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

Discussion paper for the prioritisation of dietary components and xenobiotics for future papers on their effects on maternal health – Part 1

Introduction

1. The Scientific Advisory Committee on Nutrition (SACN) last considered maternal diet and nutrition in relation to offspring health in its reports on 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life' (SACN, 2011) and on 'Feeding in the first year of life' (SACN, 2018). In the latter report, the impact of breastfeeding on maternal health was also considered.

2. In 2019, SACN agreed to conduct a risk assessment on nutrition and maternal health focusing on maternal outcomes during pregnancy, childbirth and up to 24 months after delivery; this would include the effects of chemical contaminants and excess nutrients in the diet.

3. SACN agreed that, where appropriate, other expert Committees would be consulted and asked to complete relevant risk assessments e.g. in the area of food safety advice. This subject was initially discussed during the horizon scanning item at the January 2020 meeting with a scoping paper being presented to the Committee in July 2020. This included background information on a provisional list of chemicals proposed by SACN. It was noted that the provisional list of chemicals was subject to change following discussion by COT who would be guiding the toxicological risk assessment process: candidate chemicals or chemical classes can be added or removed as the COT considered appropriate. The list was brought back to the COT with additional information in September 2020[1]. Following a discussion at the COT meeting in September 2020, it was agreed that papers on a number of components should be prioritised and to this end, papers on iodine, vitamin D and dietary supplements have been or will be presented to the Committee. The remaining list of compounds were to be triaged on the basis of toxicity and exposure. The current paper presents information intended to aid this process.

[1] COT Contribution to SACN review of nutrition and maternal health: proposed scope of work and timetable. Available at: https://cot.food.gov.uk/sites/default/files/2020-09/TOX-2020-%2045%20%20Maternal%20diet%20scoping%20paper_0.pdf

4. The list of remaining chemical and food entities for consideration in this paper is: **mycotoxins, phytoestrogens**, **resveratrol, vitamins A, C and E and caffeine.** These are either endogenous substances or of biological origin. A second paper will consider exogenous and process contaminants: heavy metals (including arsenic), selenium**,** heterocyclic amines, acrylamide, dioxins and dioxin-like PCBs, non-dioxin-like PCBs, bisphenol A, and constituents of oily fish.

Mycotoxins

5. Mycotoxins are secondary fungal metabolites that can cause biochemical, physiological and pathological changes in many species. Harmful effects of mycotoxins observed in humans and animals include carcinogenicity, teratogenicity, immune toxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive and developmental toxicity, although different compounds have different spectra of effects. In this paper, the mycotoxins being considered are aflatoxins (from *Aspergillus spp*), nivalenol, deoxynivalenol, fumonisins, fusaranon-X, T-2, and HT-2 from *Fusarium spp*, ochratoxin A from *Penicillium* and *Aspergillus,* citrinin, patulin (from various fungi) and ergot alkaloids from *Claviceps spp*.

6. In addition to parent mycotoxins, modified or “masked” forms are known to exist these are undetected by conventional analytical techniques and which can also contribute to the overall dietary intake if the masking ligand is removed from the parent molecule during digestion. Kovač *et al* (2018) reviewed the masked mycotoxins from a range of common fungi. Berthiller *et al* (2013) describe methods for analysing masked forms of mycotoxins in food matrices.

7. Mycotoxins usually occur in combination in a food commodity, either because of multiple fungal contamination or because the fungus, for example *Fusarium spp,* produces more than one toxin. A paper addressing combined dietary exposure to mycotoxins is currently being considered by the COT. In this present paper, mycotoxins are considered individually.

Aflatoxins

Toxicity

8. Aflatoxins (AF) B1, AFG1 and AFM1 are carcinogenic when delivered orally via the diet or by gavage. There is limited evidence for the carcinogenicity of AFB2 and inadequate evidence for carcinogenicity of AFG2. AFB1 is more potent than AFG1 with respect to liver carcinogenicity but AFG1 induces a higher incidence of kidney tumours than AFB1. AFB1 is also more potent than AFM1 with respect to liver carcinogenicity by approximately 10-fold. The epidemiological studies reported since 2006 have added to the weight of evidence that aflatoxin exposure is associated with a risk of developing hepatocellular carcinoma (HCC), with a higher risk for people infected with either hepatitis B or C virus (HBV or HCV). (EFSA, 2020).

9. AFB1 becomes toxic through metabolic activation by various cytochrome P450 enzyme families including CYP1A2, CYP3A4, and CyP3A5, which are mainly in the liver, but also in other tissues including the placenta, intestine, and spleen. The reactive epoxide species, AFB1-8,9-exo-epoxide and AFB1-8,9-endo-epoxide are produced by oxidation, along with other metabolites such as aflatoxin M1 (AFM1). The aflatoxin epoxides and AFM1 are toxic and AFB1 exo-epoxide binds to DNA forming mutagenic lesions (Smith *et al*, 2017).

10. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (2001) Reviewed the toxicity of aflatoxins B1 and M1, highlighting the association of hepatitis B infection and increased incidence of liver cancer elicited by AFB1. The same paper pointed out that humans appear to be less sensitive to the carcinogenic effects of AFB1 than are other species such as rats due to differences in the rates of metabolism of the parent compound to its 8,9-exo-epoxide, even though human livers do not express the detoxifying enzyme glutathione-S-transferase (GST) with high activity towards the epoxide.

