 fed groups when compared to the control. The T2 group did not differ significantly from that of control. The stimulation index of splenocytes to concavalin A revealed a significant ($p < 0.01$) decrease in all toxin fed birds. A significant ($p < 0.01$) decrease was observed for the haemagglutination inhibition titres to Newcastle disease virus vaccine F strain of birds fed CPA, T2 and in combination. Significant ($p < 0.01$) interaction was found for lymphocyte subsets, SI and HI titres to NDV. The study indicated the immunosuppressive effect of these toxins either alone or in combination in broiler chicks. (Kamalavenkatesh *et al.*, 2005).

66. Kubena *et al.* (1994) investigated the effects of feeding 6 mg T2 toxin and 34 mg CPA/kg of diet singly and in combination were characterized in male broiler chicks from 1 day to 3 weeks of age. Body weights were depressed by CPA (from week 1 to 3) and T2 and the combination of T-2 and CPA (weeks 2 and 3). When compared with controls, relative weights of the kidney and pancreas were significantly increased only in the T2-CPA group, the relative weights of the proventriculus increased for CPA alone or in combination with T2, relative weights of the gizzard were increased in T2 alone or in combination with CPA, relative weights of the bursa were decreased in T2 alone, CPA alone or T2-CPA combination and the relative weights of the liver, heart and spleen were not altered by any of the treatments. There was a significant synergistic interaction between T2 and CPA for relative liver and kidney weights and serum cholesterol and triglyceride concentrations and a significant interaction between T2 and CPA for 3-week body weights and relative bursa of Fabricius weights, which were less than additive.

67. Dietary treatment effects on serum biochemical values, serum enzyme activities and haematology values varied. Neither the efficiency of feed utilization nor mortality was affected by dietary treatments. Oral lesions were present in a majority of the chicks fed diets containing T2 with or without CPA. When compared with controls, other variables measured exhibited additive or less than additive toxicity. These data demonstrate that T-2 and CPA alone and in combination can cause reduced performance and adversely affect broiler health. The effects of these mycotoxins may be exacerbated by other factors when under field conditions; hence, the potential detrimental effects of these two mycotoxins when present alone or in combination cannot be dismissed. (Kubena *et al.*, 1994).

68. In a study by Morrissey *et al.* (1987) male rats were divided into 9 groups and were administered 0, 0.1 or 4.0 mg CPA/kg bw/day intragastrically (three groups per dose level) for three consecutive days. Thirty minutes after each of these CPA doses, AFB1 was administered to the rats by gavage at 0, 0.1 or 2.0 mg AFB1/kg bw/day. Of the 12 rats given each of these nine treatments, 6 were killed on day 4, after the initial dosing, and the rest were allowed a recovery period of 4 days prior to termination. All groups except

those dosed with 2 mg/kg bw/day AFB1 gained weight. Weight loss in the three groups receiving 2.0 mg AFB1/kg/day occurred within 24 hours of the first doses. Feed consumption by these rats was about 60% of that in the other groups. The groups with a 4 week recovery period which had received 2 mg/kg bw/day AFB1 and high dose CPA had higher feed consumption (75 % controls) than those with low dose or no CPA (50 % controls). By the end of the recovery period, rats in these three groups had lost an average of 31-38 g.

69. Gross pathological findings were primarily limited to rats in the high AFB1 group. Prior to termination some of the rats receiving high CPA, in addition, were jaundiced. All groups with high AFB1 had shrunken liver and lesions in the kidney at the cortico-medullary junction. Microscopic changes were characteristic of aflatoxicosis in rats. At both dose levels CPA produced swollen endoplasmic reticulum (ER), and at the high dose, a loss of ribosomes from the ER. Glycocholic acid assays indicated liver damage only in those groups that received the high AFB1 dose. The authors concluded that neither toxin potentiates the action of the other at the dose levels used in this study. (Morrissey *et al.*, 1987)

70. Pier *et al.* (1989) divided guinea pigs into 4 groups of 8 or 9 animals which were dosed orally with 2.2 mg/kg CPA or 0.045 mg/kg AFB1⁵ singly or in combination in gelatin capsules. Doses were calculated according to mean group weights at the start of the experiment. On the 3rd day of toxin treatments a sensitising agent was injected into the guinea pigs and on the 10th day phytohaemagglutinin (PHA) was administered. On the 11th day cutaneous induration to PHA was measured. On the 20th day a sensitising agent was again injected and delayed cutaneous hypersensitivity calculated on day 21.

71. Clinical signs of intoxication (reduced emotive behaviour and mild dehydration) were first seen on day 3. There was a marked loss in body weight in the combined group by day 8 and 20 of these animals died on day 8 and by day 11, 6/9 guinea pigs in this group had died or been euthanized when considered moribund. Gross pathologic changes were generally confined to the liver and were predominantly in animals dosed with aflatoxins. Histopathologic changes were most notable in the combined group and consisted of cytoplasmic vacuolation of hepatocytes. This group also showed moderate thymic atrophy and 1 animal showed signs of acute tubular necrosis of the kidney. CPA treated animals had a lower degree of vacuole changes in centrilobular hepatocytes. Intracutaneous injections of PHA showed a significant reduction in proliferative response in aflatoxin, but not CPA-treated animals. An apparently reduced response was achieved in the combined group but did not achieve statistical significance. Tests for delayed cutaneous hypersensitivity showed a significant difference between the responses of aflatoxin and CPA. Animals treated with CPA showed a greater response but this was not statistically verified. AFB1 significantly suppressed the lymphoblastogenic response to PHA while CPA alone had no detectable effect. In combination, CPA appeared to neutralise the effect of AFB1 and

⁵ AFB2, AFG1 and AFG2 were also present. The mixture contained 50 mg B1, 25 mg G1, 2.5 mg B2 and 2.5 mg G2. The activity equivalence of AFB1 in the mixture was 79.3 %.

restored the count to normal levels. There were significant reductions in haemolytic complement titers in serum from the groups of animals that had received aflatoxin. (Pier *et al.*, 1989).

72. Smith *et al.* (1992) arranged eighty 1 day old male broiler chicks into 4 dosing groups of control, 3.5 mg/kg diet AF, 50 mg/kg diet CPA and 3.5 mg/kg AF and 50 mg/kg CPA for 3 weeks. The treatments were replicated 4 times. All treatments significantly reduced bodyweights. The reduction in bodyweight of birds dosed with AF alone in the 3rd week was 12.5 %, in birds dosed with CPA at weeks 1, 2 and 3 was 22.5, 20.5 and 32.6 %, respectively and those on combined diet at weeks 1, 2 and 3 was 15.8, 33.2 and 35.6 %, respectively. One bird died in each of the AF and CPA treatment groups and 3 birds died in the combined group. The relative liver weights were significantly increased by CPA alone and the combination. The relative weight of the kidney was significantly increased by all treatment groups. The relative pancreas weight was significantly increased only in the combination group. CPA alone and the combination significantly increased the relative weight of the proventriculus. The relative weight of the gizzard was not affected by any group and the relative bursa of Fabricius weights were significantly reduced by CPA alone and the combination. Values for serum total protein, albumin and cholesterol were significantly lower in the AF group than in controls, but values for uric acid and cholesterol in the CPA group were higher than controls. Significant interaction of AF and CPA altered serum albumin concentration. Serum glutamic transaminase activity was significantly increased by CPA alone, serum triglyceride values for AF were significantly lower than controls and other groups. Lactate dehydrogenase was significantly decreased by AF. AF and CPA singly and in combination lowered levels of serum phosphorous significantly when compared to controls. Blood urea nitrogen was significantly increased by AF and the combination over controls. (Smith *et al.*, 1992).

73. In a study by Hill *et al.* (1986) 24 SD rats were separated into 4 groups of 3 male and 3 female rats each and dosed ip with 0.1, 1 and 5 mg/kg bw CPA once daily. The control group was given 0.5 ml of 1N sodium bicarbonate. Effects of CPA on cell-mediated immunity were assessed by a delayed-type hypersensitivity test. Humoral immunity was also assessed by measuring antibody production after ip injection of sheep red blood cells (RBCs).

74. There were no clinical signs directly related to CPA, however 1 rat died in the 5 mg/kg group of diarrhoea, dehydration and weakness on day 28. Mean weight gains of the treated rats were lower than those of the controls, however the decrease was significant ($P < 0.05$) only in the 5 mg/kg group. All rats in the 5 mg/kg group had livers that appeared large with round edges when compared to the controls and lower dose groups. Mean liver, kidney and spleen weights did not differ significantly between control and treated groups. Microscopic lesions of the liver and kidneys were present in all treatment groups. Total protein, albumin, packed cell volume, and haemoglobin values were not significantly altered by CPA. The 1 and 5 mg/kg groups had higher mean neutrophil counts and lower mean eosinophil and lymphocyte counts

than controls. On days 3, 5 and 7 after sheep RBCs had been injected the geometric mean titers (GMT) were lower in treated rats than controls. By day 14 the GMTs of treated rats had exceeded the control group. Results of the delayed-type hypersensitivity test were inconclusive. (Hill *et al.*, 1986)

75. Malekinejad *et al.* (2011) divided 10 day old male broiler chicks into control and 10, 25 and 50 µg/kg CPA groups. CPA doses or control (saline) was given daily by crop gavage for 28 days. Body weight gain, serum level of alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), uric acid, creatinine, and blood urea nitrogen (BUN) were measured after 2 and 4 weeks exposure. Moreover, the total thiol molecules (TTM) and malondialdehyde (MDA) content of the liver and kidneys were assessed.

76. No birds died in any of the control or treatment groups. There were no significant differences in body weight gain between the control and test groups after 28 days. The authors were unable to collect the whole kidney from birds and weigh them, therefore only liver weights were assessed. After 28 days, elevation of hepatic weight was significant in the 25 and 50 µg/kg groups in what appeared to be a dose-dependent response. ALP and GGT were significantly increased in 25 and 50 µg/kg groups at 28 days compared to the controls. Serum uric acid, creatinine and BUN increased significantly in a time and dose-dependent manner. TTM content in both the kidney and liver was significantly reduced after 28 days exposure to 25 and 50 µg/kg CPA. After 28 days MDA content in the liver of birds dosed with 25 and 50 µg/kg was significantly higher than controls.

77. CPA-exposed birds showed dose-dependent pathologic alterations in the liver such as congestion, cell swelling, fatty degeneration, inflammatory cell infiltration, disintegration of the cells and necrosis. The necrotic reactions tended to appear around the bile ducts and were more apparent at the 50 µg/kg dose. The main pathological findings of the kidneys included tubular degeneration and necrosis, haemorrhages in the renal parenchyma, tubular cell swelling and ureter epithelial cells hyperplasia. (Malekinejad *et al.*, 2011).

78. In a study by Hinton *et al.* (1985) male SD rats were assigned to groups of 0, 0.2, 2, 4 and 8 mg/kg bw/day CPA with 8 animals in each group. Rats were dosed orally each day for 4 consecutive days by gastric intubation, fasted overnight on the fourth day and anaesthetised with ether and decapitated on day 5. Rats dosed with 8 mg/kg bw/day died prior to the fixation of tissues and are therefore not considered further. Some rats receiving 4 mg/kg bw/day exhibited non-specific toxic signs such as rough coat and sunken eyes, within 24 hours of the first dose. No clinical signs of toxicity were observed in other dose groups or controls. At 4 mg/kg bw/day there was a relatively large amount of cytoplasmic vesiculation which was not as apparent at lower doses. Every cell examined from the livers dosed with 2 and 4 mg/kg bw/day had this vesiculated appearance. Only about 25 % of the cells in the 0.2 mg/kg group were affected. Of 2 hepatocytes from the 0.2 mg/kg group 1 was similar in appearance to control cells and the other to hepatocytes from higher dose groups. There was dilatation of the ER in the affected cell with the formation of vesicles, apparently from the ER. Nuclei,

bile canaliculi, and associated membranes all appeared normal at this and higher doses. Higher doses had increased dilatation of the ER and more extensive distribution of vesicles throughout the cytoplasm. There was a dose-related increase in the width of the ER and degree of vesiculation. At the high dose level more ribosomes had been shed into the cytoplasmic matrix than at lower doses. Mitochondria of rats dosed with 2 or 4 mg/kg were swollen, with increased swelling at the higher dose. Mitochondrial membranes were intact in all sections examined. Lysing cells were only observed at 4 mg/kg. (Hinton *et al.*, 1985).

79. In a study by Lomax *et al.* (1984) 5- to 6-week-old crossbred pigs were given CPA at doses of 0, 0.01, 0.1, 1 and 10 mg/kg bw/day, orally for 14 days. Clinical signs observed by day 7 in pigs given 10 mg/kg bw were weakness, inactivity, anorexia, rough hair coats, and reduced body weights. These pigs also developed diarrhoea during week 2. The pigs given 1.0 mg/kg bw had roughened hair coats and were moderately inactive during week 2. At necropsy, lesions were observed only in pigs given 10 and 1.0 mg/kg bw of CPA. Gross lesions in 7/8 pigs dosed with 10 mg/kg bw were serosal and mucosal hyperemia, and hemorrhage throughout the small and large intestine. The pigs also had yellow, fibrin and necrotic cellular material in the lumen of the small intestine and pale livers, 2 with raised red foci on the surface of the hepatic lobes and extending into the hepatic parenchyma. The only gross lesion observed in pigs given 1 mg/kg bw was gastric ulceration, observed in only 1 pig. Microscopic gastroenteric lesions increased in severity with increasing dose. Lesions in pigs given 10 mg/kg body weight were necrotizing gastroenteritis, focal hepatocellular necrosis, hepatic peripheral lobular fatty change, and focal renal tubular nephrosis with focal suppurative tubulointerstitial nephritis. Pigs given 1.0 mg/kg body weight of cyclopiazonic acid had necrotizing gastritis and villous blunting in the jejunum and ileum. (Lomax *et al.*, 1984).

Subchronic

80. A study by Jaskiewicz *et al.* (1988) investigated the toxicity of CPA alone and in combination with AFB1 through 3 experiments in 16 vervet monkeys of both sexes. 1) One female monkey was given 1 mg/kg bw/day of CPA intragastrically and the dose was doubled every third day to eventually achieve 60 mg/kg bw/day. 2) Two males and 3 female monkeys (including female from experiment 1) were fed 20 mg/kg bw/day CPA. After 60 and 120 days 2 males and 1 female, and 2 females and a male and a female control animals, respectively were terminated. 3) Six monkeys received AFB1 0.1 mg/kg per day, 2 received only this, 2 received 1 mg/kg per day CPA in addition and 2 received 20 mg/kg per day CPA in addition and 2 control animals received solvent only. Results of experiments 1 and 2 showed low toxicity of CPA in non-human primates with mild changes of epithelial cells of the biliary and pancreatic ducts and renal medullary tubules, and minute tubular atrophy. More pronounced pathological changes were in hepatocyte rough endoplasmic reticulum, small vessels and myocardium. Combined treatment with CPA and AFB1 indicated lack of a synergistic cumulative effect of both toxins. Animals treated with AFB1 only or AFB1 and low dose CPA

developed more advanced liver lesions and died earlier than those which also received high doses of CPA. (Jaskiewicz *et al.*, 1988).

