**TOX/2017/45**

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

## Review of potential risks from ochratoxin A (OTA) in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

### *Background*

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that will inform the Government dietary recommendations for infants and young children. The SACN is examining the nutritional basis for the advice. The COT was asked to review the risk of toxicity of chemicals in the diets of infants and young children. The reviews will identify new evidence that has emerged since the Government 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. The Food Standards Agency (FSA) has completed a survey of 36 mycotoxins in the 2014 Total Diet Survey (TDS) – mycotoxins analysis (FSA, to be published). The results of the survey provide information on the concentrations of aflatoxins (B1, B2, G1, G2 and M1), ochratoxin A, zearalenone, fumonisins (B1, B2 and B3), 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, deoxynivalenol, diacetoxyscirpenol, fusarenon-X, HT2 toxin, neosolaniol, nivalenol, T2 toxin, sterigmatocystin, citrinin, cyclopiazonic acid, moniliformin, patulin and ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine) in relevant foods. Estimates of dietary exposures have been calculated for each mycotoxin for UK infants and young children aged 4 to 60 months using food consumption data taken from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey (NDNS).

3. Details of the concentration data derived from this survey, and the subsequent exposure assessments, were presented to the Committee in a scoping paper (TOX/2017/30) at the July meetings. To aid the discussions, brief toxicology summaries for each of the mycotoxins surveyed were included, along with available health based guidance values, a risk assessment, where possible and possible conclusions. The Committee commented on the concentration data and the results of the exposure assessments, and suggested that certain mycotoxins be reviewed in more detail. This paper (TOX-2017-45) provides more toxicological information for ochratoxin A (OTA), an in depth description of the previously established Health Based Guidance Value (HBGV) by JECFA and EFSA and new in vivo toxicological data published since the 2006 EFSA opinion. In addition this paper provides an exposure assessment for OTA in breast milk using European data from the literature and an updated exposure assessment for OTA in the diet of infants and young children aged 1 to 5 years.

4. OTA is a mycotoxin produced by several fungi species in the *Penicillium* and *Aspergillus* genera, primarily *Penicillum verrucosum*, *Aspergillus ochraceus* and *Aspergilli* of the section *Nigri*, especially *A. carbonarius*. OTA has been reported in a variety of plant products such as cereals and cereal products, coffee beans, beans, pulses, cocoa products, nuts and spices 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, 2006; EFSA, 2010).

5. OTA has been assessed previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001 and the European Food Safety Authority (EFSA) in 2006[1].

6. In 2010[2] EFSA evaluated 5 research articles providing recent data on the toxicity of OTA and concluded that the data did not alter the evaluation carried out in 2006 (EFSA, 2010).

7. The current discussion paper provides a summary of the toxicity of OTA, where data is available. The paper draws on the EFSA opinion (2006) and the EFSA statement (2010) and includes a literature review (Annex A) to identify any relevant in vivo toxicity studies published since the 2006 EFSA Opinion. The derivation of the health based guidance values (HBGVs) for the above evaluations is detailed. Exposure assessments have been carried out and risk characterisations and conclusions/discussions provided.

***Toxicokinetics***

8. The toxicokinetics of OTA have been previously reviewed by EFSA (2006).

9. OTA is rapidly absorbed following ingestion. Animal studies showed absorptions between 40 % (chickens) and 66 % (pigs). OTA reaches the systemic circulation, where it is primarily bound to plasma proteins. The unbound fraction of OTA is as low as 0.02 % in humans.

10. A two-compartment open model, consisting of a fast distribution and elimination phase followed by a slow elimination phase (plasma clearance) and a long half-life has been described for in vivo data. Based on limited data the longest half-life has been described in humans of 35 days based on one individual. The half-life in other species ranges from 5 days in Wister rats, 6 days in pigs and 9-21 days in non-human primates.

11. In many species, including monkeys and humans, renal elimination is the major route of excretion. In rodents, biliary excretion seems to prevail. Differences in the degree of serum protein binding and its effect on renal clearance, as well as the

[1] EFSA opinion available at: https://www.efsa.europa.eu/en/efsajournal/pub/365

[2] EFSA statement available at: https://www.efsa.europa.eu/en/efsajournal/pub/1626

rate of conjugation and extent of entero-hepatic re-circulation contribute largely to the inter-individual and interspecies variability of kinetic parameters.

## *Toxicity*

*Summary from previous evaluations*

12. The toxicity of OTA has previously been evaluated by JECFA. JECFA concluded that none of the new data that had become available indicate any reason for the HBGV established by JECFA in 1991 and rounded in 1995 to be altered. The PTWI of 100 ng/kg bw was retained (FAO/WHO, 2007). The Scientific Committee for Food (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 (SCF, 1998).

*Studies used in the derivation of the HBGV*

13. Both, JECFA and EFSA used two experimental pig studies for the derivation of their HBGVs.

14. In a study by Elling (1979) female pigs were exposed to OTA at 5 mg/kg feed (calculated by the authors to a corresponding exposure of 400 µg/kg bw) for 5 days or at 1 mg/kg feed (no corresponding µg/kg bw exposure was calculated by the authors) for 3 months or 2 years. In the higher dose group, desquamation and focal necrosis of epithelial cells were detected in the proximal tubule of some nephrons. The activity of various enzymes was reduced in the area of the proximal tubules that showed morphological changes. In the lower dose group the histopathological changes observed were focal degeneration and necrosis of the proximal tubular cells. The lesions after 2 years were the same as after 3 month, except the tubular atrophy and the interstitial fibrosis were more widely distributed in the renal cortex. The authors concluded that OTA induced a reduction in enzyme activity, thus appearing to cause the impairment of proximal tubular function and morphological changes observed in porcine nephropathy (Elling 1979).

15. In a study by Krogh et al. (1988) female pigs received a gelatine capsule daily containing OTA at 0.25 mg and 1.17 mg, corresponding to a feed level of 1 ppm and 0.2 ppm. Renal impairment was observed in both experimental groups after 5 weeks of exposure. Further, the activities of both cytosolic phosphoenolpyruvate carboxykinase (PEPCK) and gamma-glutamyl transpeptidase were decreased after 1 week of exposure and the enzyme activities stayed inhibited for the duration of the experiment. No light-microscopic changes could be detected in kidneys of any of the animals. Due to the correlation of an increased renal impairment and decreased enzyme activity, the authors concluded that these enzymes are sensitive indicators of OTA induced porcine nephropathy (Krogh et al. 1988).

## *HBGV established by JECFA and EFSA*

*JECFA*

16. JECFA first evaluated OTA in 1991, establishing a provisional tolerable weekly intake (PTWI) of 112 ng/kg bw per day based on the deterioration of renal function in pigs as given in the studies by Elling (1979) and Krogh et al. (1988) (Paragraphs 15 and 16). The LOEL was 8 µg/kg bw per day to which the Committee applied an uncertainty factor of 500.

17. Since then JECFA has re-evaluated OTA in 1995, 2001, 2006 and 2008 due to new toxicological data becoming available. JECFA confirmed the PTWI in each evaluation, rounding it to 100 ng/kg bw per day in 1995. For the 2006 evaluation JECFA also considered the EFSA opinion from earlier that year.

18. In order to provide additional information for the risk assessment JECFA performed BMD modelling in their 2008 evaluation, using carcinogenicity data from the rat bioassay performed by the National Toxicology Program (NTP) in 1989. JECFA considered this to be the most appropriate data for modelling due to the occurrence of combined adenomas and carcinomas in kidneys and male rats being the most sensitive species and sex for kidney carcinogenicity.

19. The lowest $BMDL_{10}$ had a value of 15 µg/kg bw per day and the model having the best fit had a value of 25 µg/kg bw per day. JECFA therefore concluded that for establishing the PTWI, the $BMDL_{10}$ does not provide a lower point of departure than the previously used LOEL for minimal renal toxicity in the pig.