11. Sriwattanapong *et at* (2017) found that pregnant C57BL/6 J mice accumulated 2-fold higher AFB1-N7 -guanine DNA adducts in the liver following a single IP dose of 6 mg/kg AFB1, when compared with nonpregnant controls at 6 h post-exposure. This effect paralleled elevated hepatic protein expression of mouse CYP1A2 and mouse homologs of human CYP3A4, phase I enzymes capable of bioactivating AFB1. The authors concluded that pregnancy may constitute a critical window of susceptibility for maternal health. AFB1 exposure has been correlated with maternal anaemia in experimental animals (Shuaib *et al*, 2010), possibly by haemolysis, although Smith *et al* (2017) point out that this mechanism may only occur at intakes higher than experienced by human populations. Other mechanisms may also occur, such as malabsorption of iron, chronic inflammation leading to decreased erythropoiesis, or increased hepcidin production, leading to decreased iron absorption, and iron release from macrophages. Maternal anaemia has been linked to pre-term delivery.

12. EFSA (2020) derived a the 95th percentile lower limit of a benchmark dose eliciting a 10% increase in effect ($BMDL_{10}$) of 0.4 μg/kg bw per day for the induction of HCC by AFB1 in male rats.

Exposure

13. For adults, EFSA (2020) reported that the mean lower bound (LB) exposure to AFB1 ranged from 0.22 to 0.49 ng/kg bodyweight (bw) per day and the mean upper bound (UB) exposure from 1.35 to 3.25 ng/kg bw per day. The LB 95th percentile (P95) exposure to AFB1 ranged from 0.62 to 1.36 ng/kg bw per day for adults. The UB 95th percentile exposure to AFB1 ranged from 2.76 to 6.78 ng/kg bw per day.

14. From their $BMDL_{10}$ of 0.4 μg/kg bw per day, EFSA (2020) calculated Margin of Exposure (MOE) values (minimum to maximum) ranging from 5,000 to 225 for the mean LB exposure to AFB1 and from 690 to 58 for the mean UB exposure to AFB1 across dietary surveys and age groups. For the P95 LB exposure to AFB1, the MOE

values range from 1,143 to 64 and from 145 to 29 for the P95 UB exposure to AFB1 across the same dietary surveys and age groups. The calculated MOEs are below 10,000, which raises a health concern.

15. For AFM1, based on the $BMDL_{10}$ of 0.4 μg/kg bw per day derived for AFB1 and a potency factor of 0.1, MOE values ranged from 100,000 to 2,564 for the mean LB exposure estimates, and from 66,667 to 2,020 for the mean UB exposure estimates. For the P95 LB exposure from 33,333 to 642 estimates, and from 25,000 to 508 for the 95th percentile UB exposure estimates across dietary surveys and age groups have been calculated.

16. Following EFSA guidance for substances that are both genotoxic and carcinogenic, an MOE of 10,000 or higher between the reference point and the estimated dietary exposure would be of low health concern. The calculated MOEs for neoplastic effects in most of the surveys, in particular those for high consumers and for breastfed infants in all scenarios were below 10,000 and thus indicate a possible health concern for these consumer groups.

17. A Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of aflatoxin B1 for adults (aged 19+) was a mean of 0.000 – 0.007μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.0018 μg/kg bw per day (LB–UB).
https://www.food.gov.uk/sites/default/files/media/document/measurement-of-the-concentration-of-mycotoxins-from-the-uk-tds_0.pdf

18. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of aflatoxin G1 for adults (aged 19+) was a mean of 0.000 – 0.006 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.021 μg/kg bw per day (LB–UB).

19. However, in the absence of information on the levels associated with maternal effects, the implications of current exposure to aflatoxins is unclear. It is also unknown whether the current HBGV based on HCC would be protective for such effects.

Nivalenol (NIV) and Deoxynivalenol (DON)

Nivalenol (NIV)

20. Nivalenol belongs to the group of trichothecene mycotoxins. Trichothecenes have a common tetracyclic, sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system and are divided into four structural groups (A-D). Type A is has a functional group other than a ketone at the C-8 position, and trichothecenes that have a carbonyl function at this position are classed as type B. Type C, has a second epoxide ring at C-7,8 or C-9,10, and type D contains a macrocyclic ring system between C-4 and C-15 with ester linkages (Aupanun *et* al 2016). The epoxide group between C12 and C13 seems to account for many of the typical toxic effects of

trichothecenes. Type A and type B tricothecenes are predominant in food. Nivalenol is a Type B toxin. Others include fusarenon-X, deoxynivalenol, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol. These toxins are produced by plant pathogenic *Fusarium* fungi that grow on the cereals such as wheat, barley, oats, rye and maize.in temperate climates.

Toxicity

21. In 2000, the Scientific Committee on Food (SCF) published an opinion on nivalenol. The SCF concluded that the general toxicity, haematotoxicity and immunotoxicity of nivalenol are the critical effects and established a temporary tolerable daily intake (t-TDI) of 0-0.7 µg/kg bw per day. This t-TDI was confirmed by SCF in their opinion on group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol in 2002. In 2010 The Food Safety Commission in Japan (FSCJ) established a TDI for nivalenol of 0.4 µg/kg bw per day based on decreased white blood cell (WBC) counts observed in a 90-day rat study.