81. In a study by Voss *et al.* (1990) groups of 12 male Sprague-Dawley rats were given oral (gastric intubation) doses of 0, 0.2, 0.6, 2.0 or 4.0 mg CPA/kg bw/day for 13 consecutive weeks. No dose-related mortality or morbidity occurred. General appearance, behaviour, body weight gain and food consumption of all groups were similar. CPA had no definite adverse hematologic or serum chemistry effects, although serum creatinine concentrations of rats given 2.0 and 4.0 mg CPA/kg bw/day were increased after 7 and 13 weeks. Histopathologic effects were confined to the stomach. Acute inflammation of the lamina propria and submucosa of the glandular epithelium was found in 8/12, 11/12 and 11/12 rats dosed with 0.6, 2 and 4 mg/kg bw/day CPA, respectively. The neutrophilic infiltrate in rats receiving 2 mg/kg bw/day or greater was mild to moderate intensity and minimal to mild intensity in animals given less than or equal to 0.6 mg/kg bw/day. No other dose-related microscopic lesions were found. Changes in hepatic ultrastructure were a subtle disruption of the cisternal pattern of the endoplasmic reticulum with ribosomal detachment in animals receiving 4 but not 2 mg/kg bw/day. (Voss *et al.*, 1990).

82. Voss *et al.* (1990) repeated the 4 day study carried out by Morrissey *et al.* (1985) to compare the toxicity of the batches of CPA used. Rats were dosed with 8 mg/kg bw/day or vehicle, orally for 4 consecutive days. Clinical signs of toxicity and slight transient decreases in body weight were apparent in only 1 animal treated with 8 mg/kg bw/day CPA. The remaining 4 rats dosed with 8 mg/kg bw/day showed weight gain and food consumption comparable to controls. This suggested a difference in toxicity of the batches of CPA used. Voss *et al.* also noted that epimerisation of CPA occurs under basic conditions and differences in absorption and/or toxicity of CPA epimers may exist and influence the outcomes of studies. (Voss *et al.*, 1990).

83. In a study by Nuehring *et al.* (1985) CPA in gelatin capsules was administered to five groups of dogs of unknown age and breeding status twice a day for 90 days at 0, 0.05, 0.25, 0.5, and 1.0 mg/kg bw/day (final doses are 0, 0.1, 0.5, 1 and 2 mg/kg bw/day). All dogs administered the 0.5 and 1.0 mg of CPA/kg doses and 1 dog given the 0.25 mg of CPA/kg dose died or were humanely killed before the scheduled termination of the study. Clinical signs of intoxication were not observed in the remainder of the dogs during the 90 days trial. Clinical signs of intoxication appeared 2 to 44 days after dosing was started and consisted of anorexia and, in 1 to 2 days, vomiting, diarrhoea, pyrexia, dehydration, weight loss, and CNS depression. Grossly, the entire alimentary tract had diffuse hyperemia with focal areas of hemorrhage and ulceration. Other lesions were renal infarcts, necrotizing epididymitis, and ulcerative dermatitis. Microscopic lesions included ulceration, necrosis, vasculitis, lymphoid necrosis, karyomegaly in several organs including the liver, kidneys, bladder and skin, and decreased mitotic activity in intestinal crypt epithelium. Ulcerative and necrotic lesions were usually associated with vascular lesions. Clinical pathologic changes were increased numbers of white blood cells, neutrophils and monocytes and a decrease in the number of

lymphocytes, and increased serum alkaline phosphatase activity. (Nuehring *et al.*, 1985).

Chronic

84. No chronic studies for CPA were identified in the literature search.

Reproductive/Developmental Toxicity

85. Morrissey, Cole and Dorner (1984) randomly assigned sperm-positive female Fischer rats to 1 of 4 dose groups. Daily doses of 0, 1, 5 or 10 mg/kg bw CPA in 1 N sodium bicarbonate were given by gastric intubation to 64 females on days 8 – 11 of pregnancy and to 53 females on days 12 – 15 of pregnancy. Sacrifice took place on day 21 of pregnancy. Dams in both groups receiving 10 mg/kg bw CPA showed clinical signs of toxicity of rough fur coats and diarrhoea. One rat of each group died with additional signs including uncoordinated movements, inability to maintain posture, closed eyes and decreased feed consumption. In both groups there was no dose effect on mean body weight gained and no effect on the number of pregnant animals. There were no significant differences in pup weights, percentage pre- or postimplantation losses, or fetal deaths, compared to controls. Skeletal malformations and aberrations were present in pups from dams treated with 10 mg/kg bw but these defects and variations were not significantly increased compared to controls. Significant differences in skeletal development included retardation of ossification of cervical centra (d 12-15) and caudal vertebrae (d 8-11) in the two highest dose groups. Retardations of development were the most common manifestations of embryotoxicity. There were no statistically significant post-mortem gross pathologic findings in dams sacrificed at term. Although high dose rats had abnormal livers and spleens with coagulative necrosis and single cell necrosis. The authors concluded that since significant maternal toxicity occurred at the highest dose level in the absence of fetal malformations, the teratogenic potential of CPA is low. (Morrissey, Cole and Dorner, 1984).

86. Nishie, Cole and Dorner (1987) determined an approximate oral LD50 using 16 nonpregnant mice (4 per dose) which were maintained for 7 days. These animals were also checked for body temperature, spontaneous motor activity and pain reflex time. The approximate oral LD50 of nonpregnant mice was 63 ± 4.4 mg/kg bw. The clinical signs preceding death are identical to those observed with ip administration (Nishie *et al.*, 1985): hypothermia, ptosis, gait disturbance, hypokinesia, dyspnea and action tremor. Delayed death was preceded by prostration and cessation of water and feed intake. In nonpregnant mice surviving near lethal doses (50, 60, 70 mg/kg bw, orally) the estrous cycle returned at the expected time interval. (Nishie, Cole and Dorner, 1987).

87. Pregnant mice were dosed (15, 20, 30, 45 or 50 mg/kg) with CPA in the early phase of pregnancy (day 2-8). Male mice used in this study were untreated. A limited number of pregnant mice were treated with 66 mg/kg ergonovine maleate (orally, subcutaneous) to compare its effect with that of

an equivalent dose of CPA (50 mg/kg). Among control sperm-positive mice treated with oral 1 M sodium bicarbonate solution, 97.5% were gravid on necropsy day (pregnancy day 12). A single oral dose of CPA (15-50 mg/kg) given on days 2 to 8, decreased the pregnancy rates significantly. In general the pregnancy rates decreased with increasing dose of CPA. In groups treated with a single dose of 50 mg/kg CPA on pregnancy day 4 to 8, vaginal hemorrhage was observed 1 to 7 days after treatment, and it usually resulted in termination of pregnancy (abortion). Fetal resorption rates were higher than the control rate only in the groups treated with 30 mg/kg CPA on day 4 or 8. CPA decreased body weight gains and the weights of uteri with fetuses. The ovary weights were generally not changed. Ergonovine maleate (66 mg/kg) had no significant effect on all of the parameters examined. (Nishie, Cole and Dorner, 1987).

88. Khera *et al.* (1985) randomly assigned 15 to 20 mated females per test group (4, 8 or 16 mg/kg bw in 1 N sodium bicarbonate) and a control group. Doses were administered once daily by oesophageal intubation for 4 consecutive days from days 9 – 12 of pregnancy. Necropsy occurred on day 19 of pregnancy. There were no overt signs of toxicity or body weight suppression in dams during pregnancy or at necropsy at daily doses of up to 16 mg/kg bw CPA. A slightly decreased incidence of pregnancy was observed at all doses, but was not dose-related or statistically significant. At all test doses, the incidence of live, runted and dead fetuses, resorptions and male/female fetuses were within the control range. Mean fetal weight failed to show a dose-related effect. The number of malformations and aberrations in fetuses of all treated groups were not statistically different from control values. (Khera *et al.*, 1985).

Genotoxicity

89. In a study by Kuilman-Wahls (2002) CPA was applied at 225, 450 and 900 nmol/plate in the Ames test, using TA98 and TA100 *Salmonella typhimurium* strains. Testing was performed with and without metabolic activation by S9-liver fractions obtained from Arochlor treated rats. Cyclopiazonic acid was not mutagenic in the presence or absence of metabolic activation. However in the presence of AFB1, cyclopiazonic acid was shown to dose-dependently inhibit the mutagenic activity of AFB1. (Kuilman-Wahls *et al.*, 2002).

90. Sorensen (1984) tested 0.01, 0.03, 0.1, 0.3 and 1.0 $\mu\text{mol/plate}$ CPA, with and without S9 activation in *Salmonella typhimurium* strains TA98 and TA100. CPA was mutagenic to *S. typhimurium* TA98 and TA100 strains in the presence of metabolic activation. The correlation coefficients between concentration and the number of revertants per plate were 0.974 and 0.898 with metabolic activation for TA98 and TA100, respectively. In the absence of metabolic activation there was no correlation between concentration and mutagenic response. A specific activity of 140.74 revertants/ μmol was estimated for CPA, compared to a specific activity of 6.24 revertants/ μmol for AFB1. (Sorensen, Tucker and Simpson, 1984).

91. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were incubated with 0.25, 2.5, 25 and 250 µg/plate in the presence and absence of S9-mix obtained from Aroclor treated rats. There was no mutagenic activity for any of the CPA concentrations in the presence or absence of S9. (Wehner *et al.*, 1978).

HBGV

92. There is currently no HBGV for CPA.

Exposure Assessment

93. Most food samples analysed were below the LOD (0.5 µg/kg) or between the LOD and LOQ. “Brown bread” and “herbs and spices” samples contained levels of 0.79 and 0.89 µg/kg, respectively which are below the LOQ but above the LOD. One sample, “other snacks, not potato” contained CPA at 4.27 µg/kg. There are currently no limits for CPA in foods in legislation. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 1a-c).

94. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.004 and 0.001 – 0.011 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.007 and 0.005 – 0.018 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.002 – 0.009 and 0.007 – 0.022 µg/kg bw/day.

Table 1a. Estimated CPA chronic exposures from the TDS for infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.001	0.001- 0.003	0.000- 0.002	0.003- 0.009	0.001- 0.004	0.004- 0.011

Table 1b. Estimated CPA chronic exposures from the TDS for young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.001- 0.006	0.005- 0.015	0.001- 0.007	0.007- 0.018

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

Table 1c. Estimated CPA chronic exposures from the TDS for young children aged 18 to 60 months ($\mu\text{g}/\text{kg}$ bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.002- 0.009	0.008- 0.019	0.002- 0.009	0.007- 0.022

Risk characterisation

95. There is currently no HBGV against which to compare these exposures. Most food samples analysed were below the LOD ($0.5 \mu\text{g}/\text{kg}$) or between the LOD and LOQ.

Conclusions

Questions on which the views of the Committee are sought

96. Members are invited to consider the following questions
- i). Do Members think that data from these studies could be used to derive an HBGV? And if so are there any studies for which Members would like more details provided?
 - ii). A number of the published studies are in broiler chickens. How relevant is this for Man?
 - iii). Is there any other information that Members would like provided?

Secretariat

June 2017

References

- Burdock GA and Flamm WG. (2000). Review article: Safety assessment of the mycotoxin cyclopiazonic acid. *International Journal of Toxicology*. 19: 195-218. Available at:
<http://journals.sagepub.com/doi/abs/10.1080/10915810050074964?journalCode=ijtb>
- Byrem TM, Pestka JJ, Chu FS, Strasburg GM. (1999) Analysis and pharmacokinetics of cyclopiazonic acid in market weight pigs *J Anim Sci*. Jan;77(1):173-9. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/10064041>
- Chang PK1, Ehrlich KC and Fujii I. (2009). Cyclopiazonic acid biosynthesis of *Aspergillus flavus* and *Aspergillus oryzae*. *Toxins (Basel)*. **1(2)**: 74-99. doi: 10.3390/toxins1020074. Epub 2009 Nov 6. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/22069533>
- Cullen JM, Wilson M, Hagler WM Jr, Ort JF, Cole RJ. (1988) Histologic lesions in broiler chicks given cyclopiazonic acid orally. *Am J Vet Res*. 1988 May;49(5):728-31. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/3395020>
- De Waal EJ. (2002). Safety assessment of cyclopiazonic acid. *Int J Toxicol*. **21(5)**: 425-7; discussion 429, 431. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/12396689>
- Dorner JW, Cole RJ, Lomax LG, Gosser HS, Diener UL. (1983) Cyclopiazonic acid production by *Aspergillus flavus* and its effects on broiler chickens. *Appl Environ Microbiol*. 1983 Sep;46(3):698-703. Available at:
<http://aem.asm.org/content/46/3/698.long>
- Gentles A, Smith EE, Kubena LF, Duffus E, Johnson P, Thompson J, Harvey RB, Edrington TS. (1999) Toxicological evaluations of cyclopiazonic acid and ochratoxin A in broilers. *Poult Sci*. 1999 Oct;78(10):1380-4. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/10536785>
- Hill JE, Lomax LG, Cole RJ, Dorner JW. (1986) Toxicologic and immunologic effects of sublethal doses of cyclopiazonic acid in rats. *Am J Vet Res*. 1986 May;47(5):1174-7. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/3717743>
- Hinton DM, Morrissey RE, Norred WP, Cole RJ, Dorner J. (1985) Effects of cyclopiazonic acid on the ultrastructure of rat liver. *Toxicol Lett*. 1985 May;25(2):211-8. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/4002250>
- Jaskiewicz K, Close PM, Thiel PG, Cole RJ. (1988) Preliminary studies on toxic effects of cyclopiazonic acid alone and in combination with aflatoxin B1 in non-human primates. *Toxicology*. Nov 30;52(3):297-307. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/3142106>

Kamalavenkatesh P, Vairamuthu S, Balachandran C, Manohar BM, raj GD. (2005) Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. *Mycopathologia*. 2005 Feb;159(2):273-9. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/15770454>

Khera KS, Cole RJ, Whalen C, Dorner JW. (1985) Embryotoxicity study on cyclopiazonic acid in mice. *Bull Environ Contam Toxicol*. Mar;34(3):423-6. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/3978280>

King ED, Bobby Bassi AB Jr, Ross DC and Druebbisch B. (2100). An industry perspective on the use of "atoxigenic" strains of *Aspergillus flavus* as biological control agents and the significance of cyclopiazonic acid. *Toxin Rev*. **30(2-3)**: 33-41. Epub 2011 Jul 19. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/22844262>

Kubena LF, Smith EE, Gentles A, Harvey RB, Edrington TS, Phillips TD, Rottinghaus GE. (1994) Individual and combined toxicity of T-2 toxin and cyclopiazonic acid in broiler chicks. *Poult Sci*. 1994 Sep;73(9):1390-7. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/7800637>

Kuilman-Wahls ME, Vilar MS, de Nijs-Tjon L, Maas RF and Fink-Gremmels J. (2002). Cyclopiazonic acid inhibits mutagenic action of aflatoxin B(1). *Environ Toxicol Pharmacol*. **11(3-4)**:207-12. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/21782604>

Lomax LG, Cole RJ, Dorner JW. (1984) The toxicity of cyclopiazonic acid in weaned pigs. *Vet Pathol*. 1984 Jul;21(4):418-24. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/6464302>

Malekinejad H, Akbari P, Allymehr M, Hobbenaghi R, Rezaie A. (2011) Cyclopiazonic acid augments the hepatic and renal oxidative stress in broiler chicks. *Hum Exp Toxicol*. 2011 Aug;30(8):910-9. doi: 10.1177/09603271110384285. Epub 2010 Sep 27. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/20876160>

Morrissey RE, Cole RJ, Dorner JW. (1984) The effects of cyclopiazonic acid on pregnancy and fetal development of Fischer rats. *J Toxicol Environ Health*. 1984;14(4):585-94. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/6512883>

Morrissey RE, Norred WP, Cole RJ, Dorner J. (1985) Toxicity of the mycotoxin, cyclopiazonic acid, to Sprague-Dawley rats. *Toxicol Appl Pharmacol*. 1985 Jan;77(1):94-107. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/3966246>