20. JECFA concluded that the new data on nephrotoxicity, developmental toxicity, neurotoxicity or immunotoxicity do not indicate any reason to modify the previously taken approach and retained the previous PTWI of 100 ng/kg bw.

*EFSA opinion, 2006*

21. In its 2006 opinion, EFSA considered that there was an absence of conclusive evidence that OTA binds to DNA and therefore 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.

22. The selected (female) pig studies were based on the 2001 JECFA opinion. The LOAEL for progressive nephropathy was 40 µg OTA/kg bw per day, whereas the NOAEL in the same study was 8 µg/kg bw/day in the diet for 2 years. In a 90-day feeding study in female pigs 8 µg OTA/kg bw/day was reported to produce effects on renal enzymes and renal function tests (Elling 1979; Krogh et al. 1988).

23. EFSA concluded that 8 µg/kg bw per 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 (EFSA, 2006; JECFA 2001).

24. The default factor of 2.5 was used to account for toxicodynamic effects of interspecies differences; the Panel noting that there were significant differences between species, especially with regard to protein binding. A factor of 6 was applied to account for kinetic differences in consideration of the plasma half-life. The

common uncertainty factor (UF) of 10 was used to extrapolate from the general population to a potentially sensitive sub-population. In addition EFSA used a factor of 3 to take into account the extrapolation from a LOAEL to a NOAEL.

25. Using the LOAEL of 8 µg/kg bw/day and applying a total UF of 450 EFSA established a tolerable daily intake of approximately 18 ng OTA/kg bw per day.

26. However, given the relatively long half-life of OTA, approximately 20 days in monkeys, 5 days in Wistar rats, 6 days in pigs and 35 days in one individual (human), EFSA considered that a tolerable weekly intake (TWI) of up to 120 ng/kg bw was more appropriate.

*EFSA statement, 2010*

27. In 2010, EFSA was asked to assess five publications with recent scientific information on the toxicity of OTA. EFSA noted that four of these publications addressed the possible co-exposure of OTA and aristolochic acid of populations in areas previously associated with higher prevalence of Balkan Endemic Nephropathy.

28. The Panel concluded that the information provided was not relevant to the overall assessment and thus would neither contradict nor change the conclusions reached in the 2006 opinion. The TWI of 120 ng/kg bw was retained.

*In vivo toxicity studies published since the 2006 EFSA opinion*

29. A literature search has been performed to obtain any new *in vivo* toxicity studies since the EFSA opinion in 2006. A total number of 31 studies were identified, 7 in pigs, 20 in rats, 3 in mice and one study in rabbits. Overall, the findings of these studies were in line with the 2006 EFSA opinion. Some of the studies focused their research in more detail on molecular mechanisms and mode of action than histopathological findings, some investigated carcinogenicity or teratogenicity of OTA. A more detailed overview of the studies retrieved can be found in Table 7 in Annex A. A summary of the literature search can be found in Annex B

30. None of the studies proposed new/different HBGVs. However, some of the studies such as Balogh et al. (2007), Tanaka et al. (2016), Rached et al. (2007), Patil et al. (2006) and Prabu et al. (2013) provided NAOELs/LOAELS, all of which were higher than the LOEL of 8 µg/kg bw used by EFSA in 2006.

***Human breast milk***

31. Literature searches were performed to identify applicable data for OTA in breastmilk. As no data for the UK could be identified, the search was expanded to include EU studies which are summarized in Table 1 below. A summary of the literature search can be found in Annex B.

32. Most levels found in breast milk from European member states (Scandinavia, Italy, Slovakia) were in the range of 1.1 – 182 ng OTA/L. Two Italian studies however reported higher levels of OTA, from maximum levels of 405 ng/L to ranges of 1200/1700 – 6600 ng/L.

33. Three of the reported breast milk studies included questionnaires reporting dietary and consumption habits, including frequency and portion size (Biassuci et al. 2011; Galvano et al. 2008; Skaug et al 2001). All of the studies noted a correlation of OTA concentration in breast milk and (cured) pork meat (Biasucci et al, 2011; Glavano et al.; 2008) or processed meat products Skaug et al., 2001). Other food commodities correlating with OTA concentration were soft drinks, sweets and red wine (Biasucci et al., 2011), bread and bakery products (Galvano et al., 2008) and breakfast cereal, and cheese (Skaug ey al.; 2001).

Table 1 Concentrations of OTA in breastmilk available from the published literature

| **Country Years of sampling** | **Number of samples** | **Average concentration (ng/L)** | **Minimum Concentration (ng/L)** | **Maximum Concentration (ng/L)** | **Reference** |
|---|---|---|---|---|---|
| Italy | 57 | 10 ± 15.6 | 1.1 | ≥ 75.1 | Biasucci 2011 |
| Slovakia<br><br>03/2007 - 08/2007 | 76 | | 2.3 ± 0.99 | 60.3 ± 25.93 | Dostal et al 2008 |
| Italy<br><br>01/2006 - 12/2006 | 82 | 30.43 ± 66.89 | < 5 | 405 | Galvano et al 2008 |
| Eastern Europe<br><br>10/1998 - 04/1999 | 13 | 5.6 ± 4 | 5.3 | 17 | Postupolski et al 2006 |
| Norway<br><br>07/1995 - 09/1996 | 80 | 30 (16*) | 10 | 182 | Skaug 2001 |
| Norway<br><br>05/1994 - 08/1994 | 115 | | 10 | 130 | Skaug et al 1998 |
| Sweden<br><br>Late 1990 - Early 1991 | 40 | | 10 | 40 | Breitholtz-Emanuelsson 1993 |
| Italy*<br><br>1989 - 1990 | 50 | | 1200<br>1700 | 6600<br>6600 | Micco et al 1991 |
| North of the Alps (Switzerland)<br><br>08/1992 - 02/1993 | 40 9from 10? Women) | 14 ± 2 pg/g (1 sample)<br><br>5 pg/g (estimated in 3 other samples) | | | Zimmerli & Dick 1995 |

[a] Average concentration is the mean or median, where it is the median this has been indicated with *. Where it has been available, the standard deviation has also been provided as ±….

*unclear from the study which one of the values is correct, one reported in abstract, the other reported in the main text of the paper

### *Exposure Assessment*

34. Levels of various mycotoxins were measured in the Total dietary Survey (TDS), including OTA. Only five food categories contained measurable residues of OTA, the rest were all below the LOQ. OTA was found in the fruit and vegetable juices, dried fruit, herbs and spices and bread (granary, brown) food categories. While the data obtained from the TDS could be used as a qualitative indicator of mycotoxins present in various food categories, it is not possible to use it for a quantitative estimation of dietary exposures for the following reasons:

35. Since analysis of the TDS samples involved a wide range of matrices (some of which have not been routinely examined previously), existing validated methods were adapted/extended to some of the new matrices. For example, the method used for grape juice or wine was used to analyse the composite sample of fruit and vegetable juices. However, the presence of other fruit & vegetables (e.g. orange, carrot etc.) in addition to grape has led to some analytical difficulties, with poor recoveries and consequently high results. The low recovery meant that the large correction inflated the result and will impact on calculated dietary exposures. Given the low recovery of 32 %, this method is not considered suitable for this food matrix. Also, the reported level of 5.62 µg/kg OTA in the fruit and vegetable juice category was much higher than the average based on EFSA survey data (0.55 µg/kg).

36. Further, a range of matrices was included in a single batch in the analysis of mycotoxins. This has had an impact on recoveries, since usually in a given batch, similar matrices are included and a batch average recovery is applied and the spiked samples are the same food matrix. In the above example, three very different matrices - dried fruit and herbs and spices were included in the batch with fruit and vegetable juices (each individually spiked). If a batch recovery average which is not specific to the fruit juice matrix and grape juice method is applied, it would give an average recovery of 72 % when applied to this sample. This would give a lower result but is also not a reliable approach given the differences in sensitivities of the methods and food matrices used in the TDS.