22. In 2013, the CONTAM panel found that there was no evidence since the SCF evaluation that other toxic effects occur at doses lower than those inducing immunotoxicity and haematotoxicity. Studies in mice, fed diets containing nivalenol for 24 days, 6 months, 12 months and 24 months, and a 90-day study in rats reported neutropenia, leukopenia, erythropenia and thrombocytopenia as critical effects of nivalenol. These are known adverse effects of trichothecenes and have been associated with bone marrow depression (a decreased number of haematopoietic cells) n. The Panel considered that the decrease of white blood cell (WBC) count in rats fed diets containing nivalenol for 90 days was most appropriate for benchmark dose (BMD) modelling. The $BMDL_{05}$ of 0.35 mg nivalenol/kg bw per day was calculated for combined data for males and females, and was used as a reference point for the risk characterisation. Since nivalenol was unlikely to be genotoxic the CONTAM Panel used an uncertainty factor of 3 to account for gaps in the database in addition to the default uncertainty factor of 100, giving a TDI of 1.2 µg/kg bw per day.

23. In 2017 the EFSA The CONTAM Panel re-examined NIV and selected the emetic effects for acute hazard characterisation. Benchmark dose (BMD) analysis was performed using a benchmark response (BMR) of 10%, resulting in a benchmark dose 95% lower and upper confidence interval ($BMDL_{10}$–$BMDU_{10}$) of 0.14–0.23 mg NIV/kg body weight (bw) per day. Using the acute $BMDL_{10}$ of 0.14 mg/kg bw for NIV and an uncertainty factor of 10 for intraspecies differences, an ARfD of 14 µg NIV/kg bw was established. No interspecies variability factor was applied because humans were not considered more sensitive than mink to the acute emetic effect of NIV, as supported by studies showing that similar doses of emetine were effective both in humans and mink.

24. Ito *et* al (1986) injected NIV intraperitoneally into pregnant mice at dose levels of 0, 0.1, 0.5 or 1.5 mg/kg b.w./day on days 7-15 of gestation. The highest dose caused stillbirths after vaginal hemorrhage in six of ten animals. High percentages of embryo deaths were found in the two highest dose groups (87.8 and 48.4%), but no fetal malformations were seen in the test groups. A single administration of 3 mg/kg

on day 7 affected the embryos within 10 hrs, damaged the placenta within 24 hrs, and revealed stillbirths at 48 hrs. In a similar study, maternal and embryo toxicity which resulted to intrauterine growth retardation was reported when pregnant mice (GD: 7-15) were either fed with 6-30 mg/kg/diet of NIV or 1-20 mg/ kg bw of NIV by oral gavage, particularly at the highest doses (Ito et al., 1988). Teratogenic effects were not observed in any of the concentrations of NIV used either in contaminated feed or oral gavage (Ito et al., 1988).

Exposure

25. All chronic dietary exposures to nivalenol estimated, based on the available occurrence data in food, are below the TDI of 1.2 µg/kg bw.per day, and are therefore not of health concern.

26. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of nivalenol for adults (aged 19+) was a mean of 0.000 – 0.162 µg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.535 µg/kg bw per day (LB–UB).

Deoxynivalenol (DON)

27. Deoxynivalenol DON usually co-occurs with other mycotoxins from Fusarium and other genera. The main plant metabolite of DON, DON-3-glucoside also occurs and is a modified or “masked” form of this toxin. Other modified forms of DON have been reported

Toxicity

28. Yu *et al* (2019, from abstract) administered DON at 0, 1.0 and 2.5 mg/kg/day to mice during gestation. DON (1.0 and 2.5 mg/kg/day) slightly increased the blood levels of the liver enzymes ALT and AST on gestational day (GD)12.5 . Oxidative stress and anti-oxidation systems were both activated as shown by increased levels of the biomarkers ROS and GSH. Nrf2 translocation and increased haem oxidase-1 (HO-1) expression, which are protective against oxidative stress, were also observed following DON exposure. The authors concluded that DON-induced ROS accumulation may cause maternal liver damage in the initial stages, but the activated Nrf2/HO-1 pathway led to the removal of ROS and decreased the level of oxidative stress thereby protecting the liver from further oxidative damage.

29. Human poisoning outbreaks from acute exposure to DON have been repeatedly reported in Asia, with symptoms including nausea, vomiting, diarrhoea, abdominal pain, headaches, dizziness, fever, and in severe cases, bloody stool. No lethality was reported. However, the evidence of adverse health effects in humans due to chronic exposure to DON is lacking.

30. A TDI of 1 µg/kg bw per day, that was established for DON based on reduced bodyweight gain in mice, was used as a group-TDI for the sum of DON and its

derivatives 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside. In order to assess acute human health risk, epidemiological data from mycotoxicosis were assessed and a group acute reference dose (ARfD) of 8 μg/ kg bw per eating occasion was calculated.

Exposure

31. The CONTAM Panel assessed the exposure to the sum of DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside. The mean acute human exposure across 39 different dietary surveys and all age groups ranged from 0.2 to 2.9 μg/kg bw per day for the minimum LB to the maximum UB. The 95th percentile acute exposure ranged from 0.7 to 6.7 μg/kg bw per day. Infants showed the highest acute dietary exposure. The mean chronic human exposure to the sum of DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside across 33 different dietary surveys and all age groups was 0.2 to 2.0 μg/kg bw per day (minimum LB–maximum UB), and the 95th percentile chronic exposure was 0.5 to 3.7 μg/kg bw per day (minimum LB–maximum UB). The acute exposure estimated above is below the ARfD and would thus not be a concern for health, but the chronic exposure may exceed the TDI.

32. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of deoxynivalenol for adults (aged 19+) was a mean of 0.127 – 0.153 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.300 – 0.387 μg/kg bw per day (LB–UB).