Morrissey RE, Norred WP, Hinton DM, Cole RJ, Dorner JW. (1987) Combined effects of the mycotoxins aflatoxin B1 and cyclopiazonic acid on Sprague-Dawley rats. *Food Chem Toxicol*. 1987 Nov;25(11):837-42. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/3121482>

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

Nishie K, Cole RJ, Dorner JW. (1985) Toxicity and neuropharmacology of cyclopiazonic acid. *Food Chem Toxicol.* 1985 Sep;23(9):831-9. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/4043883>

Nishie K, Cole RJ, Dorner JW. (1987) Toxic effects of cyclopiazonic acid in the early phase of pregnancy in mice. *Res Commun Chem Pathol Pharmacol.* Mar;55(3):303-15. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/3575873>

Norred WP, (1990) Cyclopiazonic acid: toxicity and tissue distribution. *Vet Hum Toxicol.* 1990;32 Suppl:20-5; discussion 25-6. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/2092402>

Norred WP, Morrissey RE, Riley RT, Cole RJ, Dorner JW. (1985) Distribution, excretion and skeletal muscle effects of the mycotoxin [¹⁴C]cyclopiazonic acid in rats. *Food Chem Toxicol.* 1985 Dec;23(12):1069-76. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/4076936>

Nuehring LP, Rowland GN, Harrison LR, Cole RJ, Dorner JW. (1985) Cyclopiazonic acid mycotoxicosis in the dog. *Am J Vet Res.* Aug;46(8):1670-6. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/3929655>

Pier AC, Belden EL, Ellis JA, Nelson EW, Maki LR. (1989) Effects of cyclopiazonic acid and aflatoxin singly and in combination on selected clinical, pathological and immunological responses of guinea pigs. *Mycopathologia.* 1989 Mar;105(3):135-42. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/2761608>

Porter JK, Norred WP, Cole RJ, Dorner JW. (1988) Neurochemical effects of cyclopiazonic acid in chickens. *Proc Soc Exp Biol Med.* 1988 Mar;187(3):335-40. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/2894672>

Purchase IF, (1971). The acute toxicity of the mycotoxin cyclopiazonic acid to rats. *Toxicol Appl Pharmacol.* **18(1)**:114-23. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/5542818>

Smith EE, Kubena LF, Braithwaite CE, Harvey RB, Phillips TD, Reine AH. (1992) Toxicological evaluation of aflatoxin and cyclopiazonic acid in broiler chickens. *Poult Sci.* 1992 Jul;71(7):1136-44. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/1641377>

Sorenson WG, Tucker JD and Simpson JP. (1984). Mutagenicity of tetramic mycotoxin cyclopiazonic acid. *Appl Environ Microbiol.* **47(6)**:1355-7. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/6430233>

van Rensburg SJ. (1984) Subacute toxicity of the mycotoxin cyclopiazonic acid. *Food Chem Toxicol.* 1984 Dec;22(12):993-8. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/6542548>

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

Venkatesh PK, Vairamuthu S, Balachandran C, Manohar BM, Raj GD. (2005) Induction of apoptosis by fungal culture materials containing cyclopiazonic acid and T-2 toxin in primary lymphoid organs of broiler chickens. *Mycopathologia*. 2005 Apr;159(3):393-400. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/15883725>

Voss KA, Norred WP, Hinton DM, Cole RJ, Dorner JW. (1990) Subchronic oral toxicity of cyclopiazonic acid (CPA) in male Sprague-Dawley rats. *Mycopathologia*. 1990 Apr;110(1):11-8. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/2352547>

Wehner FC, Thiel PG, van Rensburg SJ and Demasius IP. (1978). Mutagenicity to *Salmonella typhimurium* of some *Aspergillus* and *Penicillium* mycotoxins. *Mutat Res*. 1978 Nov;58(2-3):193-203. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/370570>

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

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

Ergot alkaloids

Background

97. An EFSA opinion (2012⁶) has been used as the basis for this risk characterisation of ergot alkaloids.
98. Ergot alkaloids (EAs) infest plant species including commercially important grains such as rye, wheat, rice, corn, barley, millet and oat. More than 50 different EAs have been identified but the total amounts and patterns vary between fungal strains, geographic regions and host plants. The disease which infects more than 400 plant species is known as ergot. (EFSA, 2012).
99. Fungal hyphae invade the ovule of the host grass and colonize the whole ovary. Three to four weeks after infection it becomes visible and replaces the kernels of the grain ears. These sclerotia are dark, crescent shaped, protruding from regular grains of the ear and are the final stage of the disease. (EFSA, 2012).
100. EAs show a broad spectrum of pharmacological effects and have been used in medical applications. EAs or EA-derived products have been applied or tested for prolactin inhibition, treatment of Parkinsonism, cerebrovascular insufficiency, venous insufficiency, thrombosis, emboli, stimulation of cerebral and peripheral metabolism, and are still applied for migraine and uterine stimulation. In addition, lysergic acid diethylamide (LSD), a semi synthetic derivative of the EA-family was legally introduced as a pharmaceutical in the mid-1950s. (EFSA, 2012).
101. The following EAs have been measured in the TDS: ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine. Although the -inine forms are described to be biologically inactive on the neuroreceptor sites, an interconversion under alkaline or acidic conditions can take place and thus both forms have to be considered in the risk assessment (EFSA, 2012).
102. Human data are available for the naturally occurring alkaloids used as pharmaceuticals, ergometrine and ergotamine.

⁶ EFSA, 2012 available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2798>

Previous evaluations

103. Ergot alkaloids have previously been evaluated by WHO (IPCS, 1990), for the risk as contaminants in food for human consumption. The Committee for Veterinary Medicinal Products of the European Agency for the Evaluation of Medicinal Products (EMA, 1999) evaluated the veterinary use of ergometrine maleate for the control of postpartum uterine haemorrhages. BfR (2004) assessed the risk of the consumption of rye flours contaminated with EAs based on the estimated intakes of ergotamine and ergometrine. EFSA (2005) assessed EAs as undesirable substances in animal feed in the food chain. The French Food Safety Agency (AFSSA) assessed qualitatively the risk arising from the presence of EAs in the food chain for human and animal consumption (AFSSA, 2009).

HBGV

104. There is inadequate data for the genotoxic potential of EAs other than ergotamine. The data available did not indicate bacterial or mammalian cell mutation. There is inconsistent *in vivo* data but there is some evidence of clastogenicity. In a 2-year carcinogenicity study, crude ergot induced neurofibromas were found on the ears at 5 % and 2 %, but not 1 % in the diet, and these regressed if the ergot was withdrawn. The doses of ergot causing ear neurofibromas also produced significant decreases in body weight gains. It appeared that the tumorigenicity was exacerbated by a low protein diet. The absence of carcinomas and the regression indicate aetiology related to a non-genotoxic mode of action. (EFSA, 2012).

105. EAs can act on a number of neurotransmitter receptors particularly adrenergic, dopaminergic and serotonergic receptors. The effects of these receptor interactions may be acute or long-term therefore EFSA established an ARfD and a TDI. Vasoconstriction was considered to be the critical effect and selected for the derivation of the HBGVs. Dose response analyses, in the form of bench mark dose (BMD) analysis, were performed on the data from the subchronic study on ergotamine (Speijers *et al.*, 1993) and the subacute study on α -ergocryptine (Janssen *et al.*, 2000a, b).

Derivation of an ARfD

106. The lowest BMDL₁₀ for incidence of tail muscular atrophy was 0.33 mg/kg bw/day and was calculated from the 13-week rat feeding study of ergotamine (Speijers *et al.*, 1993). To establish an ARfD, EFSA concluded that an uncertainty factor of 3 was required to take into account deficiencies in the database, such as incomplete information on reproductive toxicity. The default uncertainty factor of 100 for intra and inter species differences was also applied. Therefore an overall uncertainty factor of 300 was applied to the BMDL₁₀, to produce a group ARfD of 1 μ g/kg bw for the sum of ergot alkaloids.

Derivation of a TDI

107. In considering a TDI EFSA concluded that an additional uncertainty factor of 2, should be applied to the uncertainty factor of 300 used in the derivation of the ARfD, for extrapolation from sub-chronic to chronic studies. Overall an uncertainty factor of 600 was applied to the BMDL of 0.33 mg/kg bw/day to establish a group TDI of 0.6 µg/kg bw/day for the sum of ergot alkaloids.

108. EFSA noted that the group ARfD is 2-fold below the lowest single dose of 2 µg/kg bw ergometrine used to induce uterine contractions. EFSA concluded that this value is close to a NOEL and that the margin between this dose in a sensitive subpopulation and the group ARfD is adequate.

109. The lowest prescribed dose of ergotamine used in the treatment of migraine is approximately 10 to 20 times higher than the group ARfD and 20 to 40 times higher than the group TDI. The group TDI (0.6 µg/kg bw) is 13 times lower than the maximum recommended dose for therapeutic use of ergotamine, which should not exceed 8 µg/kg bw/day over a period of 30 days in order to avoid severe side effects.

Exposure Assessment

110. Total ergot alkaloid chronic exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. Acute exposures (Tables 1a-c) used occurrence data from the TDS and a portion-size approach.

111. All bread samples contained some or all of the 12 ergot alkaloids. Levels found ranged from <1 - 60.1 µg/kg for individual ergot alkaloid in the samples. Brown bread contained a total of 322 µg/kg ergot alkaloids. Ergot alkaloids were also detected in sandwiches, at a similar level to bread samples, and at lower levels in other cereal products such as flour, breakfast cereals, biscuits and pizza. The alkaloids were not detected in branded food drinks, beer, cider or alternatives to milk. (Stratton *et al.*, 2015).

112. Once the exposures had been calculated the main contributing groups for total ergot alkaloid exposures were “miscellaneous cereals breakfast cereals”, “white sliced bread” and “wholemeal and granary bread”.

Acute

113. Mean and 97.5th percentile acute total ergot alkaloid exposures for infants aged 4 to 12 months ranged from 0.01 – 0.108 and 0.050 – 0.283 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.134 – 0.172 and 0.321 – 0.404 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.148 – 0.167 and 0.329 – 0.345 µg/kg bw/day.

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Table 1a. Estimated acute total ergot alkaloids exposures from the UK TDS in infants aged 4 to 12 months

4 to 6 month-olds ¹ (n=116)		6 to 9 month-olds ¹ (n=606)		9 to 12 month-olds ¹ (n=686)	
µg/kg bw/day		µg/kg bw/day		µg/kg bw/day	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.010 - 0.011	0.050 - 0.063	0.052 - 0.060	0.249 - 0.252	0.098 - 0.108	0.282 - 0.283

Table 1b. Estimated acute total ergot alkaloids exposures from the UK TDS in young children aged 12 to 18 months

12 to 15 month-olds ¹ (n=670)		15 to 18 month-olds ¹ (n=605)	
µg/kg bw/day		µg/kg bw/day	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.134 - 0.148	0.321 - 0.325	0.156 - 0.172	0.393 - 0.404

Table 1c. Estimated acute total ergot alkaloids exposures from the UK TDS in young children aged 18 to 60 months

18 to 24 month-olds ^{2,3} (n=118)		24 to 60 month-olds ^{2,3} (n=688)	
µg/kg bw/day		µg/kg bw/day	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.148 - 0.166	0.329 - 0.345	0.156 - 0.167	0.332 - 0.345

Chronic

114. Chronic exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 2a-c). Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0.005 – 0.064 and 0.030 – 0.179 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.081 – 0.107 and 0.205 – 0.257 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.093 – 0.108 and 0.204 – 0.234 µg/kg bw/day, respectively.

Table 2a. Estimated total ergot alkaloids chronic exposures from the TDS: infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.005	0.030 - 0.031	0.027 - 0.032	0.118 - 0.133	0.056 - 0.064	0.169 - 0.179

Table 2b. Estimated total ergot alkaloids chronic exposures from the TDS: young children aged 12 to 18 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.081 - 0.091	0.205 - 0.213	0.094 - 0.107	0.243 - 0.257

Table 2c. Estimated total ergot alkaloids chronic exposures from the TDS: young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.093 - 0.106	0.219 - 0.234	0.098 - 0.108	0.204 - 0.227

Risk characterisation

115. Total ergot alkaloid calculated mean and 97.5th percentile acute exposures for infants and young children ranged from 0.010 to 0.40 $\mu\text{g}/\text{kg bw}/\text{day}$, all of which are below the ARfD of 1 $\mu\text{g}/\text{kg bw}$ established by EFSA in 2012.

116. Total ergot alkaloid mean and 97.5th percentile chronic exposures ranged from 0.005 to 0.26 $\mu\text{g}/\text{kg bw}/\text{day}$, all of which are below the TDI of 0.6 $\mu\text{g}/\text{kg bw}/\text{day}$ established by EFSA in 2012.

Conclusions

117. Exposures to total ergot alkaloids at the levels measured in the TDS are unlikely to be of toxicological concern in either the acute or the chronic situation in infants and young children aged 0 to 5 years.

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References

- AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (2009). Évaluation des risques liés à la présence de mycotoxines dans le chaînes alimentaires humaines et animale. Rapport Final, May 2009. Available at: <https://www.anses.fr/en/system/files/RCCP-Ra-Mycotoxines2009.pdf>
- BfR (Federal Institute for Risk Assessment) (2004). Mutterkornalkaloide im Roggenmehl. Stellungnahme des BfR vom 22.01 2004. Available from http://www.bfr.bund.de/cm/208/mutterkornalkaloide_in_roggenmehl.pdf
- EFSA (2005). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission related to ergot as undesirable substance in animal feed. The EFSA Journal, 225, 1-27. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/225>
- EFSA (2012). Scientific Opinion on Ergot alkaloids in food and feed. *EFSA Journal* **10(7)**:2798. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2798>
- EMA (European Agency for the Evaluation of Medicinal Products) (1999). Committee for veterinary medicinal products – Ergometrine maleate Summary Report. Veterinary Medicines Evaluation Unit. EMA/MRL/237/97-FINAL, June 1999. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014181.pdf
- WHO-IPCS (World Health Organization - International Programme on Chemical Safety), 1990. Selected Mycotoxins: Ochratoxins, Trichothecenes, Ergot. Environmental Health Criteria, 105. Available at: <http://www.inchem.org/documents/ehc/ehc/ehc105.htm>

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

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

Fumonisin

Background

118. A JECFA technical report (2017⁷) has been used as the basis for this risk characterisation of fumonisins.

119. Fumonisin are produced by *Fusarium verticillioides*, *F. proliferatum* and *F. fujikuroi*, as well as some less common *Fusarium* species, for example *F. anthophilum*, *F. dlamini*, *F. napiforme* and *F. thapsinum*. There are 4 main B fumonisins: Fumonisin B1, B2, B3 and B4 and these are the major forms found in food. Fumonisin B1 (FB1), Fumonisin B2 (FB2) and fumonisin B4 (FB4) are also produced by *Aspergillus niger*. Fumonisin are common contaminants of maize and have also been found in rice, grapes, green coffee beans, onions, mango, corn and other cereals, peanuts and dried fruits. (FAO/WHO, 2017; FAO/WHO, 2012).

Previous evaluation

120. JECFA have previously evaluated fumonisins in 2001 (FAO/WHO, 2001) and 2012 (FAO/WHO, 2012). The same HBGV was derived on both occasions.