37. A multi-mycotoxin method was used in the analysis for various food groups, which is normally a screening method rather than a sensitive technique. This is reflected in generally poor recoveries, higher Limits of Quantification/Detection (LOQ/LOD) and when these were corrected for recovery, led to artificially inflated occurrence levels in some cases. This also indicates the unsuitability of using the TDS data for quantitative exposure estimates.

38. Therefore, occurrence data from the TDS are not suitable for estimating dietary exposures quantitatively. They are not sensitive enough and the methods are not sufficiently standardised and validated for this purpose.

39. Alternative OTA survey data were therefore considered for calculating dietary exposures for infants and young children. Exposures were calculated using data from foods analysed in years 1 and 2 of the four-year retail survey (FSA, 2010; FSA

2011) and consumption data from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey rolling programme (NDNS) years 1 – 6 (Bates et al., 2014; Bates et al., 2016). Exposures were assessed for infants aged 0 - < 4, 4 0 < 6, 6 - < 9 and 9 - < 12 months, and for young children aged 12 - < 15, 15 - < 18, 18 - < 24 and 24 - < 60 months. Consumption data from DNSIYC was used for children aged 4 – 18 months and from NDNS for children aged 10 – 60 months.

*Human breast milk*

40. Due to improved detection methodologies in recent years and the high LOD of 200 ng/L in the study by Micco et al. (1991), this study was considered of limited relevance and has therefore not been used in the exposure assessment.

41. The study by Galvano et al. (2008) has been selected for the exposure assessment. The minimum concentration (< 5 ng/L) detected is in agreement with a range of other studies, while the maximum (405 ng/L) concentration detected is the highest value reported and can therefore be considered conservative.

42. Based on the levels given by Glavano et al. (2008) OTA exposure levels were estimated for exclusively breastfed infants consuming average (800 mL) and high-level (1200 mL) volumes of breast milk (Table 2). Using the average OTA concentration of 30.43 ng/L, exposures in the 0 to < 4 month olds were 4.1 and 6.2 ng/kg bw per day for average and high consumers, respectively; for 4 to < 6 months old exposures were 3.1 and 4.7 ng/kg bw per day for average and high consumers, respectively.

Table 2 OTA exposure (ng/kg bw per day) from exclusive breastfeeding estimated for average and high level consumption of breast milk

| OTA concentration (ng/L) | Exposure (ng/kg bw/day) | | | |
|---|---|---|---|---|
| | Average consumer (800 mL/day) | | High consumer (1200 mL/day) | |
| | 0 to < 4 months | 4 to < 6 months | 0 to < 4 months | 4 to < 6 months |
| Minimum < 5 | < 0.68 | < 0.51 | < 1.02 | < 0.77 |
| Maximum 405 | 55 | 42 | 82 | 62 |
| Average 30.43 | 4.1 | 3.1 | 6.2 | 4.7 |

Infant exposure is based on consumption of 800 mL or 1200 mL per day, and expressed on a bodyweight (5.9 kg for infants aged 0-4 months and 7.8 kg for infants aged > 4-6 months) basis
Values rounded to 2 significant figures (SF)

43. Based on the levels given by Glavano et al. (2008) OTA exposure levels were also calculated for non-exclusive breastfed infants using consumption data from DNSIYC. An OTA average concentration in breast milk of 30.43 ng/kg could lead to mean OTA exposures of 0.77 to 2.8 pg/kg bw per day and 97.5$^{th}$ percentile exposures of 1.8 to 4.7 µg/kg bw per day (Table 3) in infants aged 4 to 18 months.

Table 3 OTA exposure (pg/kg bw per day) from non-exclusive breastfeeding.

| OTA | Exposure (pg/kg bw/day) |
|---|---|

| concentration (ng/L) | 4 to < 6 months | | 6 to < 9 months | | 9 to < 12 months | | 12 to < 15 months | | 15 to < 18 months | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | 97.5th | Mean | 97.5th | Mean | 97.5th | Mean | 97.5th | Mean | 97.5th |
| Minimum < 5 | <0.46 | <0.77 | <0.33 | <0.80 | <0.19 | <0.58 | <0.12 | <0.38 | <0.13 | <0.26 |
| Maximum 405 | 37 | 63 | 27 | 64 | 15 | 47 | 12 | 30 | 10 | 21 |
| Average 30.43 | 2.8 | 4.7 | 2.02 | 4.8 | 1.2 | 3.5 | 0.89 | 2.3 | 0.77 | 1.8 |

Values rounded to 2 SF

*Other dietary sources*

44. The FSA recognises the need to monitor foodstuff for the levels of mycotoxins and undertook two FSA retail surveys in 2010 and 2011 (FSA, 2010; FSA, 2011). A total of 225 products, were collected on behalf of FSA, samples were purchased from major and independent supermarkets, independent and specialist retailers and online. The products tested comprised of foods based on maize, wheat, rye and barley and commercial infant and young children foods.

45. Year 1 of the UK retail survey investigated the presence of mycotoxins in a range of cereals and cereal based products. 75 samples of maize (corn) products were analysed for OTA, 92 % (69 samples) were below the LOQ (0.1 µg/kg). 75 samples of wheat products were analysed for OTA, 47 % (35 samples) were below the LOQ (0.1 µg/kg). 35 samples of rye and barley products were analysed for OTA, 66 % (23 samples) were below the LOQ (0.1 µg/kg).

46. Year 2 of the UK retail survey investigated the presence of mycotoxins in food produced for infants and young children. 77 samples of commercial infants and young children foods were analysed for OTA. 83% (64 samples) were below the LOQ (0.05 µg/kg); 2.6% (2 samples) were above the LOD (0.02 µg/kg) but below the LOQ and 14% (11 samples) were at or above the LOQ. These analytical data were used for calculating the exposures provided in Tables 4, 5 and 6 as lower bound (LB) and upper bound (UB) estimates.

47. For children aged 4 to 18 months (Table 4 and 5) mean total UB exposures ranged from 0.63 – 1.3 ng/kg bw per day; the 97.5th percentile total UB exposures ranged from 2.5 – 3.2 ng/kg bw per day (UB).

48. For children aged 18 to 60 months (Table 6) the total mean UB exposures ranged from 0.99 – 1.3 ng/kg bw per day (UB). The corresponding 97.5th percentile UB total exposures ranged from 2.3 – 3.4 ng/kg bw per day.

49. Wheat products made the main contribution to total exposure in all age groups.

Table 4 Estimated OTA chronic exposure to children aged 4 to 12 months using data from foods analysed in years 1 and 2 of the four - year surveillance programme (retail survey)

| Food Groups | Exposure LB-UB (ng/kg bw/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4 to <6 m-olds (n=116) | | | 6 to <9 m-olds (n=606) | | | 9 to <12 m-olds (n=686) | | |
| | Number of consumers | Mean | 97.5th Percentile | Number of consumers | Mean | 97.5th Percentile | Number of consumers | Mean | 97.5th Percentile |
| Commercial Infant & young children Foods (77 samples) | 100 | 0.12-0.58 | 0.49-2.4 | 578 | 0.14-0.72 | 0.53-2.6 | 618 | 0.14-0.69 | 0.51-2.6 |
| Maize (corn) products (75 samples) | 15 | 0.0041-0.041 | 0.024-0.24 | 150 | 0.0025-0.025 | 0.011-0.11 | 250 | 0.0035-0.035 | 0.014-0.14 |
| Wheat products (75 samples) | 21 | 0.13-0.18 | 0.47-0.66 | 383 | 0.36-0.50 | 1.4-1.9 | 607 | 0.52-0.73 | 1.5-2.1 |
| Rye and barley products (35 samples) | 7 | 0.016-0.020 | 0.041-0.053 | 32 | 00.036-0.045 | 0.18-0.23 | 65 | 0.060-0.076 | 0.22-0.28 |
| **TOTAL of 4 groups above** | **100** | **0.15-0.63** | **0.53-2.5*** | **599** | **0.37-1.0** | **1.2-2.9*** | **685** | **0.58-1.3** | **1.6-3.2*** |