Fusarenon-X (FX; 4-acetylnivalenol)

Toxicity

33. FX is another tricothecene toxin produced by *Fusarium spp*, found in cereals for human and animal consumption.

34. Exposure to FX is associated with diarrhoea, extensive intestinal haemorrhaging and damage to the intestinal mucosa. The few available studies on FX suggest a higher acute toxicity of FX compared with other trichothecenes. These authors analysed the intestinal toxicity of FX by comparison to DON in order to establish whether both toxins have similar modes of action or if their toxicities are related to different mechanisms.

35. Exposure to FX resulted in more severe histomorphological alterations than exposure to DON. The responses to both mycotoxins were primarily linked to a pro-inflammatory effect. The FX doses associated with the deleterious pro-inflammatory effects were below the known effective doses for the toxicity of other type B trichothecenes targeting other critical functions. In addition to their overlapping inflammatory effects, the toxicity of DON and FX significantly deviated from one another, especially regarding signalling pathway activation.

36. A report by RIVM (Pronk *et al* 2002) reviewed the toxicology and occurrence of six type A and type B tricothecenes, including fusarenone-X, which covered *inter alia* reproductive and developmental toxicity. In pregnant female mice injected with FX at 0.63 – 4.1 mg/kg bw, all of the animals given the highest dose died within one day of dosing and in the others, spontaneous abortion was noted. with a frequency that increased with the dose, as well as fetal reabsorptions. These effects were also seen in a separate feed dosing study (approximately 25, 50 and 100 μg FX/animal/day). There was, however, no observation of teratogenic effects.

37. Alassane-Kpembi *et al* (2017) state that little is known about the effects of FX, and the available toxicity data are too limited to support derivation of a TDI.

38. EFSA (2017) calculated TDI values for nivalenol and its derivatives and noted that although FX is a derivative, it is also a mycotoxin in its own right and suggested that, instead of it being included in the group TDI, future work should be undertaken to derive a TDI of its own.

Exposure

39. RIVM (2003) estimated dietary exposure in Finland from wheat, rye, oats and barley to be about 15 ng/kg bw/day. The major contributor to this intake was wheat at 10 ng/kg bw/day and then rye at 4 ng/kg bw/day.

40. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of fusarenon-x for adults (aged 19+) was a mean of 0.001 – 0.011 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.005 – 0.034 μg/kg bw per day (LB–UB).

Ochratoxin A (OTA)

41. OTA is a mycotoxin produced by several fungal species in the *Penicillium* and *Aspergillus* genera, primarily *Penicillum verrucosum, Aspergillus ochraceus* and *Aspergilli* of the section *Nigri,* especially *A. carbonarius*. It has been detected in a variety of plant commodities such as cereals and cereal products, coffee beans, beans, pulses, cocoa products, nuts and spices and dried fruit, as well as in a number of plant-derived products such as coffee, wine, beer and grape juice It has also been found in kidney, liver and blood from farm animals, where it occurs by transfer from animal feed.

Toxicity

42. The EFSA CONTAM Panel (2020) found no evidence suggesting that OTA is acutely toxic. OTA causes DNA breaks as well as guanosine hydroxylation that is indicative of an oxidative stress mechanism in cells *in vitro*. The toxin causes kidney tumours in animals, especially pigs and rats. EFSA recognised that this may be due to both direct and indirect genotoxic effects. A MOE approach was taken, with a $BMDL_{10}$ of 14.5 μg/kg bw, based on the production of kidney tumours in a 2-year

study in male rats. A MOE of low concern of 10,000 was applied although the Panel regarded this as conservative due to the uncertainties surrounding the mode of action.

43. OTA has been suspected of being the causative agent in cases of human chronic interstitial nephropathy of unknown aetiology that has appeared in populations where it is present in food at relatively high concentrations in Tunisia. However, on considering the data, the CONTAM Panel notes that the studies all had cross-sectional designs, and thus did not allow a conclusion to be reached on a possible causal association between OTA exposure and chronic interstitial nephropathy or other renal diseases.

44. EFSA (2020) state that: "… reproduction studies show that OTA induces developmental toxicity. However, the doses at which effects are observed differ by orders of magnitude in the different investigations and thus LOAELs derived in these studies (albeit for different endpoints and from studies with deviating designs) vary from 33.9 to 2,750 μg OTA/kg bw per day. Notably, adverse effects on offspring are usually paralleled by pronounced maternal toxicity. *In vitro* data suggest that OTA developmental effects might be mediated via its general toxicity which would support the in vivo findings. Overall, the new studies corroborate the conclusion of EFSA (2006) that OTA is a developmental toxicant but that developmental effects are generally observed at doses higher than those causing adverse effects in the kidney of pigs."

Exposure

45. Absorption of OTA is rapid following oral ingestion and most of the compound present in the blood is bound to plasma proteins. It has been detected in human blood, urine and breast milk.

46. The mean chronic exposure estimates ranged from 0.64 (minimum LB)/2.53 (minimum UB) to 9.13(maximum LB)/17.79 (maximum UB) ng/kg bw per day across dietary surveys and age groups. The high (95th percentile) chronic dietary exposure estimates ranged from 2.40 (minimum LB)/5.13(minimum UB) to 30.36 (maximum LB)/51.69 ng/kg (maximum UB) bw per day.

47. The calculated MOEs for non-neoplastic effects were above 200 in most of the dietary surveys for average and high consumers, including breast fed infants, and were therefore of low health concern.