HBGV

121. In 2011 data from a short term dose-response study of liver toxicity in male transgenic mice fed diets containing purified FB1 was used to derive an HBGV. A BMDL₁₀ of 0.165 mg/kg bw/day was calculated for megalocytic hepatocytes and after application of an uncertainty factor of 100 a group PMTDI of 2 µg/kg bw was derived for FB1, FB2 and FB3, alone or in combination. As this was the same as the PMTDI derived at the 2001 meeting, the group PMTDI was retained. (FAO/WHO, 2017; FAO/WHO, 2012)

122. For the most recent evaluation, a final report had been published of the study which had been used in the evaluation in 2012. (In 2012, a preliminary report had been provided.) The final report contained a number of changes but the committee concluded that these slight differences would not

⁷ JECFA technical report available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

change the overall toxicological assessment performed by the committee of 2011. (FAO/WHO, 2017)

123. In light of this updated report the committee reviewed the previous dose-response analysis and confirmed it.

124. The committee reaffirmed that fumonisins are associated with a wide range of toxic effects but the liver and kidneys are the most sensitive target organs. The committee concluded that the updated study of the 2012 evaluation was still the most relevant for the evaluation of fumonisins. Having reviewed the updated data the committee concluded that it would not change the overall toxicological evaluation. The PMTDI of 2 µg/kg bw, established previously for FB1, FB2 and FB3, alone or in combination, was retained. (FAO/WHO, 2017).

Exposure Assessment

125. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. The results from almost all of the food sample groups that were analysed for fumonisins were below the LOD⁸. These exposures provided in Table X are LB and UB. FB1 was detected in the sample of “herbs and spices” at a level of 5.53 µg/kg, below the LOQ of 7.15 µg/kg.

126. For fumonisin exposures, for all age groups, the mean values were all below 0.089 µg/kg bw/day and the 97.5th percentile exposures were all below 0.188 µg/kg bw/day (Table 1).

⁸ LODs for FB1 ranged from 3.87 µg/kg, for beers and cider to 7.95 µg/kg for flour, and chocolate biscuits which had a much higher LOD of 37.1 µg/kg. For FB2 LODs were in the range 3.4 to 8.9 µg/kg apart from chocolate biscuits where it was 36.8 µg/kg. For FB3 LODs were in the range 3.1 to 6.5 µg/kg, with an LOD in chocolate biscuits of 31.5 µg/kg.

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Table 1. Estimated fumonisin chronic exposures from the TDS in infants and young children aged 4 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

Fumonisin	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)		12 to 15 month-olds		15 to 18 month-olds		18 to 24 month-olds		24 to 60 month-olds	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
B1	0.000- 0.008	0.000-0.032	0.000- 0.030	0.001- 0.107	0.000- 0.052	0.001- 0.135	0.000- 0.071	0.001- 0.156	0.000- 0.083	0.001- 0.188	0.000- 0.089	0.002- 0.152	0.000- 0.080	0.001- 0.153
B2	0.000- 0.007	0.000-0.026	0.000- 0.027	0.000- 0.103	0.000- 0.048	0.000- 0.128	0.000- 0.067	0.000- 0.150	0.000- 0.078	0.000- 0.172	0.000- 0.085	0.000- 0.150	0.000- 0.077	0.000- 0.143
B3	0.000- 0.006	0.000-0.025	0.000- 0.024	0.000- 0.088	0.000- 0.042	0.000- 0.112	0.000- 0.058	0.000- 0.130	0.000- 0.068	0.000- 0.157	0.000- 0.073	0.000- 0.127	0.000- 0.066	0.000- 0.125

Risk characterisation

127. Total fumonisin results were not provided to the FSA as part of the TDS and due to the inconsistent reporting of the total fumonisins across the EU it is not certain whether total exposures could be calculated from the data available. Therefore in this paper comparison to the PMTDI of 2 µg/kg bw has been used for individual fumonisins.

128. All calculated mean and 97.5th percentile exposures of FB1, FB2 and FB3 for all age groups are below the PMTDI of 2 µg/kg bw.

129. It is uncertain whether summing of the fumonisin exposures would change the risk characterisation.

Conclusions

130. In this current risk assessment the calculated mean and 97.5th percentile fumonisin exposures for all age groups were below the TDI of 2 µg/kg bw and it is unlikely that there would be a toxicological concern.

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References

FAO/WHO (2001). WHO Food Additives Series 47. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm>

FAO/WHO (2012). WHO Food Additives Series 65. Safety evaluation of certain food additives and contaminants. Prepared by the Seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: http://apps.who.int/iris/bitstream/10665/44813/1/9789241660655_eng.pdf

FAO/WHO (2017). WHO Technical Report Series. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

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

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

Moniliformin

Introduction

131. There are currently no EU or international evaluations of moniliformin. However EFSA are due to publish an opinion on this mycotoxin in December 2017. All moniliformin TDS sample results are below the LOD or LOQ. It was decided that a risk characterisation should be completed once EFSA have published an opinion.

Exposure Assessment

132. Moniliformin is a very small, charged analyte which is highly soluble in water. The analysis of moniliformin in the TDS was not straightforward, especially due to the number of food matrices analysed for the TDS. Recovery results were very low, however “the sensitivity of the LC-MS/MS method and the fact that every sample was overspiked at 25 µg/kg meant that even with very low recovery reasonable LOQs could be determined, and if moniliformin had been present in the samples it would have been detected” (Stratton *et al.*, 2015).

133. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. The results from all of the food sample groups that were analysed for moniliformin were below the LOD or LOQ. The exposures provided in Tables 1a-c are LB and UB.

134. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.072 and 0 – 0.178 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.134 and 0 – 0.309 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.149 and 0 – 0.289 µg/kg bw/day.

Table 1a. Estimated moniliformin chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile

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0.000-0.010	0.010-0.047	0.000-0.037	0.000-0.136	0.000-0.072	0.000-0.178
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Table 1b. Estimated moniliformin chronic exposures from the TDS young children aged 12 to 18 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000-0.110	0.000-0.238	0.000-0.134	0.000-0.309

Table 1c. Estimated moniliformin chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000-0.148	0.000-0.289	0.000-0.149	0.000-0.283

Risk characterisation

135. There is currently no HBGV against which to compare these exposures. EFSA are due to publish an opinion on moniliformin in December 2017 after which, a risk characterisation will be carried out. However all values are below the LOD and LOQ.

Conclusions

136. It is currently not possible to determine the level of risk to infants and young children, however the levels in all food samples analysed were below the LOD or LOQ for moniliformin. A risk characterisation will be performed after EFSA have published their evaluation in December 2017.

Questions on which the views of the Committee are sought

137. Members are invited to consider the following questions

- i). Do Members agree that the EFSA opinion on moniliformin, once published, should be used in the risk characterisation?

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References

Stratton J., Anderson S., Leon I., Hepworth P., Chapman S., Christy J., Jardine S., Philips D., Setter R., Clough J. and MacDonald S. (2015) Final report diet study (TDS) – mycotoxin analysis. FS102081. Unpublished report.

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

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

Ochratoxin A (OTA)

Background

138. An EFSA opinion (2006⁹) and an EFSA statement (2010¹⁰) have been used as the basis for this risk characterisation of OTA.

139. In 2010 EFSA evaluated 5 research articles providing recent toxicity of OTA and concluded that the data did not alter the evaluation carried out in 2006. EFSA published a statement to this effect (EFSA, 2010).

140. OTA is produced by several fungi (*Penicillium* and *Aspergillus* species), and occurs naturally in a variety of plant products such as cereals, coffee beans, beans, pulses and dried fruit all over the world. It has also been detected in products such as coffee, wine, beer and grape juice and occurs in kidney, liver and blood from farm animals by transfer from animal feed. (EFSA, 2010).

Previous evaluations

141. OTA has also been evaluated by JECFA (FAO/WHO, 2001; FAO/WHO, 2007). In 2007 JECFA concluded that new data that had become available on the mode of action of OTA on the kidney did not indicate any reason for the HBGV established by JECFA in 2001 to be altered. The PTWI of 100 ng/kg bw was retained (FAO/WHO, 2007). The SCF evaluated OTA in 1996 and 1998 (EC, 1996; EC, 1998). In 1998, with concerns about potential genotoxicity of OTA the SCF recommended that exposures should be reduced as much as possible and kept to the lower end of a range of TDI's of 1.2 – 14 ng/kg bw/day, preferably below 5 ng/kg bw/day.

HBGV

142. In its 2006 opinion, EFSA considered that there was an absence of conclusive evidence that OTA binds to DNA therefore EFSA concluded that the hazard characterisation should be based on nephrotoxicity. The most sensitive and pivotal effects of OTA are its effects on the kidneys in rats and pigs.

⁹ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/365>

¹⁰ EFSA statement available at: <https://www.efsa.europa.eu/en/efsajournal/pub/1626>

143. The studies selected by EFSA were in female pigs. The LOAEL for progressive nephropathy was 40 µg ochratoxin A/kg bw/day in the diet for 2 years. The NOAEL in the same study was 8 µg/kg bw/day. In a 90-day feeding study in female pigs 8µg ochratoxin A/kg bw/day was reported to produce effects on renal enzymes and renal function tests.

144. EFSA concluded that 8 µg/kg bw/day was a LOAEL representing an early marker of renal toxicity in experimental animals (i.e. female pigs) and likely to be close to a NOAEL as the observed changes in biochemical markers indicated transient changes in the kidneys.

145. EFSA used the LOAEL of 8 µg/kg bw/day for risk characterisation and applied uncertainty factors to derive a tolerable intake of ochratoxin A for humans. A default factor of 2.5 was used to account for the toxicodynamic effects of interspecies differences and an additional uncertainty factor of 6 was applied for kinetic differences. A factor of 10 was used for the extrapolation from average to potentially sensitive human populations and an additional uncertainty factor of 3 was used to take into account the use of a LOAEL instead of a NOAEL.

146. The total uncertainty factor applied to the LOAEL of 8 µg/kg bw/day was 450 resulting in a TDI of approximately 18 ng/kg bw.

147. However, given the relatively long half-life of ochratoxin A in humans, EFSA considered that a tolerable weekly intake (TWI) of up to 120 ng/kg bw was more appropriate.

148. EFSA (2010) reviewed new toxicological data for OTA and concluded that the information provided would neither contradict nor change the conclusions reached in the 2006 opinion. The TWI of 120 ng/kg bw was retained.

Exposure Assessment

149. Exposures (calculated on a weekly basis for ease of comparison with the TWI) were calculated using data from the TDS and consumption data from DNSIYC and NDNS. The results from almost all of the food sample groups that were analysed for OTA were below the LOQ. These exposures provided in Tables 1a-c are lower bound (LB) and upper bound (UB). OTA was detected in some food groups. Levels of 5.6, 1.65, 0.63 0.53 and 0.45 µg/kg were detected in “fruit and vegetable juices”, “dried fruit”, “herbs and spices”, “brown bread” and “granary bread” samples, respectively. The main food group contributing to total OTA exposure in infant and young children was “fruit and vegetable juices”.

150. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0.007 – 0.028 and 0.056 – 0.22 µg/kg bw/week, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.042 – 0.077 and 0.44 – 0.48 µg/kg bw/week.

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Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.13 – 0.18 and 0.71 – 0.90 µg/kg bw/week.

Table 1a. Estimated OTA chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
µg/kg bw/day	0.001	0.008-0.009	0.002	0.018	0.003-0.004	0.030-0.031
µg/kg bw/week	0.007	0.056-0.063	0.014	0.126	0.021-0.028	0.21-0.22

Table 1b. Estimated OTA chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

	12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
µg/kg bw/day	0.006-0.008	0.063-0.065	0.008-0.011	0.065-0.068
µg/kg bw/week	0.042-0.056	0.44-0.46	0.056-0.077	0.46-0.48

Table 1c. Estimated OTA chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

	18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
µg/kg bw/day	0.018-0.021	0.102-0.105	0.023-0.026	0.125-0.129
µg/kg bw/week	0.13-0.15	0.71-0.74	0.16-0.18	0.88-0.90

Risk characterisation

151. All mean exposures for children under the age of 18 months and the 97.5th percentile exposures for children aged 4 to <6 month-olds were below the TWI. These exposures are not of toxicological concern.

152. Mean exposures for children aged 18 to 60 months were 110 – 150 % of the TWI. These exceedences of the TWI only minor exceedences and

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exposures would be unlikely to remain at these levels for a prolonged period of time. These exposures are not of toxicological concern.

153. Exposures at the 97.5th percentile for infants aged 6 to <12 months range from 120 – 180 % of the TWI. Again, these exceedences of the TWI are only minor exceedences, and exposures would be unlikely to remain at these levels for a prolonged period of time. These exposures are not of toxicological concern.

154. Exposures at the 97.5th percentile for young children aged 12 to <18 months range from 370 to 400 % of the TWI. If exposure to OTA were to remain at these levels over a period of time it would not be possible to rule out a risk to health.

155. Exposures at the 97.5th percentile for children aged 18 to 60 months were 590 - 750 % of the TWI. If exposures to OTA remained at this level for a prolonged period there could be some risk to health.

Conclusions

156. Mean exposures for infants and young children aged 4 to <18 months and 97.5th percentile exposures for infants aged 4 to <12 months are less than or only slightly exceed the TDI and are unlikely to be of toxicological concern.

157. Exposures at the 97.5th percentile for young children aged 12 to <18 months, if maintained it would not be possible to rule out a risk to health.

158. Exposures at the 97.5th percentile for children aged 18 to 60 months if maintained, could be of some risk to health.

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References

EFSA (2006). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to ochratoxin A in food. *The EFSA Journal* **3**(5): 1 – 56. Available at:

<https://www.efsa.europa.eu/en/efsajournal/pub/365>

EFSA (2010). Statement on recent scientific information on the toxicity of Ochratoxin A. EFSA Panel on Contaminants in the Food Chain. *EFSA Journal* **8**(6): 1626. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/1626>

FAO/WHO (2001). WHO Food Additives Series 47. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm>

FAO/WHO (2007). WHO Food Additives Series 59. Safety evaluation of certain food additives and contaminants. Prepared by the Sixty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Available at:

http://apps.who.int/iris/bitstream/10665/43823/1/9789241660594_eng.pdf

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

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

Patulin

Background

159. A JECFA monograph (FAO/WHO, 1995¹¹) has been used as the basis for this risk characterisation of OTA.

160. Patulin is produced by certain species of the genera *Aspergillus* and *Penicillium*, including *A. clavatus*, *P. expansum*, *P. patulum*, *P. aspergillus* and *P. byssochlamys*. *P. expansum* is a common spoilage microorganism in apples. The major potential dietary sources of patulin are apples and apple juice made from affected fruit (FAO/WHO, 1995).

Previous evaluation

161. The most recent evaluation of patulin was conducted by JECFA (FAO/WHO, 1995). Prior to that JECFA evaluated patulin in 1990. In this evaluation JECFA established a PTWI of 7 µg/kg bw (FAO/WHO, 1990). The SCF, in 1994 agreed with the PTWI of 7 µg/kg bw established by JECFA, in 1990 (SCF, 1994). In 2000, the SCF produced a minute statement and endorsed the PMTDI of 0.4 µg/kg bw established by JECFA in 1995 (SCF, 2000).