* Determined from a distribution of consumption of any combination of categories rather than by summation of the respective individual 97.5th percentile consumption value for each of the three food categories

**NOTE**: Please note that consumption or exposure estimates made with a small number of consumers may not be statistically reliable. As a guide, estimates based on less than 60 consumers should be treated with extreme caution

Table 5 Estimated OTA chronic exposures to children aged 12 to 18 months using data from foods analysed in years 1 and 2 of the four - year surveillance programme (retail survey)

| Food Groups | Exposure LB-UB (ng/kg bw/day) | | | | | |
|---|---|---|---|---|---|---|
| | 12 to <15 m-olds (n=670) | | | 15 to <18 m-olds (n=605) | | |
| | Number of consumers | Mean | 97.5th Percentile | Number of consumers | Mean | 97.5th Percentile |
| Commercial Infant & young children Foods (77 samples) | 471 | 0.10-0.48 | 0.41-2.0 | 338 | 0.066-0.33 | 0.26-1.3 |
| Maize (corn) products (75 samples) | 302 | 0.0045-0.045 | 0.016-0.16 | 296 | 0.0049-0.05 | 0.018-0.18 |
| Wheat products (75 samples) | 649 | 0.68-0.96 | 1.9-2.7 | 597 | 0.75-1.1 | 1.7-2.4 |
| Rye and barley products (35 samples) | 47 | 0.16-0.20 | 0.71-0.90 | 25 | 0.15-0.19 | 0.62-0.78 |
| **TOTAL of 4 groups above** | **667** | **0.74-1.3** | **2.0-3.2*** | **602** | **0.79-1.3** | **1.8-2.9*** |

* Determined from a distribution of consumption of any combination of categories rather than by summation of the respective individual 97.5th percentile consumption value for each of the three food categories

**NOTE**: Please note that consumption or exposure estimates made with a small number of consumers may not be statistically reliable. As a guide, estimates based on less than 60 consumers should be treated with extreme caution

Table 6 Estimated OTA chronic exposure to children aged 18 to 60 months using data from foods analysed in years 1 and 2 of the four - year surveillance programme (retail survey)

| Food Groups | Exposure LB-UB (ng/kg bw/day) | | | | | |
|---|---|---|---|---|---|---|
| | 18 to 24 m-olds (n=118) | | | 24 to 60 m-olds (n=688) | | |
| | Number of consumers | Mean | 97.5th Percentile | Number of consumers | Mean | 97.5th Percentile |
| Commercial Infant & young children Foods (77 samples) | 43 | 0.058-0.29 | 0.21-1.0 | 78 | 0.028-0.14 | 0.19-0.94 |
| Maize (corn) products (75 samples) | 56 | 0.0059-0.059 | 0.025-0.25 | 301 | 0.0056-0.056 | 0.019-0.19 |
| Wheat products (75 samples) | 118 | 0.81-1.1 | 2.3-3.2 | 678 | 0.68-0.96 | 1.6-2.2 |
| Rye and barley products (35 samples) | 6 | 0.08-0.10 | 0.19-0.24 | 27 | 0.08-0.10 | 0.20-0.26 |
| **TOTAL of 4 groups above** | **118** | **0.84-1.3** | **0.23-3.4*** | **685** | **0.68-0.99** | **0.16-2.3*** |

* Determined from a distribution of consumption of any combination of categories rather than by summation of the respective individual 97.5th percentile consumption value for each of the three food categories

**NOTE**: Please note that consumption or exposure estimates made with a small number of consumers may not be statistically reliable. As a guide, estimates based on less than 60 consumers should be treated with extreme caution

## *Risk characterisation*

*Human breast milk*

50. Assuming a low or average concentration of OTA, all estimated exposures for infants under the age of 6 month, feed exclusively on breast milk are below the TWI. These exposures are not of toxicological concern.

51. Assuming a high OTA concentration (405 ng/L) exposures for low consumption (800 mL) are 2.5 – 3 times the TWI and for high consumption (1200 mL) 3.5 – 5 times the TWI. The high concentration used here might not be representative, other studies report maximum occurrence values well below 405 ng/L. The likelihood of exposure to such concentrations is therefore unlikely. In addition, the TWI is set on lifetime consumption and due to the relatively short duration of breastfeeding, infants would not be expected to be exposed to these levels for a prolonged period of time.

52. All mean and 97.5$^{th}$ percentile exposures for infants under the age of 18 months, fed non-exclusively on breast milk are below the TWI. These exposures are not of toxicological concern.

*Other dietary sources*

53. In infants and young children all mean and 97.5$^{th}$ percentile exposures were below the TWI. These exposures are not of toxicological concern.

*Uncertainties in the risk characterisation*

54. The study by Galvano et al. (2008) found concentrations of OTA in breastmilk ranging from $<$ 5 to 405 ng/L, with an average of 30.43 ng/L. High levels of OTA in other studies were in the range of 60 -182 ng/L, the chosen highest value for exposure might therefore be conservative and not representative. It is also unlikely that infants would be continuously exposed to high levels.

55. No information on LODs or LOQs were given in the study and while the higher concentrations in breast milk were linked to high consumption of bread and cereal based foods as well as alcoholic beverages in a few cases, no clear reason was provided for the high maximum value or it's possible link to consumption.

## *Conclusions*

56. Assuming a low or average concentration of OTA, all estimated exposures for infants under the age of 6 month, fed exclusively on breast milk are below the TWI. Assuming a high concentration, infants exceed the TWI by up to 5 fold.

57. The high concentration used here might not be representative, as other studies report maximum occurrence values well below 405 ng/L. The likelihood of exposure to such concentrations is therefore unlikely. In addition,

it is unlikely that infants would be exposed to such high values continuously throughout their life. Overall, an effect on health cannot be entirely excluded in the short term; chronic effects on health are unlikely.

58. Respective exposures for infants up to 6 months, fed non-exclusively with breast milk and mean and 97.5$^{th}$ percentile dietary exposures for infants at all age groups are below the TWI. These exposures are of no toxicological concern.

***Questions to be asked to the Committee***

i) Do the Committee endorse the TWI established by EFSA in 2006 and re-confirmed in 2010?
ii) If not, do the Committee think the studies used by EFSA and JECFA are the most appropriate studies or are there any newer studies that might be more appropriate?
iii) Do the Committee have any other comments on this discussion paper?

**Secretariat**

**November 2017**

***Abbreviations***

| | |
|---|---|
| DNSIYC | Diet and Nutrition Survey of Infants and Young Children |
| FSA | Food Standards Agency |
| NDNS | National Diet and Nutrition Survey |
| TDS | Total Diet Survey |
| OTA | Ochratoxin A |
| EFSA | European Food Safety Authority |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| FAO | Food and Agriculture Organization of the United Nations |
| WHO | World Health Organisation |
| HBGV | Health based guidance value |
| PTWI | Provisional tolerable weekly intake |
| SCF | Scientific Committee for Food Standards Agency |
| EC | European Community |
| TDI | Tolerable daily intake |
| PEPCK | Phosphoenolpyruvate carboxykinase |
| NTP | National Toxicology Program |
| BMD | Benchmark dose modelling |
| BMDL | Benchmark dose lower confidence limit |
| bw | Body weight |
| LOEL | Lowest observed effect level |
| LOAEL | Lowest observed adverse effect level |
| NOAEL | No observed adverse effect level |
| UF | Uncertainty factor |
| TWI | Tolerable weekly intake |
| LOQ | Limit of quantification |
| LOD | Limit of detection |
| UB | Upper bound |
| LB | Lower bound |
| IL | Interleukin |
| NF-kB | Nuclear factor kappa beta |
| eNOS | Endothelial nitric oxide synthase |
| iNOS | Inducible nitric oxide synthase |
| SOD | Superoxide dismutase |
| FB1 | Fumonisin B1 |
| TNF-α | Tumour necrosis factor α |
| HO-1 | Heme oxygenase |
| ASAT | Aspartate aminotransferase |
| ALAT | Alanine aminotransferase |
| MDA | Malondialdehyde |
| AST | Aminotransferase |
| ALP | Alkaline phosphatase |
| TP | Total serum protein |
| BUN | Blood urea nitrogen |
| CREA | Creatinine |
| TG | Triacylglycerol |
| LDH | Lactate dehydrogenase |
| GLU | Glucose |
| ALB | Albumin |