48. EFSA concluded that more studies in appropriate animal models were needed to identify the sequence of critical events leading to kidney tumour development and the sites of OTA-specific gene mutations

49. EFSA also recommended additional studies into :

- the differential toxicokinetics of OTA in laboratory animals and humans, including transfer of OTA to the fetus;
- the relationship between biomarkers of exposure (OTA in plasma/serum) and external daily intakes for more reliable estimates of OTA exposure;

- the levels of OTA in human breast milk;
- the occurrence of OTA in cheese paste vs. cheese rind; and
- the occurrence and toxicity of modified OTA.

50. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of ochratoxin A for adults (aged 19+) was a mean of 0.004 – 0.008 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.025 – 0.029 μg/kg bw per day (LB–UB).

Fumonisins (FB)

51. Fumonisins are mycotoxins produced by fungi of the *Fusarium* genus found largely in plant products. Fumonisins are often found to be covalently bound to the food matrix and thus can be difficult to analyse, but are released during digestion.

Toxicity

52. Fumonisins are carcinogenic to laboratory animals, and in humans, consumption of fumonisin-contaminated maize is associated with higher rates of oesophageal cancer and neural tube defects (NTDs).

53. Gelineau-van Waes *et al* (2005, from abstract) found that administration of FB1 (20 mg/kg of body weight) to pregnant LM/Bc mice during early gestation resulted in 79% NTDs in exposed fetuses. The mechanism for this appeared to involve a GPI-anchored folate receptor (Folbp1) and its association with the lipid ganglioside GM1. Maternal folate supplementation partially rescued the NTD phenotype whereas GM1 significantly restored folate concentrations and afforded almost complete protection against FB1-induced NTDs. The authors concluded that maternal FB1 exposure altered sphingolipid metabolism and folate concentrations in LM/Bc mice, resulting in a dose-dependent increase in NTDs that could be prevented by maintenance of folate levels.

54. In an 2018 EFSA Opinion on FBs and their modified forms, it was stated that: “Following the guidance of EFSA (EFSA Scientific Committee, 2017) that recommends use of the lowest BMDL derived for a compound to set a HBGV, the CONTAM Panel decided to use the BMDL10 of 0.1 mg/kg bw per day derived for induction of megalocytic hepatocytes in mice for establishing a TDI for FB1. An Uncertainty Factor of 100 for intra and interspecies variability was applied resulting in a TDI of 1.0 lg FB1/kg bw per day.” Based on similarity of toxicological profiles, the related toxins FB2 – 4 were included in the TDI for FB1, but due to lack of *in vitro* and *in vivo* data, FB5 and FB6 were not.

55. EFSA (2005) observed that maternal toxicity was observed in rabbits at a dose of 0.25 mg/kg b.w./day, and foetal toxicity occurred at the lowest dose tested (0.1 mg/kg b.w.), but no major malformations could be observed

56. The CONTAM panel decided that the available data did not require an acute reference dose to be derived for the FBs or their modified forms.

57. JECFA (2016) retained a PMTDI of 2 µg/kg bw for FB1, FB2 and FB3, alone or in combination that was derived by them in 2011 https://apps.who.int/food-additives-contaminants-jecfadatabase/chemical.aspx?chemID=747

58. Humans consuming mouldy sorghum and maize containing fumonisins have shown acute adverse effects such as gastrointestinal symptoms but there was no information on the dose or type of fumonisin and presence of other mycotoxins in the food consumed. Therefore, any effects cannot be clearly attributed to fumonisin alone and hence it is not possible based on these studies to decide on acute effects of FBs in humans. Several clinical effects have been discussed in humans (such as oesophageal cancer, liver cancer, neural tube defects, growth impairment), but so far none of these have been causally related to fumonisin exposure.

Exposure

59. EFSA (2018) reviewed several biomarker studies for human exposure to the FBs in different countries. Consumers of maize-based staple food in Guatemala are a particularly well studied group. FB1-levels in maize in high-exposure communities were much higher (average: 3.69 µg/g) compared with low-exposure communities (0.69 µg/g).

60. In order to calculate an intake corresponding to 0.5 ng FB1/mL in urine, it was assumed that excretion is 0.5% of FB intake, that total urine output in the Guatemalan women is 1,000 mL, and the average weight was 60 kg. Based on these assumptions, 0.5 ng/mL urinary FB1 represents a total intake of 1.67 µg/kg bw per day (i.e. if 0.5 µg/L is 0.5% FBs daily intake, 100% is 100.2 µg/day, assuming 60 kg bw these are 1.67 µg/kg bw).

61. Estimates of dietary intakes of fumonisin based on national estimates have been presented by FAO/WHO, indicating an exposure from 0.02 µg/kg b.w./day to 0.2 µg/kg b.w./day, thus remaining below the PMTDI of 2 µg/kg b.w./day set by JECFA (FAO/WHO, 2001) In the JECFA evaluation only the consumption of contaminated maize or maize-containing food products was considered, as the contributions of other commodities to the intake of fumonisins were too low and too variable to affect long-term exposure significantly.

62. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of fumonisin B1 for adults (aged 19+) was a mean of 0.000 – 0.038 µg/kg bw per day (LB–UB) and a 97.5th percentile of 0.002– 0.102 µg/kg bw per day (LB–UB).

63. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of fumonisin B2 for adults (aged 19+) was a mean of 0.000 – 0.035 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.093 μg/kg bw per day (LB–UB).

64. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of fumonisin B3 for adults (aged 19+) was a mean of 0.000 – 0.031 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.083 μg/kg bw per day (LB–UB).

65. Exposure to fumonisins B1, 2 and 3 in the UK are below the JECFA PMTDI.

Zearalenone

66. Zearalenone (ZEN) is a mycotoxin that is also produced by *Fusarium* species of fungi and is found in plant-based foods, especially grain-based products. This mycotoxin, like others, can be found in various related derivatives or “masked” forms.

Toxicity

67. ZEN is known to have hormonal effects. For example, Bauer *et al* (1987) found that pigs readily exhibited signs of hyperoestrogenism, such as swelling and thickening of the vagina and vulva within a few days of onset of exposure to zearalenone (0.25 mg ZEN /kg diet (equivalent to 10 µg/kg b.w. per day) for 11 days, followed by 5 days of feed without zearalenone). There was also uterine hypertrophy and disturbance of oestrous cycles, ovulation, conception and implantation.

68. In their evaluation, JECFA (2000) noted that hepatocellular adenomas and pituitary tumours were observed in carcinogenicity studies in mice, but only at doses greatly in excess of the concentrations known to have hormonal effects (≥ 8-9 mg/kg bw/d.). The Committee concluded that these tumours were due to estrogenic effects and that the safety of ZEN should be evaluated on the basis of the dose that had no hormonal effect in the most sensitive species which was pigs. The Committee established a PMTDI for ZEN of 0.5 µg/kg bw/d, based on the NOEL of 40 µg/kg bw/d and using a safety factor of 100. The Committee also considered the LOEL of 200 µg/kg bw per day in a study on the metabolite alpha-zearalanol and recommended that the total intake of ZEN and its metabolites should not exceed the previously established TDI of 0-0.5 µg/kg bw derived for alpha-zearalanol.

69. In the same year, the EU Scientific Committee on Food (SCF) established a temporary TDI (t-TDI) of 0.2 µg/kg bw. This t-TDI included an additional uncertainty factor because of some deficiencies in the data base’, for example, whether adolescent pigs were more sensitive than adult pigs, as suggested by the study of Bauer *et al* (1987).

70. In 2011, EFSA published an Opinion on the risks for public health related to the presence of ZEN in food. A study by Döll *et al*. (2003b), was considered the critical study for establishment of a TDI. In the selection of an appropriate uncertainty factor to apply to the NOEL of 10 µg/kg b.w. per day, the comparative sensitivity of the female pig and human to oestrogens and to zearalenone and its metabolites was considered. It was thought unlikely that the human female would be more sensitive to oestrogens in general, or zearalenone and its metabolites in particular, than the female pig and thus it was not necessary to include an uncertainty factor of 2.5 for toxicodynamic differences between pigs and humans. An uncertainty factor of 40 (4 for interspecies differences in toxicokinetics and 10 for interhuman variability) was therefore used to derive a TDI of 0.25 µg/kg b.w.

71. In a more recent study, Gao *et al* (2017) investigated the effects of dietary ZEN on feed consumption, body weight gain, and reproductive performance in 4 groups of rats treated with 0, 5, 10 and 20 mg ZEN/kg bw /day on gestational days 0-21. Compared to the controls, the bw was decreased in the pregnant rats and female offspring of in groups receiving 10 and 20 mg/kg bw ZEN ($p < 0.05$). During gestation, the average daily food intake decreased significantly in the 20 mg/kg bw ZEN group, and average daily weight gain decreased in both the 10 and 20 mg/kg bw ZEN groups ($p < 0.05$). Pregnant rats treated with ZEN at concentrations up to 20 mg/kg bw showed no significant effect on litter size. But a dose of 20 mg/kg ZEN significantly decreased the birthweight and viability of pups ($p < 0.05$) compared to that of the controls. Treatment with 10 or 20 mg/kg bw ZEN increased FSH levels and decreased estrogen levels in both the maternal animals and adults of the F1 generation. No pathological changes were found in the ovaries or uteri of weaned rats, but significant follicular atresia and uterine thinning were observed in adult F1 rats in the top dose group.

Exposure

72. The EFSA CONTAM Panel (2011) estimated total chronic dietary exposures to zearalenone across 19 European countries, using LB and UB mean concentrations of zearalenone in foods, and consumption data for different age groups. For adults the minimum LB to maximum UB was 2.4 to 29 ng/kg bw per day for average consumers (average consumption in total population), and 4.7 to 54 ng/kg bw for high consumers (95th percentile consumption in total population).

73. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of zearalenone for adults (aged 19+) was a mean of 0.003 – 0.007 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.025 – 0.032 μg/kg bw per day (LB–UB).

T-2 and HT-2

74. T-2 and HT-2 are mycotoxins produced by *Fusarium spp*

Toxicity

75. T-2 toxin inhibits protein, RNA and DNA synthesis and, *in vitro*, appears to induce apoptosis, and, in some cell types, necrosis as well as lipid peroxidation. T-2 toxin induces haematotoxicity and myelotoxicity associated with impairment of haematopoiesis in bone marrow toxicity. T-2 toxin is rapidly metabolised to a large number of products, a major one of which is HT-2, which affects membrane integrity.
76. Doi *et al* (2008) reviewed studies on maternal and fetal toxicity. The review highlighted the study of Sehata *et al* (2003), where a single oral dose of T-2 toxin (2 mg/kg) on day 13 of gestation induced apoptosis in lymphoid, hematopoietic and gastrointestinal tissues and liver in pregnant rats. Other studies reported placental haemorrhage as a result of the direct cytotoxic effect of T-2 toxin on the vasculature in the labyrinth zone, mediated by T-2 toxin on the clotting system, either by depression of clotting factors, disturbance of platelet function, or both. Prolongation of both prothrombin time and a decrease in the expression of blood coagulation-related genes had been reported in adult mice exposed to a single oral dose of T-2 toxin (10 mg/kg). Conversely, Rousseaux *et al* (1987) reported that no long-term reproductive and teratological effects of low dose dietary T-2 toxin (1.5 and 3.0 ppm, equivalent to 0.075 and 0.15 mg/kg bw/day) were found in a two-generation female reproduction and teratology study.