HBGV

162. The pivotal study used by JECFA (1995) to determine an HBGV was a combined reproductive toxicity, long-term toxicity/carcinogenicity study published by Becci *et al.*, (1981). Concentrations of patulin in citrate buffer of 0, 0.1, 0.5 or 1.5 mg/kg bw/day were administered to groups of 70 FDRL Wistar rats of each sex, by gavage, 3 times/week for 2 years. The rats, derived from the F1 generation showed increased mortality in both sexes at the highest dose. All males had died by 19 months whereas 19% of females survived until termination at 2 years. Body weights of males were reduced at the mid and high dose, but females body weights were comparable in all groups. No difference in tumour incidence was observed. The NOEL in this study was 0.1 mg/kg bw, administered 3 times weekly, equivalent to 43 µg/kg bw/day. (FAO/WHO, 1995)

¹¹ JECFA monograph available at:
<http://www.inchem.org/documents/jecfa/jecmono/v35je16.htm>

163. Based on this NOEL and with an additional uncertainty factor of 100 applied, JECFA established a PMTDI of 0.4 µg/kg bw was established. (FAO/WHO, 1995).

Exposure Assessment

164. Patulin exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 1a-c). The results from all of the food groups that were analysed for patulin were below the LOD. Individual LODs calculated for the samples analysed ranged from 1.7 µg/kg for the mushroom sample to 13.6 µg/kg for the cereal sample.

165. The mean total lower bounds in all age groups for patulin are zero and thus it is not possible to attribute any food group contributing to total exposure.

166. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.114 and 0 – 0.293 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.171 and 0 – 0.364 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.177 and 0 – 0.324 µg/kg bw/day.

Table 1a. Estimated patulin chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.023	0.000- 0.099	0.000- 0.072	0.000- 0.242	0.000- 0.114	0.000- 0.293

Table 1b. Estimated patulin chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.151	0.000- 0.318	0.000- 0.171	0.000- 0.364

Table 1c. Estimated patulin chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th	Mean	97.5 th

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	percentile		percentile
0.000- 0.177	0.000- 0.324	0.000- 0.164	0.000- 0.299

Risk characterisation

167. Mean and 97.5th percentile exposures of infants aged 0 to 12 months and young children aged 12 to 60 months (Tables X, X and X) are all below the PMTDI of 0.4 µg/kg bw/day.

Conclusions

168. The levels of patulin measured in the food groups in the TDS are not of toxicological concern for infants and young children aged 1 to 5 years.

Secretariat

June 2017

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It does not reflect the views of the Committee and should not be cited.

References

Becci PJ, Hess FG, Johnson WD, Gallo MA, Babish JG, Dailey RE and Parent RA. (1981). Long-term carcinogenicity and toxicity studies of patulin in the rat. *J Appl Toxicol.* **1(5)**: 256-61.

FAO/WHO (1990). WHO Food Additives Series 26. Toxicological evaluation of certain food additives and contaminants. Prepared by: The 35th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v26je01.htm>

FAO/WHO (1995). WHO Food Additives Series 35. Toxicological evaluation of certain food additives and contaminants. Prepared by: The 44th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v35je16.htm>

SCF (1996). Reports of the Scientific Committee for Food (Thirty-fifth series). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_35.pdf

SCF (2000). Minute statement on patulin. Expressed by the Scientific Committee on Food during the plenary meeting on 8 March 2000. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out55_en.pdf

TOX/2017/30 ANNEX I

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

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

Sterigmatocystin (STC)

Background

169. A JECFA Technical Report (2017¹²) and an EFSA opinion (EFSA, 2013¹³) have been used as the basis for this risk characterisation of STC.

¹² JECFA monograph available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

¹³ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/3254>

170. STC is mainly produced by more than a dozen species of *Aspergillus* as well as by a number of phylogenetically and phenotypically different fungal genera. *A. versicolor* is the most common source. STC shares its biosynthetic pathway with aflatoxins (AF). Wild-type *A. nidulans* and *A. versicolor* are apparently unable to biotransform STC into O-methylsterigmatocystin, the direct precursor of aflatoxin B1 (AFB1) and G1 (AFG1). Consequently, substrates colonised by these fungi can contain high amounts of STC, while substrates invaded by *A. flavus* and *A. parasiticus* contain only low amounts of STC as most is converted into AFs. (FAO/WHO, 2017; EFSA, 2013).

171. STC is generally produced in storage, rather than in the field, and has been found in grains and grain-based products, green coffee beans spices, beer, peanuts, crispbread, rye wholemeal, rice, white bread, muesli, chilli and cheese. (FAO/WHO, 2017; EFSA, 2013).

Previous evaluation

172. There is no evaluation, prior to the EFSA opinion (EFSA, 2013).

HBGV

173. Sterigmatocystin exhibits genotoxic effects *in vitro*, *in vivo* and *ex vivo* and carcinogenicity of sterigmatocystin has been demonstrated after oral, intraperitoneal, subcutaneous and/or dermal exposure in the animal species tested. EFSA evaluated the dose-response effects of using data from available carcinogenicity bioassays using oral administration of sterigmatocystin in mice, rats and monkeys. (EFSA, 2013)

174. After consideration of a number of studies EFSA concluded that only one study was suitable for dose-response assessment (Maekawa *et al.*, 1979). This study reported a dose-dependent incidence of liver tumours in rats. EFSA noted that the study was not suitable as the basis for risk characterisation as the tumours were of different origins (hepatocellular carcinomas (HCC) and haemangiosarcomas and one tumour was not specified). The incidence of HCC could not be analysed for dose-response assessment (only one tumour-bearing animal in the highest dose group). EFSA concluded that it was appropriate to perform BMD analysis on the incidence of haemangiosarcomas (0 animals with tumours in the control and low dose groups, 1 animal with tumours in the mid dose group and 3 animals with tumours at the high dose group). The lowest BMDL₁₀ value obtained from the fitted models was 0.16 mg/kg bw/day with a BMD₁₀ of 0.36 mg/kg bw/day. (EFSA, 2013)

175. EFSA noted that the number of animals with haemangiosarcomas was only 11 % of the total number of animals with tumours and the overall incidence of tumours in the control group was 64 %. This BMD₁₀/BMDL₁₀ pair is based on a limited tumourigenicity database. (EFSA, 2013)

176. A dose-response relationship was also observed for necrosis and hyperplastic foci and/or hyperplastic areas; these were not subjected to dose-

response analysis since it is not current practice to use such data in risk characterisation of substances that are genotoxic and carcinogenic. (EFSA, 2013)

177. The study by Maekawa was also considered the pivotal study by JECFA. The critical end point selected was hepatic hemangiosarcoma in male rats. As for the EFSA evaluation, when JECFA used the US EPA’s BMDS software a BMDL₁₀ of 0.16 mg/kg bw/day was calculated. JECFA calculated a BMDL₁₀ model-average estimate of 0.30 mg/kg bw/day. JECFA selected the BMDL₁₀ of 0.16 mg/kg bw/day as the value to use as the POD for MOE assessment. (FAO/WHO, 2017).

Exposure Assessment

178. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 1a-c). Three samples contained sterigmatocystin below the LOQ but above the LOD at levels from 0.46 to 2.17 µg/kg. These were chocolate biscuits (0.46 µg/kg), white unsliced bread (0.58 µg/kg) and herbs & spices (2.17 µg/kg). These 3 results are not quantitative as they are outside the reliable quantification range but indicates a low level presence of this analyte. However once the exposures to all the food groups, for all age ranges had been calculated it was not possible to attribute any food groups contributing to total exposure

179. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.002 and 0 – 0.007 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.004 and 0.001 – 0.009 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.005 and 0.001 – 0.011 µg/kg bw/day.

Table 1a. Estimated sterigmatocystin chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000	0.000- 0.001	0.000- 0.001	0.001- 0.005	0.000- 0.002	0.001- 0.007

Table 1b. Estimated sterigmatocystin chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile

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0.000-0.003	0.001-0.008	0.000-0.004	0.001-0.009
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Table 1c. Estimated sterigmatocystin chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000-0.005	0.001-0.011	0.000-0.005	0.002-0.011

Risk characterisation

180. MOEs for sterigmatocystin have been calculated (Tables 2a-c) based on the fold-difference between the lowest BMDL₁₀ value of 0.16 mg/kg bw/day (EFSA, 2013; FAO/WHO, 2017) and the chronic exposures. All values are greater than 10,000.

Table 2a. MOEs calculated for estimated sterigmatocystin chronic exposures from the TDS in infants aged 4 to 12 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
	> 160000	>160000	160000-32000	> 80000	160000-23000

Table 2b. MOEs calculated for estimated sterigmatocystin chronic exposures from the TDS young children aged 12 to 18 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
> 53000	160000-20000	> 40000	160000-18000

Table 2c. MOEs calculated for estimated sterigmatocystin chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile

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> 32000	160000- 15000	> 32000	80000- 15000
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Conclusions

181. EFSA (2005) is of the view that in general an MOE of 10,000 or higher, if it is based on the BMDL₁₀ from an animal study, would be of low concern from a public health point of view. It is therefore likely that the infant and young exposures to sterigmatocystin are of low concern.

Secretariat

June 2017

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References

EFSA (2013). Scientific Opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. *EFSA Journal* **11(6)**: 3254. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/3254>

FAO/WHO (2017). WHO Technical Report Series. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

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

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

Zearalenone (ZEN)

Background

182. An EFSA opinion (EFSA, 2011¹⁴) has been used as the basis for this risk characterisation of ZEN.

183. ZEN is produced by several *Fusarium* species, particularly *F. graminearum* and also *F. culmorum*, *F. equiseti* and *F. verticillioides*. ZEN is commonly found in maize but also in wheat, barley, sorghum and rye in various countries. Generally, the *Fusarium* species can grow and invade crops in moist cool field post-harvest under poor storage conditions. *F. graminearum* also produces trichothecenes, such as deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, 4-acetyl nivalenol and FUS-X.

Previous evaluation

184. ZEN was previously evaluated by JECFA (2000) and a PMTDI of 0.5 µg/kg bw was established. The SCF also evaluated ZEN in 2000 and established a temporary TDI of 0.2 µg/kg bw (SCF, 2000). EFSA also reviewed ZEN in 2004 as a substance in animal feed. It was concluded that animal-derived foods would only contribute marginally to dietary exposure. The temporary TDI was not reviewed on this occasion.

HBGV

185. Zearalenone does not cause gene mutations in bacterial test systems but is clastogenic and aneugenic *in vitro* and clastogenic *in vivo*, in the mouse (EFSA, 2011). Based on limited evidence in experimental animals for the carcinogenicity of zearalenone, IARC has classified it as group 3 (not classifiable as to carcinogenicity in humans) (IARC, 1993).

186. Since limited evidence has shown carcinogenicity of zearalenone, and it is clastogenic EFSA modelled the dose response. Liver and pituitary adenomas were observed in an NTP study (NTP, 1982). EFSA selected the incidence of pituitary adenomas in male B6C3F1 mice for dose-response modelling. The incidence of pituitary adenomas was 0/40, 4/45 and 6/44 for zearalenone doses of approximately 0, 8 and 17 mg/kg bw/day. The BMR was

¹⁴ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2197>

chosen as 10 % extra risk. BMD modelling was carried out using US EPA BMDS 2.1.2 software from which EFSA selected the lowest BMDL₁₀ of 6.39 mg/kg bw/day as the POD to be used in calculating the MOEs between the BMDL₁₀ and the TDI.

187. EFSA acknowledged that there was a wide variability in the sensitivity of species to oestrogenic effects of zearalenone. These effects are observed in pigs at doses approximately 3 orders of magnitude lower than doses reported to cause clastogenicity and increases in adenomas in mice. EFSA decided to establish a TDI for zearalenone based on its oestrogenic effects. The female pig is the most sensitive species and sex to the oestrogenic effects of zearalenone.

188. EFSA made a weight of evidence assessment and considered studies on immature and mature pigs. A study by Doll *et al.*, (2003), supported by data from other studies was selected by EFSA as the critical study to use for the derivation of a TDI. The NOEL¹⁵ derived in this study was 10.4 µg/kg bw/day.

189. EFSA concluded that the human female would not be more sensitive to the effects of oestrogens than the female pig. Therefore when deriving an uncertainty factor to apply to the NOEL, EFSA decided not to use a factor of 2.5 for interspecies toxicodynamics. The overall uncertainty factor of 40 was calculated from a factor of 4 for interspecies toxicokinetics and a factor of 10 for interhuman variability. The application of this uncertainty factor to the NOEL of 10 mg/kg bw/day resulted in the derivation of a group TDI of 0.25 µg/kg bw/day.

190. The margin between the BMDL₁₀ of 6.39 mg/kg bw/day and the TDI of 0.25 µg/kg b.w. was in the region of 25,000. This exceeds the value of 10,000, established by EFSA (2009) as of low concern for a genotoxic carcinogen. Because the genotoxicity of zearalenone may be related to oxidative stress mediated mechanisms, and it is at most a weak carcinogen, EFSA concluded that the critical effects of zearalenone relate to its oestrogenicity. (EFSA, 2011).

191. The TDI of 0.25 µg/kg bw was later established by EFSA as a group TDI for zearalenone and its modified forms (EFSA, 2016).

Exposure Assessment

192. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 1a-c). Five samples contained residues above the LOD but below the LOQ, levels ranged from 0.57 to 1.92 µg/kg, although these values are not quantitative. The pizza sample contained a

¹⁵ “The CONTAM Panel considered that these are NOELs rather than NOAELs because it is not possible to conclude that the effects described above in pigs at the LOEL are adverse in terms of later fertility and reproductive performance. However, although the estrogenicity per se may not be adverse it is undesirable and indicative of adverse effects.” (EFSA, 2011).

level of 16.5 µg/kg and was the highest level measured in all the samples. All levels measured were well below the maximum permitted levels in legislation (Stratton *et al.*, 2015). The snacks group made the main contribution to total zearalenone exposure in infants over 6 months and in young children.

193. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.006 and 0 – 0.021 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.004 – 0.012 and 0.039 – 0.048 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.005 – 0.016 and 0.031 – 0.056 µg/kg bw/day.

Table 1a. Estimated zearalenone chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.001	0.000- 0.004	0.001- 0.004	0.002- 0.015	0.001- 0.006	0.012- 0.021

Table 1b. Estimated zearalenone chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.004- 0.011	0.041- 0.048	0.004- 0.012	0.039- 0.047

Table 1c. Estimated zearalenone chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.005- 0.014	0.031- 0.048	0.007- 0.016	0.042- 0.056

Risk characterisation

194. Mean and 97.5th percentile exposures of infants aged 0 to 12 months and young children aged 12 to 60 months are all below the group TDI of 0.25 µg/kg bw/day.

This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

Conclusions

195. The levels of zearalenone measured in the food groups in the TDS are unlikely to be of toxicological concern for infants aged 4-12 months and young children aged 1 to 5 years.

Secretariat

June 2017

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It does not reflect the views of the Committee and should not be cited.

References

- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. *The EFSA Journal*. **1150**: 1-72. Available at: <http://www.efsa.europa.eu/en/efsajournal/pub/1150>
- EFSA (2011). Scientific Opinion on the risks for public health related to the presence of zearalenone in food. *EFSA Journal* **9(6)**: 2197. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/2197>
- EFSA (2016). Appropriateness to set a group health-based guidance value for zearalenone and its modified forms. *EFSA Journal*. **14(4)**: 4425. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/4425>
- FAO/WHO (2000). Food Additives Series 44. Safety evaluation of certain food additives and contaminants. Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v44jec14.htm>
- IARC 1993. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 56. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf>
- NTP (National Toxicology Program), 1982. NTP 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
- SCF (2000). Opinion of the Scientific Committee on Food on *Fusarium* toxins. Part 2: Zearalenone (ZEA). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out65_en.pdf

TOX/2017/30 ANNEX K

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

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

Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-Ac-DON) and 15-acetyldeoxynivalenol (15-Ac-DON)

Background

196. A JECFA monograph (FAO/WHO, 2011¹⁶) has been used as the basis for this risk characterisation of DON, 3-Ac-DON and 15-Ac-DON.