| | |
|---|---|
| PCNA | Proliferating cell nuclear antigen |
| DSB | Double strand break |
| HDL | High density lipoprotein |
| MOA | Mode of action |
| CLU | Clusterin |
| OPN | Osteopontin |
| BdrU+ | 5-bromo-2-deoxyuridine |
| TBARS | Thiobarbituric acid-reactive substances |
| DA | Dopamine |
| TH | Tyrosine hydroxylase |
| ED50 | Median effective dose |
| ip | Intraperitoneal |
| AFB1 | Aflatoxin B1 |
| OTB | Ochratoxin B |
| OTC | Ochratoxin C |
| GD | Gestation day |
| PND | Postnatal day |

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## Annex A

Table 7 Summary of *in vivo* toxicity studies since the EFSA opinion in 2006

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| **Studies in pigs** | | | | | | | | |
| Female crossbreed weaned piglets (TOPICS-40) | Total N=12<br><br>N=6 / group | 0.05 mg OTA/kg feed<br><br>Control feed 2.52 ppb<br><br>OTA feed 49.62 ppb<br><br>OTA was purchased | Daily via diet<br><br>30 days | | Concentrations 6.25, 26.4 and 26 times higher in kidney, duodenum and colon of animals exposed to OTA<br><br>Effects on immune response and anti-oxidant self-defence at gut and kidney level<br><br>Down regulation of gene expression of markers of inflammation<br>-IL-6, IL-8, IL-12, IL-17A, IL-18 in the colon[a]<br>-IL-17A and IL-10 in the kidney<br><br>Decrease of markers for signalling pathway<br>-NF-kB[a] and eNOS[a] in duodenum<br>-iNOS[a] in colon<br>-No sign effect in kidney<br><br>OTA increased SOD[a] activity in kidney (127.6%) and duodenum (106%) | LOAEL *<br>0.05 mg/kg feed<br><br>For immune response and anti-oxidant self-defence | Other mycotoxins below LOD | Marin et al. 2017b |
| Female crossbreed weaned piglets (TOPICS-4-) | Total N=12<br><br>N=6 / group | 0.05 mg/kg feed<br><br>Control feed 2.52 ppb | Daily via diet<br><br>30 days | | Renal toxicity - correlated with activation of immune response pathways, ox stress response and early carcinogenic events<br><br>OTA was 6.25 times higher in kidney | LOAEL*<br>0.05 mg/kg feed<br><br>For renal toxicity, | All other mycotoxins below LOD, except FB1[a] (88 ppb) | Marin et al. 2017a |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | OTA feed 49.62 ppb<br><br>OTA was purchased | | | of exposed animals<br><br>In the kidney gene expression of 74 transcripts increased and 31 genes decreased<br>-expression of genes involved in renal necrosis, kidney failure, renal proliferation, renal dysplasia and renal hypoplasia<br>-expression of some genes involved in ox stress response<br><br>OTA increased the SOD activity in the kidney | immune response, ox stress and early carcinogenic events | | |
| Weaned piglets<br><br>([Landrace x Yorkshire] x Duroc) | Total N=36<br><br>N=4 / group | 0, 0.4 (OTA-L) or 0.8 (OTA-H) mg/kg<br><br>*A. ochraceus* in shredded corn ~20 mg OTA/kg corn substrate | Daily via diet<br><br>42 days | OTA-L 790.7 ± 43.71 g/day<br><br>OTA-H 724.67 ± 25.0 g/day | Polyuria & polydipsia in both groups Severe wasting<br><br>Decreased growth performance (time & dose-dependent manner)<br><br>Day 28 and 42 OTA sign increased in serum and in kidney and liver on day 42 (higher in OTA-H)<br><br>Degenerative changes in kidney & liver<br>Hyperchromatic nuclei & cytoplasm, nuclei atrophy, necrosis & exfoliation in epithelial cells of proximal tubules<br><br>Reduced antioxidant ability<br>-SOD activity was sig decreased on days 14, 28 and 42 | LOAEL*<br>0.4 mg/kg | Low concentrations of OTB[a] & OTC[a]<br><br>Exposure**<br>0.32 mg/day (OTA-L)<br>0.58 mg/day (OTA-H) | Zhang et al. 2016 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| Castrated male Large White weaned pigs | Total N=60<br><br>N=30/ group | Experimental 181 ± 34 ng/g feed<br><br>Control 0.45 ± 0.05 ng/g feed | Diet<br><br>43 days | Average daily intake<br><br>Experimental 708 ± 43 g<br><br>Control 711 ± 24 g | Growth performance was not affected<br>No pathological signs in kidney and liver<br><br>Sign decrease in level of serum protein<br>Sign increase in TNF-α[a] (12.2 fold) and IL-10 (5.2 fold) in blood<br>Significant decrease in expression of HO-1[a] in kidney<br><br>Indications for increased cell stress, possibly associated with increased systematic inflammatory status | LOAEL*<br>181 ng/g feed<br><br>For cellular stress/ochratoxicosis | Exposure**<br>Experimental<br>128 µg/day<br>0.32 µg/day | Bernardini et al. 2014 |
| Male pigs (Zegers hyprid) | Total N=8<br><br>N=4 / group | 300 ug OTA/kg of feed (300 ppb)<br><br>OTA was purchased | Daily via diet/capsule directly in pigs mouth<br><br>30 days | | Indicative of impaired liver & kidney function<br>- Changes in serum biochemical parameters<br><br>Increase in serum levels of creatinine, urea (kidney damage), potassium and ALP[a] (liver damage)<br>Levels of glucose and total protein were decreased.<br><br>Significant decrease in glucose concentration observed on day 10 pointed to early kidney damage | Changes of biochem parameters indicate kidney/liver damage as early as 10 & 20 days<br><br>LOAEL*<br>300 ug/kg feed at 10 days exposure<br><br>For biochem parameters indicating kidney and | Small number of animals<br><br>Unclear from text weather animals were exposed via diet or through capsule directly into pigs mouth | Pleadin et al. 2012 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | liver damage<br><br>No effect after 1 day | | |
| Pigs<br>Landrace x Bulgarian white | Total<br>N=24<br><br>N=6 / group<br><br>OTA<br>N=6 | 0.5 ppm<br><br>Moulded shredded wheat<br>OTA ~2mg/g | Daily via diet<br><br>3 months | | Degenerative changes in proximal tubules & fibrosis in kidneys<br><br>Slight degenerative changes in duodenum or jejunal mucosa<br><br>Liver showed staining and fatty changes<br><br>Increase in serum creatinine & urea level<br>Increased activity of enzymes (ASAT [a] and ALAT[a])<br>Disturbance in humoral immune response | LOAEL*<br>0.5 ppm<br><br>For pathological changes in the kidney<br>And<br>disturbance in humoral immune response | Separate and simultaneous exposure to OTA and fumonisin FB1[a] (10 ppm)<br><br>Data presented here is for individual exposure to OTA only<br><br>Only one group of animals (N=6) exposed to OTA only<br><br>No other mycotoxins present | Stoev et al. 2012 |
| Hungarian Large White x Hungarian Landrace F1 weaned piglets<br><br>(Sex ratio 1:1) | Total<br>N = 24<br><br>N=12 / group<br><br>2x control group<br>2x exposure group<br><br>N=6 / group | Experimental<br>379.6 & 338.1 ug/kg feed<br><br>Control<br>1.5 & 2.6 ug/kg feed<br><br>OTA extracted from maize using *Aspergillus westerdijkiae* strain NRRL | Diet<br><br>42 days | Experimental<br>0.894 kg/day/piglet<br><br>Control<br>1.117 kg/day/piglet | Daily weight gain significantly lower in OTA contaminated feed group<br><br>Increased MDA[a] in liver<br><br>Moderate level; (0.4 mg/kg) of OTA in feed does not result in clinical signs of toxicity | NOAEL*<br>0.4 mg/kg | The published tables only report control and OTA exposure group, no differentiation between the concentrations given in the methods<br>Samples/Groups pooled? | Balogh et al. 2007 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | 3174 | | | | | | |
| **Studies in rats** | | | | | | | | |
| Female Wistar rats | Total N=42<br><br>N=7 / group | 3 mg OTA/kg food<br><br>45 µg/day in their food<br><br>45 g OTA/rat/day | Daily via diet<br><br>6, 9 & 24 weeks | 20 g/rat/day | Toxic effects on endocrine pancreatic function as early as 6 weeks<br><br>Significant decrease in insulin levels<br>Significant increase in blood glucagon & glucose levels<br><br>Slight to moderate degeneration in Langerhans islet cells; vacuolization, megalocytosis, karyomegaly | | Poorly reported study.<br><br>I calculated dose, see below:<br>3 mg OTA/kg feed<br>20 g per rat<br>= 60 µg OTA/20g feed? | Mor et al. 2017 |
| Female Crl:CD (SD) rats – mated<br><br>Offspring 6 male + 2 female pups | Total N=48<br><br>N=12 / group | 0, 0.12, 0.6, 3.0 ppm<br><br>Based on mean values of food consumption:<br><br>Gestation 8.0, 76.0, 206.6 µg/kg bw per day<br><br>Lactation 16.1, 76.0, 378.6 µg/kg bw per day<br><br>OTA concentration in diet until exposure: | Diet<br><br>Gestation Day (GD) 6 to Post-natal day (PND) 21 on<br><br>Remaining offspring kept through PND 77 without OTA exposure | | At 3.0 ppm offspring showed transient bw decrease after weaning<br><br>Changes in hippocampal neurogenesis related parameters measured in male PND 21 offspring<br><br>Maternal OTA exposure reversibly disrupts neurogenesis in rat offspring | NOAEL<br>0.6 ppm<br>39.3-76 ug/kg bw/day<br><br>For offspring neurogenesis (Maternal oral exposure) | Preliminary feeding study -exposure to 0, 1 ppm (N=3) or 3 ppm (N=4)<br><br>3 ppm decreased brain weight of male offspring on PND 21<br><br>Selected as highest dose expected to show slight offspring toxicity | Tanaka et al. 2016 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | under limit of detection of 5 ppb<br><br>OTA was purchased | | | | | | |