77. In 2001, the EU Scientific Committee on Food (SCF) published an opinion on T-2 and HT-2 toxins. The SCF concluded that the general toxicity, haematotoxicity and immunotoxicity of T-2 toxin are the critical effects and established a combined temporary tolerable daily intake (t-TDI) for the sum of T-2 toxin and HT-2 toxin of 0.06 µg/kg bw. This was in line with the provisional maximum tolerable daily intake (PMTDI) established for T-2 and HT-2 toxin by JECFA.

78. In 2011, the EFSA CONTAM panel established a group TDI for the sum of T-2 and HT-2 toxins. An uncertainty factor of 100 was applied to the $BMDL_{05}$, to establish a group TDI of 100 ng/kg bw for the sum of T-2 and HT-2 toxins.

79. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of T-2 for adults (aged 19+) was a mean of 0.002 – 0.022 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.004 – 0.053 μg/kg bw per day (LB–UB).

80. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of HT-2 for adults (aged 19+) was a mean of 0.004 – 0.020 μg/kg bw per day (LB–UB) and a 97.5th percentile of 0.011 – 0.3057μg/kg bw per day (LB–UB).

Exposure

81. EFSA (2011) reported that in the adult population, the mean dietary exposure to the sum of T-2 and HT-2 toxins across dietary surveys ranged from 3.4 to 18

ng/kg bw per day (minimum LB to maximum UB). The 95th percentile dietary exposure ranged from 7.2 to 39 ng/kg bw per day (minimum LB to maximum UB). The levels of exposure are lower than those associated with reproductive effects.

Citrinin (CIT)

82. Citrinin is a mycotoxin produced by several species of the genera *Aspergillus, Penicillium* and *Monascus spp,* occurring mainly in stored grains.

83. EFSA published a statement on citrinin in 2012. Both *in vitro* and *in vivo* studies showed that citrinin exposure led to reproductive toxicity and teratogenic and embryotoxic effects. In the *in vivo* experiments there were clear signs of maternal toxicity including nephrotoxicity and the CONTAM panel suggested that the reproductive effects might be a consequence of this maternal toxicity.

84. In particular, EFSA (2012) highlighted studies by Singh *et al* (2006, 2007) *where c*itrinin was administered to pregnant Wistar rats in the diet at 10 mg/kg feed. In the same experiments, endosulfan (1 mg /kg b.w.) and endosulfan plus-citrinin were tested. Dams showed mild maternal toxicity as degenerative liver changes, multiple renal lesions and glomerular congestion when exposed to citrinin. A significant increase in the activity of intestinal global cells was noted along with mild to moderate, non-specific changes in other organs. Maternal weight gain and feed intake were reduced, and various animals showed polyuria and polydypsia, indicating renal damage. This effect was later found by Singh *et al* (2008) to be due to tubular degeneration, medullar tubular necrosis and interstitial fibrosation.

85. Hood *et al*. (1976) dosed rats with a single s.c. dose of 35 mg citrinin/kg b.w. on days 3 - 15 of gestation and found no fetal skeletal malformations but enlarged kidneys, internal hydrocephalus and cleft palates were observed and 30-50 % of the pregnant dams died and the resorption rate of fetuses in the treated group was higher than in controls.

86. The embryotoxic potential of citrinin was also investigated in the chick embryo model. Citrinin (1 - 10 µg, injected subgerminally or intra-amniotically at different days of the egg incubation period) alone and together with OTA appeared to cause morphological alteration on their heads (exencephaly, microophthalmia and cleft beak) (Vesela et al., 1983).

87. EFSA (2012) concluded that in vitro and in vivo studies provided clear evidence for reproductive toxicity and teratogenic and embryotoxic effects of citrinin and that these effects might be secondary to maternal toxicity.

88. Taking into account data on genotoxicity, and limitations and uncertainties in the database on citrinin, the CONTAM Panel decided that a HBGV, such as a TDI,

could not be derived and, due to lack of data on human dietary exposure, nor could a MOE be calculated.