197. DON and its acetylated derivatives are members of the tricothecenes family along with diacetoxyscirpenol, fusarenon-X, neosolaniol, nivalenol, and T2 and HT2, all considered in this scoping paper. DON is a type B tricothecene and is produced mainly by the *Fusarium* species. 3-Ac-DON and 15-AC-DON are also naturally occurring fungal secondary metabolites. DON is a common contaminant in cereals including wheat, maize, barley, oats, rye and rice, and their products. It has been measured beer, bread, pasta, biscuits and muesli

Previous evaluation

198. The most recent evaluation of 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol was conducted by JECFA (FAO/WHO, 2011). Prior to that JECFA evaluated DON in 2001. In this evaluation JECFA had established a PMTDI of 1 µg/kg bw (FAO/WHO, 2001). The SCF has previously published opinions on DON (1999 and 2002) with a TDI of 1 µg/kg bw/day established.

199. EFSA was due to publish an Opinion on DON in January 2017, but this is still not yet available. Once this opinion is published, should there be any significant changes in the value for a TDI or an ARfD it will be brought to the attention of the Committee.

HBGV

Acute

200. JECFA (2011) considered emesis the critical end point for acute effects as this effect has consistently been observed after DON intoxication in both experimental animals and humans. The emetic effect was considered to be dependent on the maximum plasma concentration, therefore JECFA considered that the data to be used to derive an ARfD should come from dietary studies rather than those that have used gavage.

201. JECFA combined data from 2 studies, on emesis in pigs and piglets following exposure to DON via the diet, for BMD modelling. The doses were calculated from the measured DON concentrations and the observed feed

¹⁶ JECFA monograph available at:
http://apps.who.int/iris/bitstream/10665/44520/1/9789241660631_eng.pdf

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intake. In the first study, feed intakes were drastically reduced (88 – 94 % compared with controls), and body weights decreased during the test period, at dietary concentrations above 3 mg/kg feed. For these groups it was assumed that the total intake over the 4 or 1 days was actually consumed on day 1, because it has been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg feed or more, compared with controls. The initial body weights were used for the calculations, because the emesis was observed on day 1 of exposure.

202. JECFA used the PROAST software (23.2) to perform BMD analysis with the BMR set at 10 % extra risk. The BMDL₁₀, among the accepted models, ranged from 0.21 to 0.74 mg/kg bw/day, the lowest of which was used for the POD, for establishing an ARfD.

203. The Committee derived a group ARfD for DON and its acetylated derivatives using the BMDL₁₀ of 0.21 mg/kg bw/day. JECFA considered that because “DON-induced emesis is a systemic effect and more dependent on C_{max} than on area under the plasma concentration–time curve (AUC), it would be appropriate to apply an uncertainty factor of 25 (used by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for acute C_{max}-dependent effects)” (FAO/WHO, 2011). An ARfD of 8 µg/kg bw/day was established. Limited data from human case reports indicate that dietary exposures up to 50 µg/kg bw/day are not likely to induce emesis. (FAO/WHO, 2011).

Chronic

204. Groups of 50 male and 50 female B6C3F1 mice were given diets containing deoxynivalenol at the equivalent of 0, 0.1, 0.5, and 1.1 mg/kg bw per day in males and 0, 0.1, 0.7, and 1.6 mg/kg bw per day in females for 2 years (Iverson *et al.*, 1995). Survival was not significantly affected. Average daily food consumption was unchanged in females, but that of males was significantly reduced by about 8% at the two higher doses. There were decreases in body weights. At 0.5 mg/kg bw/day the relative weight of the liver in males was decreased. When dosed at 1.1 mg/kg bw/day: the relative weight of the liver and spleen was decreased in males and the relative weight of the testis significantly increased. No increase in the incidence of preneoplastic or neoplastic changes was observed. The NOEL was 1 mg/kg of diet, equal to 0.1 mg/kg bw per day. (FAO/WHO, 2001).

205. The results of this 2-year feeding study in mice did not suggest that deoxynivalenol presents a carcinogenic hazard. The Committee considered that this study was appropriate for evaluation of other long-term effects. Although the mean body weight of animals at the lowest dose was lower than that of controls, the difference was considered not to be biologically significant, and no toxicological changes were observed at this dose. The Committee established a provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg bw on the basis of the NOEL of 100 µg/kg bw per day in this study

and a safety factor of 100. The Committee concluded that intake at this level would not result in effects of deoxynivalenol on the immune system, growth, or reproduction. (FAO/WHO, 2001)

206. Repeated-dose short-term studies considered in the 2011 evaluation indicated that this NOAEL of 100 µg/kg bw/day remains appropriate (FAO/WHO, 2011).

207. JECFA decided to convert the PMTDI for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON) because 3-Ac-DON is converted to DON and therefore contributes to the total DON-induced toxicity. In this regard, the Committee considered the toxicity of the acetylated derivatives equal to that of DON.

Exposure Assessment

208. Acute exposures used occurrence data from the TDS and a portion-size approach (Tables 1a-c). 3-Ac-DON, 15-Ac-DON and DON chronic exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 2a-c). 3-Ac-DON and 15-Ac-DON were not detected in any samples above the LOD. DON was detected in all cereal products, snack and sandwiches at levels from 11.2 to 166 µg/kg. DON was also measured at levels between the LOD and LOQ in herbs and spices, vegetable oil and beer (Stratton *et al.*, 2015).

Acute

209. Mean and 97.5th percentile acute 15-Ac-DON exposures for infants aged 4 to 12 months ranged from 0 – 0.176 and 0 – 0.455 µg/kg bw, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.259 and 0 – 0.577 µg/kg bw. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.270 and 0 – 0.584 µg/kg bw.

210. Mean and 97.5th percentile acute 3-Ac-DON exposures for infants aged 4 to 12 months ranged from 0 – 0.032 and 0 – 0.081 µg/kg bw, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.046 and 0.003 – 0.104 µg/kg bw. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.001 – 0.050 and 0.003 – 0.099 µg/kg bw.

211. Mean and 97.5th percentile acute DON exposures for infants aged 4 to 12 months ranged from 0.042 – 0.363 and 0.225 – 0.974 µg/kg bw, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.483 – 0.592 and 1.187 – 1.422 µg/kg bw. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.549 – 0.588 and 1.135 – 1.320 µg/kg bw.

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Table 1a. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol acute exposures from the TDS in infants aged 4 to 12 months ($\mu\text{g}/\text{kg}$ bw/day)

	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.038	0.000-0.159	0.000-0.119	0.000-0.393	0.000-0.176	0.000-0.455
3-Ac-DON	0.000-0.008	0.001-0.037	0.000-0.022	0.002-0.079	0.001-0.032	0.003-0.081
DON	0.042-0.047	0.225-0.243	0.196-0.208	0.856-0.869	0.345-0.363	0.964-0.974

Table 1b. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol acute exposures from the TDS young children aged 12 to 18 months ($\mu\text{g}/\text{kg}$ bw/day)

	12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.231	0.000-0.500	0.000-0.259	0.000-0.577
3-Ac-DON	0.001-0.042	0.003-0.096	0.001-0.046	0.003-0.104
DON	0.483-0.508	1.187-1.202	0.563-0.592	1.365-1.422

Table 1c. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol acute exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg}$ bw/day)

	18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.270	0.000-0.584	0.000-0.238	0.000-0.500
3-Ac-DON	0.001-0.050	0.003-0.099	0.001-0.041	0.003-0.082
DON	0.549-0.584	1.135-1.174	0.557-0.588	1.292-1.320

Chronic

212. Mean and 97.5th percentile chronic 15-Ac-DON exposures for infants aged 4 to 12 months ranged from 0 – 0.112 and 0 – 0.290 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.172 and 0 – 0.386 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.180 and 0 – 0.338 µg/kg bw/day.

213. Mean and 97.5th percentile chronic 3-Ac-DON exposures for infants aged 4 to 12 months ranged from 0 – 0.020 and 0 – 0.054 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.030 and 0.002 – 0.064 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.034 and 0.002 – 0.062 µg/kg bw/day.

214. Mean and 97.5th percentile chronic DON exposures for infants aged 4 to 12 months ranged from 0.022 – 0.222 and 0.131 – 0.592 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.296 – 0.374 and 0.711 – 0.857 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.346 – 0.385 and 0.681 – 0.768 µg/kg bw/day.

215. The food groups contributing the highest exposures to DON in infants and young children were “wholemeal and granary bread”, “miscellaneous cereals breakfast cereals” and “white sliced bread”. “Fats and oils” was the highest contributing group to 3-Ac-DON.

Table 2a. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.019	0.000-0.083	0.000-0.067	0.000-0.249	0.000-0.112	0.000-0.290
3-Ac-DON	0.000-0.004	0.000-0.018	0.000-0.012	0.001-0.043	0.000-0.020	0.001-0.054
DON	0.022-0.025	0.131-0.163	0.102-0.113	0.424-0.439	0.205-0.222	0.554-0.592

Table 2b. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

	12 to <15 month-	15 to 18 month-olds
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	olds (n=670)		(n=605)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.152	0.000-0.328	0.000-0.172	0.000-0.386
3-Ac-DON	0.000-0.027	0.002-0.061	0.000-0.030	0.002-0.064
DON	0.296-0.318	0.711-0.726	0.347-0.374	0.799-0.857

Table 2c. Estimated 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and deoxynivalenol chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg}$ bw/day)

	18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
15-Ac-DON	0.000-0.180	0.000-0.338	0.000-0.163	0.000-0.308
3-Ac-DON	0.001-0.034	0.002-0.062	0.000-0.029	0.002-0.058
DON	0.346-0.377	0.681-0.699	0.358-0.385	0.745-0.768

Risk characterisation

216. Mean and 97.5th percentile acute exposures to 15-Ac-DON, 3-Ac-DON and DON were below the group ARfD of 8.0 $\mu\text{g}/\text{kg}$ bw/day, for all age groups.

217. Mean and 97.5th percentile chronic exposures to 15-Ac-DON, 3-Ac-DON and DON were below the TDI of 1.0 $\mu\text{g}/\text{kg}$ bw/day, for all age groups.

218. A total concentration for 15-Ac-DON, 3-Ac-DON and DON was not provided to the FSA as part of the TDS and thus exposures for each compound was estimated individually. There is an inconsistency in the way total concentrations are reported across the EU. Therefore in this paper the individual toxins have been compared to the ARfD or the TDI.

Conclusions

219. The levels of 15-Ac-DON, 3-Ac-DON and DON currently present in foods lead to individual acute exposures below the group ARfD and individual chronic exposures which are below the group TDI. These are unlikely to be of toxicological concern.

Secretariat

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June 2017

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References

FAO/WHO (2001). WHO Food Additives Series 47. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm>

FAO/WHO (2011). WHO Food Additives Series 63. Safety evaluation of certain contaminants in food. Prepared by the Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: http://apps.who.int/iris/bitstream/10665/44520/1/9789241660631_eng.pdf

Iverson F, Armstrong C, Nera E, Truelove J, Fernie S, Scott P, Stapley R, Hayward S and Gunner S (1995). Chronic feeding study of deoxynivalenol in B6C3F1 male and female mice. *Teratog Carcinog Mutagen*. **15(6)**: 283-306.

SCF (1999). Opinion on *Fusarium* toxins. Part 1: Deoxynivalenol (DON). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_out44_en.pdf

SCF (2002). Opinion of the Scientific Committee on Food on *Fusarium* toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_fusarium_out123_en.pdf

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

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

Diacetoxyscirpenol (4,15-DAS)

Background

220. A JECFA Technical Report (FAO/WHO, 2017¹⁷) has been used as the basis for this risk characterisation of 4,15-DAS.

221. 4,15-DAS is a type A tricothecene and is found in cereals and cereal-based products including wheat, barley, rice, rye, maize, oats and sorghum. In addition it has been found in coffee beans.

222. EFSA are due to publish an opinion on 4,15-DAS in December 2017. If the outcomes are different from those established by JECFA, the Committee may wish to review them.

HBGV

223. JECFA (2017) concluded that there were insufficient toxicological data available to derive a POD for risk characterisation. JECFA concluded that there were limitations in the available short term toxicity studies and no data for chronic exposures or reproductive and developmental toxicity studies. (FAO/WHO, 2017).

224. 4,15-DAS is structurally similar to T2 and HT2 toxins with evidence that they cause similar effects at the biochemical and cellular levels, have similar effects *in vivo* and an additive dose when co-exposure occurs. Therefore JECFA included 4,15-DAS in the group PMTDI for T2 and HT2, established by JECFA (FAO/WHO, 2001) of 0.06 µg/kg bw. This was based on a LOAEL of 0.03 mg/kg bw/day associated with changes in white blood cell counts, following 3 weeks of dietary exposure in pigs and application of an uncertainty factor of 500. (FAO/WHO, 2017).

Exposure Assessment

225. Chronic 4,15-DAS exposures were calculated and are shown in Tables 1a-c. Levels in the majority of food samples were below the LOQ. Food

¹⁷ JECFA Technical Report available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

groups that had values between the LOD and LOQ for 4,15-DAS included pasta, pizza, vegetable oils and dried pulses.

226. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.028 and 0.001 – 0.092 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.038 and 0.004 – 0.118 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.001 – 0.043 and 0.003 – 0.118 µg/kg bw/day.

227. The food groups which contributed most highly to total exposure in infants and young children were “potatoes” and “miscellaneous cereals pasta”.

Table 1a. Estimated 4,15-DAS chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.006	0.001- 0.036	0.001- 0.019	0.003- 0.085	0.001- 0.028	0.004- 0.092

Table 1b. Estimated 4,15-DAS chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.001- 0.038	0.005- 0.118	0.002- 0.038	0.004- 0.098

Table 1c. Estimated 4,15-DAS chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.002- 0.043	0.005- 0.118	0.001- 0.034	0.003- 0.081

Risk characterisation

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228. Mean 4,15-DAS exposures for all age groups were below the T2, HT2 and 4,15-DAS group PMTDI of 0.06 µg/kg bw/day. The 97.5th percentile exposure for infants aged 4 to <6 months was also below the PMTDI.

229. However 97.5th percentile exposures for all other age groups exceeded the PMTDI of 0.06 µg/kg bw/day and ranged from 140 – 200 % of the PMTDI.

Conclusions

230. At the current levels of 4,15-DAS it is unlikely that these exposures would be of toxicological concern.

231. However it should be borne in mind that the risk characterisation of 4,15-DAS may need to be reviewed and updated by the Committee once EFSA have published their opinion in December this year.

Questions on which the views of the Committee are sought

232. Members are invited to consider the following questions

- ii). Should we consider the approach taken by JECFA (comparison of toxicity to other mycotoxins and possible establishment of a group HBGV) for other mycotoxins that don't have an HBGV (CPA, Fus-X, Neosolaniol)?
- iii). EFSA also established an ARfD for T2/HT2 in their latest Opinion so should this also be looked into for 4,15-DAS? Possibly as a group ARfD?
- iv). Does 4,15-DAS data need adding to T2/HT2 data when measured in the same food groups or should we look at them individually?
- v). EFSA are due to publish their opinion on 4,15-DAS in December 2017. Do Members want to wait until this has been published to compare the approach taken?