| Male Wistar rats | N=6 / treatment | 0, 1 (L) or 4 (H) mg/kg bw | Daily by gavage (in corn oil)<br><br>7 days | | Decrease in bw<br>-day 4 (H)<br>-day 7 (L)<br><br>(H)[a]<br>Increased levels of AST, ALP, TP, BUN, CREA, TG, LDH, GLU<br>Increase in ALP, AST, ALB indicative of hepatic injury<br><br>(L)[a]<br>Increased levels of ALB, TP, BUN<br><br>(H) and (L)<br>Impairment of renal function (indicators: CREA[a],BUN)<br>Hydropic degeneration, swelling, vacuolization & necrosis in epithelial cells of renal tubules<br><br>Dose dependent increase in positive PCNA[a] signals (indication for increased cell proliferation) in the kidneys | | Number of animals only given below tables | Zhu et al. 2016 |
| Male F344/NSlc-Tg (*gpt* | N=10 / group | Exp I<br>5 ppm | Exp I<br>Daily via basal diet | | Indication DSBs[a] induction at carcinogenic target sites<br>-DSBs predominantly repaired by HR | | 210 µg/10mL/kg/day by gavage is equivalent to 5 ppm dietary exposure | Kuroda et al. 2014 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| delta) rats | | Exp II<br>70, 210 or 630 µg/10mL/kg/day<br><br>OTA was extracted from a culture of *Aspergillus ochraceus* (BD-5)<br>Purity > 95% | Exp II<br>Daily by gavage<br><br>4 weeks | | -consequently leads to large deletion mutations<br><br>Sign reduced kidney weights (Exp I & II)<br><br>Sign decrease bw at 630 ug/kg (Exp II) | | Dosing level based on 2 year carcinogenicity study in rats | |
| Male F344 rats | N=6 / group | 0, 70 or 210 µg/kg bw | 5 days per week by gavage (in corn oil)<br><br>4 or 13 weeks | | Apparent kidney damage within 13 weeks<br>-cytoplasmic vacuolisation, karyomelagy<br><br>13 weeks<br>-decrease in HDL[a], AST, LDH<br>-some implications for carcinogenicity<br><br>Changes in AST, LDH, ALP suggest liver damage<br><br>Increase in PCNA<br><br>Limited effect on ox stress parameters | | | Qi et al. 2014 |
| Male F344/NDlc rats | Total N=16<br><br>N=4 / | 210 ug/kg bw<br><br>(see Taniani et al 2012a – not | Daily by gavage<br><br>28 days | Average 14.46 ± 0.30 g/day/animal | Increased cell proliferation activity, apoptosis & immunohistochemical cellular distributions of molecules suggestive of induction of DNA | | Combination study with antioxidants<br><br>Data presented here is for | Taniani et al. 2014 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | group | available online) | | | damage & cell cycle aberrations<br><br>Increased number of proximal tubular cells with karyomegaly<br><br>Limited effect on ox stress parameters | | individual exposure to OTA only | |
| Male Wistar rats | | 3 mg/kg<br><br>Ota was purchased | Daily by gavage (in corn oil)<br><br>1, 3 or 7 days | | Results point to an epigenetic action of OTA<br><br>Further evidence supporting non-genotoxic mechanism of carcinogenicity | | DNA array data mined from a previous study (Luhe et al 2003)<br>-results provide molecular basis for interpretation of nephrotoxicity<br>- transcriptional changes were detected for genes involved in DNA damage response and apoptosis, response to oxidative stress and inflammatory reactions<br>-culture model comparable to in vivo data<br><br>Not most useful study? | Jennings et al. 2012 |
| Fischer F344 rats | Total N=34<br><br>N=5 / cage | 50 µg OTA/kg bw<br><br>Fermentation product of *A. ochraceus* (D2306)<br><br>5-6 mg OTA/g | Daily via diet (aluminium vessel in powdered diet)<br><br>Up to 2 years | 20 g per animal | Renal carcinomas (unilateral) (4/34)<br><br>First (large) renal carcinoma at 76 weeks<br><br>Renal adenoma at weeks 93 and 105 (recognition of early tumourigenesis) | | Design virtually the same as Mantle et al. 2005<br><br>Dose calculated based on dietary intake from initial weight<br>Dietary OTA of 1 mg/kg<br><br>Mononuclear leukemia (47%) (67% in controls) | Mantle and Kulinskaya 2010 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | OTA B<br>5-10% of total OTA<br><br>No other mycotoxins were biosynthesised | | | | | - this seems to be a common appearance in rats at a higher stage in life | |
| Male Dark Agouti rats | Total N=80<br><br>N=5 / group | 5 ppm<br><br>400 ppb putative threshold diet<br><br>From initial weight dietary intake of OTA ~90 ug/day<br><br>3 month exposure ~640 ug/kg declining to 450 ug/kg daily<br><br>Fermentation product of A. ochraceus (D2306)<br><br>5-6 mg OTA/g (~5000 ppm) | Daily via diet<br><br>5 ppm for 3, 6 & 9 month, animals kept for natural life<br><br>400 ppb For up to 2 years | 90 g per group of 5 animals<br><br>18 g/animal | Renal tumours<br>- 9 months – 20% of animals<br>-None in group exposed for only 3 month<br>-Non in group given threshold dose<br><br>Latency between ceasing toxin exposure and tumour discovery was 35-97 weeks | NOEL<br>~7 ug OTA/day (feed containing 400 ppb)<br><br>Mean daily dose commenced at ~50 ug/kg For adults 30-20 ug/kg<br><br>3 month exposure - Threshold for malignant renal disease<br><br>Mean tolerable daily dose ~ 85 ug (3month exposure to 5ppm diet) – | | Mantle 2009 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | OTA B<br>5-10% of total OTA | | | | threshold exposure for renal carcinoma | | |
| Male F344 (Fisher) rats | Total N=24<br><br>N=3 / group | 100 ug<br><br>5 ppm in contaminated diet<br><br>(from 50 weeks of age on)<br><br>Rats ~2 years<br>-1 group 35 weeks exposure<br>-1 group exposure until end<br><br>Fermentation as described in previous papers | Daily via diet<br><br>35 or 51 weeks<br><br>Total of ~2 years | 20 g | No renal carcinomas occurred<br><br>4 renal adenomas were found in the 6 rats surviving for 110 weeks | | Authors comment:<br><br>First adenoma found after six months of continuous OTA exposure, at an age close to the normal endpoint of NTP toxicological studies.<br>The other three small adenomas all occurred in rats given only the basic 35 weeks of OTA exposure, but found at an age beyond a classical two year endpoint<br><br>Mononuclear leukaemia (38%)<br>-distributed across last quarter of life<br>- this seems to be a common appearance in rats at a higher stage in life | Mantle and Nolan 2009 |
| Male Fisher 344 rats | N=10 / group<br><br>5 / cage | 0.5 mg/kg bw<br><br>Purchased | Daily by gavage<br><br>7 & 21 days | | Time dependent increase of OTA in plasma, liver, kidney<br><br>Signs of nephrotoxicity<br>-Histopathological changes in the kidney<br>-single cell necrosis, karyomegalic | | Unclear from paper if N=10 is per treatment group or the total number of animals | Arbillaga et al. 2008 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | nuclei<br>-most pronounced after 21 days<br><br>Day 7 - 1 rat very slight tubulonephrosis<br>Day 21 - 3 of the 5 treated animals showed signs of tubulonephrosis<br><br>Ox stress response pathways & genes involved in mechanisms & transport inhibited<br><br>Genes implicated in cell survival/proliferation up-regulated at day 21 | | | |
| Male Fischer F-344 rats | N=5 / group | Approx. 5 ppm<br><br>Exp I<br>Initially<br>300 µg/kg bw (weight 175 g)<br>After<br>100 µg/rat (wheight 333g)<br><br>Exp II<br>50 or 300 µg OTA/kg bw for 2 years<br><br>Exp III<br>300 µg/kg bw for 10 month | Daily via diet<br><br>Up to 2 years<br><br>Exp I<br>2 years<br><br>Exp II<br>2 years<br><br>Exp III<br>10 month<br><br>Exp IV<br>9 month | 20 g | Renal tumours<br>-adenomas & carcinomas<br><br>Renal histopathological change (at dose without clinical abnormalities) – karyomegalic nuclei in proximal tubule epithelial<br><br>No evidence of necrosis, apoptosis, fibrosis<br><br>Some cases mononuclear leukemia /testicular tumours<br>-typical in some aging rats<br><br>Exp I<br>Tumour incidence 16/64<br>(rats 1-4 & 9) | | Concentration in feed was adjusted every 2 weeks according to weight changes during animal growth | Brown et al. 2007 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | from puberty<br><br>Exp IV<br>100 µg/rat starting at 1 year for 9 month<br><br>Fermentation as described in previous papers<br>5-6 mg OTA/g | | | Exp II<br>Tumour incidence 6/35 and 7/12<br>(rats 5 & 10, rats 6 & 7)<br><br>Exp III<br>Tumour incidence 5/5<br>(rat 8)<br><br>Exp IV<br>Tumour incidence 4/24<br>(rats 11 & 12) | | | |
| Male F344/N rats | N=5 / group | 0, 21, 70 or 210 µg/kg bw<br><br>OTA was purchased 99% | 5 days per week by gavage (in corn oil)<br><br>14, 28 or 90 days | | OTA produced renal alterations<br>-single cell death of lining cells<br>-enlarged nuclei, karyomegaly<br>-lesions progressed with increasing dose & time<br>-as early as 2 weeks (210 µg/kg bw)<br>-28 and 90 days for 70 µg OTA/kg bw<br><br>Increase of cell proliferation (70 & 210 µg/kg bw)<br><br>No clinical signs of toxicity were observed<br><br>No signs of nephrotoxicity were evident by serum/urine analysis<br><br>4 weeks<br>Decreased number pf reticulocytes (70 or 210 µg/kg bw); not detected | NOEL<br>21 ug/kg bw<br><br>For nephrotoxicity (kidney pathology & renal cell proliferation) | | Rached et al. 2007 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | after 90 days<br><br>≥ 4 weeks<br>Increase in leucocytes | | | |
| Female Wistar rats (sexually mature) | N=10 On each gestational day (GD)<br><br>N=11 on GD 6 & 7 (due to abortion) | 2.0, 2.5, 2.75, 3.0, 3.5 or 4 mg/kg bw<br><br>OTA extracted from *Aspergillus ochraceus* (NRRL-3174) Purity 94% | Single exposure by gavage<br><br>Between GD 6 & 15<br><br>Treatment volume 0.1mL / 100 g bw | | GD 6 & 7 most critical for induction of teratogenicity<br><br>Between GD 6 & 15<br>4 mg/kg bw<br>Maternotoxic, embryocidal, fetotoxic<br>Maternal deaths (15%)<br>Foetal resorption (up to 55.75%)<br>Post implementation loss (69.5%)<br>Reduction foetal bw (up to 54.72%)<br><br>3.0 & 3.5 mg/kg bw<br>Teratogenic<br>Reduction in maternal bw<br>Developmental defects<br><br>2.75 mg/kg bw<br>Multiple foetal abnormalities in many organ systems of individual foetuses<br><br>2.5 mg/kg bw<br>No maternal toxicity<br>No developmental embryotoxicity<br>Foetal abnormalities less severe | 2,75 mg/kg bw was found to be min effective teratogenic dose<br><br>Sub-threshold level of OTA for induction of teratogenicity by oral single dose | Authors speak of a preliminary study first thing in the result section<br>-unclear if the following results are the preliminary study or the actual study | Patil et al. 2006 |
| Female Wistar rats (adult) | Total N=30<br><br>N=5 / group | 0.5 mg/kg bw<br><br>OTA was purchased 99% | Daily by intraperitoneal injection | | Ox stress involved in mechanism of OTA toxicity<br><br>Increased protein carbonyls in kidney/liver | | No indication how many of the 6 groups are control and exposure<br><br>Focuses on ox stress only | Domijan et al. 2005 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | 7, 4 & 21 days | | -highest level was found after day 14 (kidney) & day 21 (liver) | | | |
| Male F344 rats | Total N=20<br><br>N=5 / group | 0, 0.03, 0.1 or 0.3 mg/kg bw<br><br>Purchased | Daily by gavage<br><br>4 weeks | | No effect on basic DNA damage<br><br>OTA mediated ox damage detected in kidney/liver of all dose groups<br><br>Histopathological changes in kidney (0.3 mg/kg)<br>-apoptosis & karyomegaly in epithelium of proximal convoluted tubules<br>To a lesser degree swelling, vacuolisation,<br><br>Proteinurea, Glucosuria, Polyuria (0.3 mg/kg)<br><br>Ox damage in kidney/liver at 0.03 & 0.3 mg/kg bw<br><br>Oxidative damage in 0.1 mg/kg group not stat sign | Oxidative damage in liver/kidney as low as 0.03 mg/kg bw | | Kamp et al. 2005 |
| Male F344 Fischer rats | N=3 / group | 0, 250, 500, 1000 or 2000 µg/kg bw<br><br>OTA was purchased | 5 days per week by gavage (in corn oil)<br><br>2 weeks | | Data suggest OTA may cause genetic damage in target/nontarget tissues<br>-Independent of direct covalent binding to DNA<br>-Mechanisms may involve ox stress<br><br>Significant (but small) dose-dependent increase in DNA breakage in liver and spleen; more prominent in lower doses in kidneys | | Small number<br>No mention how many animals in total/repeats of doses | Mally et al. 2005a |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | Characteristic pathological alterations in the kidney<br>-karyomegaly, polyploidy, increased apoptosis & mitosis<br><br>No histopathological changes in liver | | | |
| Male F344 rats | N=3 / group | 0, 0.25, 0,5, 1 or 2 mg/kg bw<br><br>Purchased | 5 days per week by gavage (in corn oil)<br><br>2 weeks | | Kidney pathology was present at all dose levels administered<br>(clear dose dependent increase)<br><br>Increase in expression of PCNA<br><br>Changes in clinical chemical parameters indicative of nephrotoxicity only observed at end of study (1 & 2 mg/kg bw)<br><br>No liver damage<br><br>Significant reduction in bw gain only at highest dose (2 mg/kg bw) | | Small number<br>No mention how many animals in total/repeats of doses | Mally et al. 2005b |
| Male Fisher-344 rats | N=5 / group | Approx 100 µg OTA/day<br><br>(300 µg OTA/kg bw until weight of 333 g was reached, thereafter 100 µg/rat)<br><br>Fermentation | Daily via diet<br><br>Up to 2 years | 100 g | Renal carcinoma (20%, unilateral)<br>-first renal tumor at 75 weeks (animal was losing weight and was euthanized)<br>-rate of discovery increased from ~90 weeks | | No mention of total number/replication of dosing<br><br>Mononuclear leukaemia (50%)<br>- this seems to be a common appearance in rats at a higher stage in life | Mantle et al. 2005 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | product<br>5-6 mg OTA/g<br><br>OTA B<br>5-10 % of total OTA | | | | | | |
| **Studies in mice** | | | | | | | | |
| Male p53 +/- mice (P53N5-T)<br><br>Male p53 +/+ mice (P53N5-W) | Total<br>N = 80<br><br>N=40 / strain<br><br>4 x N=10 / dose / strain | Weeks 1 & 2<br>1, 15 or 40 mg/kg diet<br><br>Due to rapid weight loss<br><br>Weeks 3-26<br>0.5, 2 or 10 mg/kg diet<br><br>OTA was purchased<br>Purity > 98% | Daily via diet<br><br>26 weeks | Mean daily OTA consumption based on recovery from diet<br><br>Week 1 & 2<br>0.8, 10.2, or 31.7 mg OTA/kg bw/day<br><br>(20-30% lower than at preparation)<br><br>Week 3-26<br>0.03, 0.20, or 1.46 mg/kg bw/day | Renal damage; no tumours<br>-renal lesions, cellular proliferation, karyomegaly, apoptosis, tubular degeneration, anisokaryosis in epithelium of proximal tubules<br>-consistent with ochratoxicosis<br><br>Lesion in the lung, consistant wirh eosinophilic crystalline pneumonia<br><br>Dose dependent increase in PCNA-positive epithelial cells on proximal tubules<br>(sign higher in 10 mg/kg diet)<br><br>Expression of kidney injury biomarkers CLU[a] & OPN[a] in proximal tubules was significantly increased in p53+/+ mice (10 mg /kg) and p53+/- mice (2 & 10 mg/kg) | LOEL<br>200 µg/kg bw per day<br><br>Equivalent to 2 mg/kg diet | LOEL – derived based on literature?<br>No mention of derivation based on experimental results in text<br><br>Authors interpreted results as suggestive of primarily nongenotoxic (epigenic) MOA<br><br>OTA not detected in control diet<br>LOQ 6 ng/g<br>LOD 1.6 ng/g | Bondy et al. 2015 |
| Male C57BL/6 mice | Total<br>N=26<br><br>OTA1-3<br>N=4<br>(for each | 3.5 mg/kg bw<br><br>In a volume of 2.8 µL/g bw<br><br>OTA was | 1, 2, 3 or 6 cumulative doses by intraperitoneal injection | | Significant alteration in the proliferation process<br><br>Suggest that OTA exposure can affect the brain development, alter the regulation of adult neurogenesis, | | | Paradells et al. 2014 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | dose)<br><br>OTA6<br>N=8<br><br>Control<br>N=6 | purchased<br>Purity 99% | (OTA1, OTA2, OTA3, OTA6)<br><br>Each dose separated by 3 days to min toxic effects | | acting as a detrimental factor of the neuroregenerative capacity of the brain<br><br>Decrease in [a]$BrdU^+$ in OTA2,3&6<br><br>Maximum proliferation impairment OTA6 | | | |
| Male Swiss ICR mice | N=70<br>Experimental<br><br>N=20<br>Control | Neurotoxicity<br>0-6 mg/kg bw<br><br>3.5 mg/kg<br><br>Purchased | Single exposure by intraperitoneal injection<br><br>Endpoints 6, 24 or 72 hours after injection | | 3.5 mg/kg i.p.<br>-Ox stress in all brain regions<br>-TBARS[a] levels increased<br>-upregulation of SOD activities (peak values after 24h)<br>-reduction of striatal DA[a], DA turnover & TH[a] immunoreactivity<br><br>0-6 mg/kg i.p.[a]<br>Dose dependent decrease in striatal content & turnover with an $ED_{50}$[a] of 3.2 mg/kg | | | Sava et al. 2006 |
| **Study in rabbits** | | | | | | | | |
| Male New Zealand white rabbits | Total<br>N=32<br><br>N=4 / group | 1 ppm | Diet<br><br>30 & 60 days | | Dullness, lethargy, marked depression, anorexia and dehydration at 60 days.<br><br>Significant decrease in bw from day 30 up to day 60 days<br><br>Significant nephrotoxicity<br>- degeneration of the proximal | | Combination study with AFB1[a] | Prabu et al. 2013 |