Secretariat

June 2017

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References

FAO/WHO (2001). WHO Food Additives Series 47. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm>

FAO/WHO (2017). WHO Technical Report Series. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food. Available at: <http://www.who.int/foodsafety/publications/technical-report-series-1002/en/>

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

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

Fusarenon-X (Fus-X)

Background

233. There is currently no evaluation of Fus-X available by EFSA or JECFA. An evaluation has been performed by The Netherlands National Institute for Public Health and the Environment (RIVM) (Pronk, Schothorst and van Egmond, 2012¹⁸). A summary of the ADME and toxicity data in the RIVM report are provided in the relevant section.

234. Fus-X is a type B tricothecene and is predominantly produced by *F. crookwellense*, *F. poae*, *F. graminearum*, *F. culmorum*, *F. nivale* and *F. equiseti*. *Fusarium* species are generally field fungi but can continue to grow on crops in storage. As a *Fusarium* mycotoxin, Fus-X is predominantly found in cereals such as wheat, barley, oats, rye, rice, sorghum, millet and maize.

ADME and Toxicity

ADME

235. Uniformly labelled 3H-FusX was administered subcutaneously at 4 mg/kg bw to mice. After 30 minutes activity was found, at the highest level, in the liver (3%), and also in the kidneys, intestines, stomach, spleen, bile and plasma. None was detected in heart, brain or testis. After 12 hours there was no dose in any of the organs and 25 % had been recovered in metabolised forms of Fus-X, in the urine. Fus-X is deacetylated to nivalenol by rat and rabbit liver carboxy deesterases. (Pronk, Schothorst and van Egmond, 2012).

Acute toxicity

236. A range of LD₅₀ values in mice, rats, guinea pigs and cats (0.1 to 5.6 mg/kg bw) were obtained for oral, subcutaneous, intraperitoneal and intravenous administrations.

237. Shortly after injection of mice with Fus-X, a rapid increase of leukocytes and lymphocytes was observed along with increased β -globulin and decreased γ -globulin. At autopsy cellular degeneration and karyorrhexis were marked in bone marrow and small intestine.

¹⁸ Available at: <http://www.rivm.nl/dsresource?objectid=130b6b3f-ec30-43ef-9295-11f3839449a5>

(Sub)Chronic toxicity/Carcinogenicity

238. A series of experiments were described in which male Donryu rats and male DDD mice received repeated oral or subcutaneous administrations of Fus-X. The description of the experiments was rather limited, which was also observed by IARC (1993). (Pronk, Schothorst and van Egmond, 2012)

239. In the first experiment 20 rats were administered weekly oral 0.4 mg Fus-X/kg bw by intubation for 50 weeks. Twelve rats survived the 50 weeks. Of these, one had developed a hepatoma. Histopathology showed that approximately 50 % of the animals had hypoplasia and atrophy of the bone marrow, thymus and spleen and some rats showed intrahepatic bile duct hyperplasia and atypical hyperplasia in the gastric and intestinal mucosa. The same dose given to 18 rats via subcutaneous injection for 22 weeks led to similar histopathologic observations. Most rats survived for more than 1 year. (Pronk, Schothorst and van Egmond, 2012).

240. Two groups of mice (16 or 18 animals) were administered weekly subcutaneous injections of 2.5 mg Fus-X/kg bw for 10 or 20 weeks. Most of the animals survived. Alopecia was observed locally to the injection site but hair regrew within a few months. Minimal pathological changes were noted except for moderate thymus atrophy. One case of leukaemia was observed. (Pronk, Schothorst and van Egmond, 2012).

241. In 20 control rats and 11 control mice no tumours occurred during the experimental periods. Atrophy of the organs was mild and only observed in a few cases. (Pronk, Schothorst and van Egmond, 2012).

242. Male Donryu rats were administered 3.5 (n = 49) or 7 (n = 25) mg FUS-X/kg in the diet for 2 years. Two additional groups of animals (n = 26, each) were given 7 mg FUS-X/kg bw for 1 year and then control diet for 1 year with one of the groups also receiving 20 oral administrations of penicillic acid (50 mg/animal) in the first year. Control animals received no treatment (n = 48) or 20 administrations of penicillic acid (n = 25). All animals were given restricted feed (15 g/day) to provide 50 and 105 µg/day/animal. Survivors were killed at 24 months. (Pronk, Schothorst and van Egmond, 2012)

243. Mean bodyweights of treated animals were generally lower than control animals but bodyweight gain recovered after removal of the experimental diet. Survival was poor and treatment-related. The major cause of death in the controls and treated groups was bronchopneumonia, but the incidence was higher in treated animals. No increase in the incidence of tumours was observed in treated rats. This study was also evaluated by IARC (1993) and they noted the poor survival of the animals. IARC (1993) concluded that there is inadequate evidence in experimental animals for the carcinogenicity of Fus-X. No human data are available, and overall IARC concluded that Fus-X is not classifiable as to its carcinogenicity to humans (Group 3). (Pronk, Schothorst and van Egmond, 2012).

Genotoxicity

244. Fus-X was tested *in vitro* and was classed as weakly clastogenic for induction of chromosomal aberrations and sister chromatid exchanges. The activity of Fus-X was not affected by metabolic activation. (Pronk, Schothorst and van Egmond, 2012). "IARC (1993) reported that Fus-X did not induce DNA damage in the Rec-assay using *Bacillus subtilis*, was not mutagenic to *Salmonella typhimurium* and did not induce gene mutations in cultured mouse mammary carcinoma FM3A cells. However, at high doses Fus-X increased the frequency of petite mutations in yeast and (weakly) induced DNA single-strand breaks in cultured human HeLa cells. For the latter, however, a negative result was reported by WHO (1990)." (Pronk, Schothorst and van Egmond, 2012).

Reproductive and developmental toxicity

245. Fus-X was administered to groups of female DDD mice by subcutaneous injection. Single doses of 0.63, 1, 1.6, 2.6 or 4.1 mg/kg bw were given on day 10 of gestation, or at a single dose of 1.6 mg/kg bw on day 6, 8 or 13 of gestation, or at multiple doses of 0.63, 1 or 1.6 mg/kg bw on days 8-12 or 8-14 of gestation. Dams given 4.1 mg/kg bw died within 24 hours of the injection and all dams given 2.6 mg/kg bw aborted 1 day after the injection. At lower doses, abortion occurred less frequently (16-20 %) with longer intervals after injection (2 - 7 days), but embryotoxicity as evidenced by a dose-dependent increase in the number of resorbed and dead fetuses was seen. A single administration of 1.6 mg/kg bw induced more abortions when given on day 6 (75 %) or 13 (50 %) than on day 10, but none when given on day 8. More fetal absorptions occurred the later the day of administration. Multiple doses of 1 and 1.6 mg/kg bw caused all animals to abort, but none aborted with multiple administrations of 0.63 mg/kg bw. Body weight and body length of the surviving fetuses were reduced compared with control fetuses, especially when given a single dose of 1.6 mg/kg bw on day 6 or 8 of gestation, or multiple doses of 0.63 mg/kg bw. Teratogenic effects were not observed.

246. Pregnant DDD mice were fed diets mixed with Fus-X at concentrations of approximately 25, 50 and 100 µg/animal/day throughout gestation or during early, mid or late pregnancy. Fus-X inhibited embryonal implantation and induced abortion, fetal absorption or fetal growth retardation when given throughout gestation or during the early stages of gestation. When Fus-X was given during mid-gestation the outcomes were abortion, fetal absorption, and growth retardation in surviving fetuses. Teratogenic effects were not observed.

Immunotoxicity

247. Immunosuppressive effects were observed in BALB/c mice after repeated daily ip administration of Fus-X. *In vivo* IgE and IgG1 antibody formation was suppressed as was *in vitro* antibody formation by splenic lymphocytes raised by T-dependent and independent mitogens.

248. Blastogenesis in cultured human lymphocytes was inhibited by Fus-X. A concentration of 18 ng/ml inhibited [³H]thymidine uptake by mitogen-stimulated human lymphocytes by 50 %.

249. T and B cell proliferation were significantly and dose-dependently inhibited by Fus-X in an *in vitro* test with human peripheral blood mononuclear cells.

HBGV

250. Due to the data insufficiencies for FUS-X, especially the limited number of oral studies, and their limitations, RIVM were unable to establish a temporary TDI. (Pronk, Schothorst and van Egmond, 2012).

Exposure Assessment

251. Chronic Fus-X exposures were calculated and are shown in Tables 1a-c. Levels in the majority of food samples were below the LOQ. Food groups that had values between the LOD and LOQ for Fus-X were vegetable oils and herbs and spices.

252. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.013 and 0.001 – 0.035 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.020 - and 0.004 – 0.041 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.001 – 0.022 and 0.004 – 0.057 µg/kg bw/day.

253. The food groups which contributed most highly to total exposure in infants and young children were “herbs and spices” and “fats and oils”.

Table 1a. Estimated Fus-X chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.002	0.001- 0.010	0.000- 0.008	0.003- 0.031	0.001- 0.013	0.003- 0.035

Table 1b. Estimated Fus-X chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th	Mean	97.5 th

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	percentile		percentile
0.001- 0.018	0.004- 0.040	0.001- 0.020	0.005- 0.041

Table 1c. Estimated Fus-X chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.001- 0.022	0.006- 0.057	0.001- 0.019	0.004- 0.040

Risk characterisation

254. There is currently no HBGV against which the dietary exposures can be compared.

Questions on which the views of the Committee are sought

255. Members are invited to consider the following questions

- i). Do Members agree that the summary of the toxicology section in the RIVM document does not provide enough information to be able to establish an HBGV?
- ii). Would Members like to see a separate paper for the possible derivation of an HBGV if there are sufficient extra data available? The available data are likely to be limited. In which case would this be a priority?

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References

Pronk MEJ, Schothorst RC and van Egmond HP (2012). Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six trichothecenes. Available at: <http://www.rivm.nl/dsresource?objectid=130b6b3f-ec30-43ef-9295-11f3839449a5>

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

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

Neosolaniol (NeoSol)

Background

256. There is currently no evaluation of NeoSol available by EFSA or JECFA. An evaluation has been performed by RIVM (Pronk, Schothorst and van Egmond, 2012¹⁹). A summary of the available data in the RIVM report are provided in the relevant section.

257. NeoSol is a type A tricothecene and is predominantly produced by *F. sporotrichioides*, *F. poae*, *F. acuminatum*, *F. equiseti*. *Fusarium* species are generally field fungi but can continue to grow on crops in storage. As a *Fusarium* mycotoxin, NeoSol is predominantly found in cereals such as wheat, barley, oats, rye, rice, sorghum, millet and maize.

ADME and Toxicity

258. There are very few data on the toxicokinetics of neosolaniol. NeoSol, in contrast to deacetoxyscirpenol, is not deacetylated by rat and rabbit liver carboxy esterases due to esterase activity interference from the hydrophilic nature of the substituent at C8 (Pronk, Schothorst and van Egmond, 2012)

259. LD₅₀ values have been obtained for mice and broiler chicks. In mice given NeoSola via the intraperitoneal route LD₅₀ values ranged from 14.5 to 17.8 mg/kg bw and via the subcutaneous route, an LD₅₀ of 9.7 mg/kg bw. The LD₅₀ for broiler chicks dosed via the intraperitoneal route was 24.87 mg/kg bw. (Pronk, Schothorst and van Egmond, 2012)

260. NeoSol has been shown to cause vomiting in ducklings (0.1 mg/kg bw) and cats. Dermal toxicity has been observed in guinea pigs at an effective dose of 10⁻⁹ mol. (Pronk, Schothorst and van Egmond, 2012).

261. Repeated subcutaneous injection of NeoSol to cats induced decreased white blood cells and ataxia in the hind legs. Extensive meningeal bleeding and lung haemorrhage were observed at autopsy. Early indications after injection of NeoSol into mice were rapid increase of lymphocytes and leukocytes accompanied by increased β-globulin and decreased γ-globulin. Autopsy findings included marked cellular degeneration and karyorrhexis in bone marrow and small intestine. (Pronk, Schothorst and van Egmond, 2012).

¹⁹ <http://www.rivm.nl/dsresource?objectid=130b6b3f-ec30-43ef-9295-11f3839449a5>

262. NeoSol administration in *in vitro* demonstrated high levels of cytotoxicity to cultured cells and protein synthesis inhibiting activity. NeoSol was also able to block viral replication (EC₅₀ of 52 ng/ml) in cells infected with herpes simplex virus type 2. (Pronk, Schothorst and van Egmond, 2012).

HBGV

263. Due to the lack of pertinent data it was not possible for a temporary TDI to be established by RIVM. (Pronk, Schothorst and van Egmond, 2012).

Exposure Assessment

264. Chronic NeoSol exposures were calculated and are shown in Tables 1a-c. Levels in the majority of food samples were below the LOQ. The only food group that had values between the LOD and LOQ for NeoSol was vegetable oils.

265. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.001 and 0 – 0.018 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.010 - and 0.001 – 0.021 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.011 and 0.001 – 0.021 µg/kg bw/day.

266. The food group which contributed most highly to total exposure in infants and young children was “fats and oils”.

Table 1a. Estimated NeoSol chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.001	0.000- 0.005	0.000- 0.004	0.000- 0.014	0.000- 0.007	0.001- 0.018

Table 1b. Estimated NeoSol chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.009	0.001- 0.020	0.000- 0.010	0.001- 0.021

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Table 1c. Estimated NeoSol chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg}$ bw/day)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.011	0.001- 0.021	0.000- 0.010	0.001- 0.019

Risk characterisation

267. There is currently no HBGV against which the dietary exposures can be compared.

Questions on which the views of the Committee are sought

268. Members are invited to consider the following questions

- i). There is currently no HBGV for neosolaniol, nor is there any indication that it is being looked at by EFSA or JECFA in the short term. There is very little data in the literature. Do members want to see all the toxicity data for neosolaniol to see whether an HBGV can be determined?

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References

Pronk MEJ, Schothorst RC and van Egmond HP (2012). Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six trichothecenes. Available at: <http://www.rivm.nl/dsresource?objectid=130b6b3f-ec30-43ef-9295-11f3839449a5>

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

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

Nivalenol

Background

269. An EFSA opinion (EFSA, 2013²⁰) has been used as the basis for this risk characterisation of nivalenol.

270. Nivalenol is a type B tricothecene and is produced by *Fusarium* genus, i.e. *F. crookwellence*, *F. poae*, *F. culmorum* and *F. graminearum*. The *Fusarium* species invade and grow on crops, and may produce nivalenol under moist and cool conditions. Nivalenol is predominantly found in cereal grains and cereal-based products (EFSA, 2013).

Previous evaluations

271. Nivalenol has previously been risk assessed by the SCF in 2000 and a temporary TDI of 0.7 µg/kg bw was established (SCF, 2000). This was retained in 2002 in another assessment by the SCF (SCF, 2002). The RIVM confirmed the temporary TDI (Pronk MEJ, Schothorst RC and van Egmond HP, 2002). A review was carried out by IARC who concluded that “There is inadequate evidence in experimental animals for the carcinogenicity of nivalenol” and that nivalenol was not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1993). The Food Safety Commission of Japan (FSCJ) established a TDI of 0.4 µg/kg bw (FSCJ, 2010). The Norwegian Scientific Committee for Food Safety (VKM, 2013) carried out a risk assessment for nivalenol and used the temporary TDI of 0.7 µg/kg bw established by the SCF.

HBGV

272. Generally, tricothecenes are immunotoxic and haematotoxic/myelotoxic and several *in vivo* studies on nivalenol have been reported since the SCF assessment (SCF, 2000) such as an increase of IgA or IgM, but at higher concentrations than those where haematotoxic effects such as neutropenia or leukopenia have been described. EFSA considered disturbances in WBC counts to be the critical effect for risk assessment of nivalenol. (EFSA, 2013).