| Species | Number | Exposure concentration & purification | Exposure | Feed Intake | Results | NOAEL/LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | convoluted tubules<br><br>Testes were atrophic | | | |

* LOAEL/NOAEL not given by the authors but derived from the information given in the paper
** Exposures not given by authors but derived from levels of OTA in feed and the feed intake
[a] Please find full names in abbreviation list on pages 15 and 16

## Annex B

59. Systematic literature searches were performed to retrieve appropriate information on OTA toxicity and occurrence in breast milk. The search engine used was pubmed.

*Toxicity data*

60. A literature search was performed in September 2017 to retrieve new *in vivo* toxicological studies since the EFSA opinion in 2006. To ensure that no publications were missed, the literature search was set from 2005 until the present date.

61. Using Boolian characters, the literature search was conducted using the search terms “ochratoxin a” AND toxic*. 791 hits were recorded and the abstracts were scanned for *in vivo* toxicity/studies. The relevant studies were retrieved or where necessary ordered.

62. The relevant studies have been summarized in Table 7 in Annex A.

*Breast milk*

63. A separate literature search was performed for ochratoxin a AND breastmilk. No limitations were set for said search and 54 hits were recorded. The abstracts were scanned and the relevant publications were retrieved or where necessary ordered. Publications from outside the European member states were excluded.

64. The relevant studies are summarized in Table 1 in the main body of the text.