²⁰ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/3262>

273. Following evaluation of 4 relevant studies EFSA decided to use a 90-day rat study by Takahashi *et al.* (2008) as the pivotal study for dose response modelling. The haematological disturbances in white blood cell counts observed in rats showed a clear dose-response curve and were therefore suitable for dose-response modelling. EFSA selected a benchmark response of 5 % extra risk when applying the BMD approach to the changes in white blood cell count data.

274. EFSA used the PROAST software for BMD modelling and used both the Exponential and Hill nested model families for the data. From the results obtained, EFSA chose the lowest BMDL₀₅ of 0.35 mg nivalenol/kg bw/day as a reference point for the risk characterisation.

275. EFSA decided that as nivalenol is unlikely to be genotoxic it was appropriate to establish a TDI. The default uncertainty factor of 100 was applied and in addition EFSA applied an additional uncertainty factor of 2 for extrapolation from sub-chronic to chronic study duration in rats and an uncertainty factor of 1.5 due to the limitations of data available on reproductive and developmental toxicity of nivalenol. The application of an overall uncertainty factor of 300 to the BMDL₀₅ resulted in a TDI of 1.2 µg/kg bw.

Exposure Assessment

276. Chronic nivalenol exposures were calculated and are shown in Tables 1a-c. Nivalenol was not detected at a level above the LOD for any food groups. LB and UB exposures were calculated which meant it was not possible to determine which food group was the highest contributor to the overall exposures.

277. Mean and 97.5th percentile chronic nivalenol exposures for infants aged 4 to 12 months ranged from 0 – 0.234 and 0 – 0.664 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0 – 0.332 and 0 – 0.790 µg/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0 – 0.337 and 0 – 0.741 µg/kg bw/day.

Table 1a. Estimated nivalenol chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
0.000- 0.048	0.000- 0.256	0.000- 0.143	0.000- 0.520	0.000- 0.234	0.000- 0.664

Table 1b. Estimated nivalenol chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000-0.311	0.000-0.790	0.000-0.332	0.000-0.771

Table 1c. Estimated nivalenol chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
Mean	97.5 th percentile	Mean	97.5 th percentile
0.000-0.337	0.000-0.741	0.000-0.310	0.000-0.604

Risk characterisation

278. Nivalenol dietary exposures for all infants aged 4 - 12 months and young children aged 12 – 60 months are below the TDI of 1.2 $\mu\text{g}/\text{kg bw}$ as established by EFSA (2013).

Conclusions

279. It is unlikely that there is a toxicological concern for the exposures calculated from the current levels of nivalenol in food groups consumed by infants and young children.

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References

EFSA (2013). Scientific Opinion on risks for animal and public health related to the presence of nivalenol in food and feed. Available at:
<https://www.efsa.europa.eu/en/efsajournal/pub/3262>

FSCJ (Food Safety Commission of Japan) 2010. Risk Assessment Report Deoxynivalenol and Nivalenol (Mycotoxin), FS/872/2010, Available online:
http://www.fsc.go.jp/english/evaluationreports/nm_toxins/rar_donniv_fs872_2010_nm.pdf

IARC 1993. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 56. Available at:
<http://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf>

Pronk MEJ, Schothorst RC and van Egmond HP (2012). Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six trichothecenes. Available at:
<http://www.rivm.nl/dsresource?objectid=130b6b3f-ec30-43ef-9295-11f3839449a5>

SCF (2000). Opinion of the Scientific Committee on Food on Fusarium toxins Part 4: Nivalenol. Available at:
https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_out74_en.pdf

SCF (2002). Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. Available at:
https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_fusarium_out123_en.pdf

Takahashi M, Shibutani M, Sugita-Konishi Y, Aihara M, Inoue K, Woo G, Fujimoto H and Hirose M (2008). A 90-day subchronic toxicity study of nivalenol, a trichothecene mycotoxin, in F344 rats. *Food and Chemical Toxicology*. **46**: 125-135. Available at:
<https://www.ncbi.nlm.nih.gov/pubmed/17765382>

VKM (Norwegian Scientific Committee for Food Safety/Vitenkapskomiteen for mattrygghet) (2013). Risk assessment of mycotoxins in cereal grain in Norway. Available at:
http://www.english.vkm.no/eway/default.aspx?pid=278&trg=Content_6444&Main_6359=6582:0:31,2556&Content_6444=6393:2016032::0:6596:1::0:0.

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

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

T2 and HT2

Background

280. An EFSA opinion (EFSA, 2017²¹) has been used as the basis for this risk characterisation of T2 and HT2.

281. T2 toxin and HT2 toxin are type A trichothecenes and are produced by a variety of *Fusarium* species. The *Fusarium* species grow and invade crops and produce the T2 and HT2 toxins under cool, moist conditions. T2 and HT2 toxins are predominantly found in cereal grains (particularly oats) and their products.

282. T2 and HT2 toxins are toxic to all animal species, including humans. The toxic effects include inhibition of protein synthesis, which includes effects on immunoglobulin synthesis and therefore humoral immunity. Acute effects are mainly due to alteration of cell membrane functions and lipid peroxidation, whilst dietary systemic toxicity is due to apoptosis of proliferating cells including bone marrow cells and cells of the immune system.

Previous evaluations

283. T2 and HT2 toxins have previously been assessed by JECFA and the Scientific Committee on Food (SCF) in 2001 and 2002. Both Committees used the LOEL of 0.029 mg/kg bw/day for changes in white and red blood cell counts identified in a 3 week dietary study in pigs (Raifa *et al.*, 1995b). An uncertainty factor of 500 was applied to the LOEL by both Committees, and JECFA derived a provisional maximum tolerable daily intake (PMTDI) and the SCF a temporary TDI, of 60 ng/kg bw (FAO/WHO, 2001; SCF, 2001).

HBGV

Acute Reference Dose (ARfD) for T2, HT2 and their modified forms

284. Recent studies have reported anorectic effects at low doses in mouse mink and pig. Acute effects were seen at the lowest dose in mink in a study by

²¹ EFSA opinion available at: <https://www.efsa.europa.eu/en/efsajournal/pub/4655>

Wu *et al.* (2016). This study was used by EFSA for the BMD analysis as the basis for an ARfD. Following oral gavage in two independent tests, one with T2 and one with HT2, each with four animals/dose groups, identical results at identical doses were seen.

285. EFSA used the BMDL₁₀ of 2.97 µg T2 or HT2/kg bw derived for emetic response in mink as a reference point for establishing a group ARfD for T2 and HT2. An UF of 10 for intraspecies variability was applied. However, no interspecies variability factor was applied because humans were not considered more sensitive than mink to the acute emetic effect of T2 or HT2. An ARfD of 0.3 µg T2 or HT2/kg bw was established. With regard to phase I metabolites, neosolaniol (NEO) was equipotent with T2 and HT2 when tested for vomiting in ducklings and was therefore included together with T2 and HT2 in a group ARfD. Phase II metabolites of T2, HT2 and NEO are assumed to be hydrolysed to their parent compounds (aglycones) after ingestion, and are therefore included in a group ARfD with the same potency as their parent compounds. (EFSA, 2017).

TDI for T2, HT2 and their modified forms

286. Since 2011, several subacute and subchronic toxicity studies have been published. In the 90-day study in rats by Rahman *et al.* (2014), dose-dependent decreases in total erythrocyte, leucocyte and thrombocyte counts as well as a decrease in the percentage of lymphocytes were observed. This effect progressed during the whole study period with no signs of reaching a plateau at the end. The exposure duration to T2 is longer (90 days) in absolute terms, but also relative to species life time than for pigs in the Rafai *et al.* (1995) study.

287. EFSA noted that, in essence, the effects observed (i.e. anorectic effects and effects on immune system and blood parameters) in the new (longer term) rat study were essentially similar to those seen in the pig study confirming the immune system and the blood cell production as target organs of T2 through species.

288. Therefore, EFSA decided, considering the longer exposure duration of the study from Rahman *et al.* (2014) and its biological relevance, to use the total leucocytes count reported from this study for calculating a new BMD for T2. EFSA used a BMR of 10%, considering such a response in leucocyte counts to be within the individual physiological variation and negligible, and further noted that the selected BMR is slightly below the control standard deviation of the controls in the Rahman *et al.* study (14%). A 95% lower confidence limit for the benchmark dose response (BMDL₁₀) of 3.3 µg T2/kg bw was derived. EFSA used this value as a reference point for establishing a chronic HBGV for T2 and HT2.

289. To this value an uncertainty factor of 200 was applied. A factor of 10 for interspecies variability, 10 for intraspecies variability and 2 for extrapolation from subchronic to chronic exposure duration and for the progression of the

toxic effect through the duration of the study with no signs of reaching a plateau at the end. EFSA established a TDI of 0.02 µg T2/kg bw.

290. Haematotoxicity with reduced production of erythrocytes, leucocytes and platelets, is the critical chronic effect of T2. The underlying mode of action is inhibition of protein synthesis, induction of ribotoxic stress and apoptosis. Based on similar toxic profile and potency, structural similarity and the fact that HT2 is an immediate metabolite of T2 in agreement with the EFSA assessment of 2011, it was concluded that T2 and HT2 can be included in a group TDI with the same potency.

Exposure Assessment

291. Acute exposures used occurrence data from the TDS and a portion-size approach (Tables 1a-c). T2 and HT2 chronic exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Tables 2a-c). T2 and HT2 were detected in a number of samples above the LOD, but below the LOQ. Most samples with detectable residues were cereal products, however they were also both detected in vegetable oils and cider and additionally, T2 was measured in potatoes and dried pulses. (Stratton *et al.*, 2015).

Acute

292. Mean and 97.5th percentile acute T2 exposures for infants aged 4 to 12 months ranged from 0.001 – 0.055 and 0.010 – 0.157 µg/kg bw, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.020 – 0.087 and 0.057 – 0.211 µg/kg bw. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.020 – 0.094 and 0.050 – 0.228 µg/kg bw.

293. HT2 exposures were also calculated. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0.001 – 0.074 and 0.003 – 0.205 µg/kg bw, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.009 – 0.107 and 0.025 – 0.250 µg/kg bw. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.008 – 0.114 and 0.020 – 0.296 µg/kg bw.

Table 1a. Estimated T2 and HT2 acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw)

	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.001- 0.005	0.010- 0.025	0.011- 0.037	0.049- 0.154	0.016- 0.055	0.056- 0.157

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It does not reflect the views of the Committee and should not be cited.

HT2	0.001- 0.008	0.003- 0.042	0.005- 0.052	0.020- 0.178	0.007- 0.074	0.020- 0.205
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Table 1b. Estimated T2 and HT2 acute exposures from the TDS young children aged 12 to 18 months ($\mu\text{g}/\text{kg}$ bw/day)

	12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.020- 0.075	0.057- 0.198	0.024- 0.087	0.067- 0.211
HT2	0.009- 0.099	0.025- 0.239	0.010- 0.107	0.025- 0.250

Table 1c. Estimated T2 and HT2 acute exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg}$ bw/day)

	18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.024- 0.094	0.063- 0.228	0.020- 0.075	0.050- 0.170
HT2	0.011- 0.114	0.029- 0.296	0.008- 0.091	0.020- 0.197

Chronic

294. T2 exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0.001 – 0.044 and 0.003 – 0.125 $\mu\text{g}/\text{kg}$ bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.005 – 0.064 and 0.014 – 0.149 $\mu\text{g}/\text{kg}$ bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.005 – 0.070 and 0.010 – 0.156 $\mu\text{g}/\text{kg}$ bw/day.

295. HT2 exposures were also calculated. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0.001 – 0.032 and 0.010 – 0.086 $\mu\text{g}/\text{kg}$ bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.011 – 0.051 and 0.032 – 0.133 $\mu\text{g}/\text{kg}$ bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.011 – 0.057 and 0.030 – 0.135 $\mu\text{g}/\text{kg}$ bw/day.

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296. The food groups contributing the highest exposures to T2 were “miscellaneous cereals pasta”, “miscellaneous cereals breakfast cereals” and “miscellaneous cereals potatoes”. “miscellaneous cereals breakfast cereals”, “miscellaneous cereals pasta” and “wholemeal and granary bread” were the highest contributing groups to HT2.

Table 2a. Estimated T2 and HT2 chronic exposures from the TDS in infants aged 4 to 12 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

	4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
	Mean	97.5 th percentile	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.001- 0.008	0.003- 0.042	0.002- 0.027	0.009- 0.101	0.004- 0.044	0.011- 0.125
HT2	0.001- 0.005	0.010- 0.025	0.005- 0.020	0.024- 0.069	0.009- 0.032	0.028- 0.086

Table 2b. Estimated T2 and HT2 chronic exposures from the TDS young children aged 12 to 18 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

	12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.005- 0.060	0.014- 0.149	0.006- 0.064	0.015- 0.145
HT2	0.011- 0.044	0.032- 0.113	0.014- 0.051	0.040- 0.133

Table 2c. Estimated T2 and HT2 chronic exposures from the TDS young children aged 18 to 60 months ($\mu\text{g}/\text{kg bw}/\text{day}$)

	18 to 24 month-olds (n=118)		24 to 60 month-olds (n=688)	
	Mean	97.5 th percentile	Mean	97.5 th percentile
T2	0.006- 0.070	0.016- 0.156	0.005- 0.058	0.010- 0.114
HT2	0.014- 0.057	0.033- 0.135	0.011- 0.046	0.030- 0.101

Risk characterisation

Acute

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297. All of the acute T2 and HT2 dietary exposures are below or approximately equal to the EFSA ARfD of 0.3 µg/kg bw.

Chronic

298. Apart from the UB exposure of 4 to <6 months, all the upper bound exposures exceed the TDI (135 – 780 % of the TDI).

299. The HT2 upper bound mean exposure for infants aged 4 to <6 months is below the TDI. All other UB exposures exceed the TDI (120 – 665 % of the TDI).

Conclusions

300. There is unlikely to be a toxicological concern for acute exposures of HT2 and T2 since they are all below or approximately equal to the ARfD of 0.3 µg/kg bw/day.

301. For chronic T2 and HT2 exposures below the TDI of 0.02 µg/kg bw/day, there is unlikely to be a toxicological concern.

302. For chronic T2 and HT2 exposures exceeding the TDI there will be an increased risk as the exposure increases. Exposures that are 5 times the TDI or greater may already pose a risk to health.

Secretariat

June 2017

References

- EFSA (2017). Appropriateness to set a group health based guidance value for T2 and HT2 toxin and its modified forms. *EFSA Journal*. **51(1)**: 4655. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/4655>
- Rafai P, Tuboly S, Bata A, Tilly P, Vanyi A, Papp Z, Jakab L and Tury E. (1995). Effect of various levels of T-2 toxin in the immune system of growing pigs. *Vet. Rec.* **136**: 511-514. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/7660548>
- Rahman S, Sharma AK, Singh ND, Telang AG, Azmi S and Prawez S (2014). Clinico-haematological changes in T-2 toxicosis in Wistar rats. *Indian Journal of Veterinary Pathology*. **38**: 22–28. Available at: <http://www.indianjournals.com/ijor.aspx?target=ijor:ijvp&volume=38&issue=1&article=006>
- SCF (2001). Opinion of the Scientific Committee on Food on Fusarium toxins. Part 5: T-2 toxin and HT-2 toxin. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_out88_en.pdf
- SCF (2002). Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_fusarium_out123_en.pdf
- Wu W, Zhou H, Bursian SJ, Link JE and Pestka JJ (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**: 997–1007. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/25855062>