89. However, the Panel determined a level of no concern for nephrotoxicity by applying a default uncertainty factor of 100 to a NOAEL of 20 µg/kg bw per day, to give a value of 0.2 µg/kg bw per day. The Panel also stated that a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

Exposure

90. Ali *et al* (2016) found that biomonitoring data in Bangladeshi adults indicated exposure to OTA and CIT. They analysed urinary biomarkers for both CIT and OTA to investigate regional and seasonal influences on mycotoxin exposure in two Bangladeshi cohorts. Urine was collected from 164 (n = 69 in summer, n = 95 in winter) residents of a rural and an urban area, of whom 62 participants enrolled in both sampling periods. Most samples had detectable biomarkers (OTA, CIT and the metabolite dihydrocitrinine, HO-CIT), with seasonal and regional differences. CIT biomarkers showed pronounced variations, with a mean of 0.10 ± 0.17 ng/ml (range 0.02-1.22 ng/mL) and HO-CIT mean of 0.42 ± 0.98 ng/mL (range 0.02-5.39 ng/mL) in summer, and CIT mean of 0.59 ± 0.98 ng/mL (range 0.05-5.03 ng/mL) and HO-CIT mean of 3.18 ± 8.49 ng/mL (range 0.02-46.44 ng/mL) in winter samples of both cohorts. In both seasons, total CIT biomarker concentrations were significantly higher in the rural cohort than in the urban cohort. A provisional daily intake for CIT was calculated and it exceeded the level of no concern set by EFSA (0.2 µg/kg/d) in 10 and 24 % of participants in summer and winter, respectively.

91. Olsen *et al* (2019) investigated consumer exposure to mycotoxins from the consumption of stored apple jam and crème fraiche that had grown surface mould in storage. Despite finding only a small colony of *Penicillium verrucosum* (8 ± 0.5 mm in diameter) following inoculation of a sample of crème fraiche, OTA and CIT were detected after 21 days at 15 °C in the top 2 cm layer (including the fungal colony), and at concentrations in a normal serving corresponding to an exposure above the HBGV established by EFSA for both mycotoxins.

92. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of citrinin for adults (aged 19+) was a mean of 0.000 – 0.019 µg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.042 µg/kg bw per day (LB–UB); this is lower than the level of no concern established by EFSA.

Patulin

93. Patulin is a small, water soluble γ-lactone-based mycotoxin that is produced by a number of moulds that infect soft fruit, particularly apples, including *P.*

expansum, Aspergillus spp, Byssochlamys fulva and *B. nivea*. It is therefore commonly found in rotting fruit and juice expressed from it. Patulin forms covalent bonds with the thiol groups of proteins and glutathione.

Toxicity

94. Acute symptoms of patulin exposure include immune toxicity, particularly inhibition of macrophage functions, neurotoxicity, and possible damage to the liver, spleen, and kidney. Chronic exposure to patulin is associated with effects on the gut and pneumonia that JECFA (1995) suggested might be due to the mycotoxin acting as an antibiotic on Gram positive bacteria, allowing overgrowth of pathogenic Gram negative bacteria.

95. Puel *et* al (2010) reviewed the biosynthesis and toxicology of patulin. Genotoxicity assays carried out with mammalian cells were generally positive while assays with bacteria were mainly negative. Some studies indicated that patulin impaired DNA synthesis. These effects were possibly related to patulin's reactivity with sulfhydryl groups and induction of oxidative damage.

96. IARC has classified patulin in the group 3:''not classifiable as to its carcinogenicity to humans" http://www.inchem.org/documents/iarc/vol40/patulin.html

97. JECFA (1995) found two reproductive toxicology studies in rats along with teratogenicity studies in mice. No effects were noted in rats or mice at up to 1.5 mg patulin/kg bw/day but maternal toxicity and an increase in fetal resorptions was seen at the higher doses.

98. A provisional maximum tolerable daily intake of 0.4 μg/kg bw/day was established based on a NOAEL of 0.43 mg/kg bw/ day and an uncertainty factor of 100 based on a combined reproductive toxicity/long term toxicity/carcinogenicity studies in rats, taking into account the fact that the compound does not bioaccumulate. The JECFA paper points out that fibrosarcomas were observed in rats dosed subcutaneously at 0.2 mg patulin/day for 61 or 64 days and that forestomach papillomas occurred in rats who had been gavage dosed at 1 mg/ kg bw for 4 weeks, and 2.5 mg/kg bw for the following 70 weeks.

99. EFSA (2000) endorsed the JECFA PTDI for patulin of 0.4 μg/kg bw/day.

Exposure

100. The EU Scientific Cooperation group (2002) surveyed patulin intake data across the EU and found that for the total adult population, 95th percentile intake was 22 ng/kg bw/day (154 ng/kg bw/week) if the data included Italy and 6.6 ng/kg bw/day (46.2 ng/kg bw/ week) if the data excluded Italy. For adult consumers only, 95th percentile intake was 57 ng/kg bw/day (399 ng/kg bw/week) if the data included Italy and 33 ng/kg bw/day (231 ng/kg bw/ week) if the data excluded Italy.

101. The report states that for the United Kingdom “Manufacturers reject any apple product that contains more than 50 µg/kg of patulin (some as low as 35 µg/kg), which are diverted into cider or vinegar production. As these particular batches would not be available for retail sale in the UK, levels above were not considered for calculation. The UK data for all ages are calculated to reflect a worst case scenario, all juice consumed being assumed to be apple juice.”

102. However, Olsen *et al* (2019) found that in apple jam inoculated with *P. expansum*, patulin was detected in all 2-cm layers of the jam at 15 °C. Concentrations in the upper two layers of the jar corresponded to exposures exceeding the health-based guidance value (HBGV) for a normal serving size. Consequently, removal of the mouldy part is insufficient to avoid unhealthy exposure.

103. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of patulin for adults (aged 19+) was a mean of 0.000 – 0.067 µg/kg bw per day (LB–UB) and a 97.5th percentile of 0.000 – 0.141 µg/kg bw per day (LB–UB). The estimated exposure is lower than the JECFA PTDI

Ergot alkaloids (EAs)

104. Ergot alkaloids are a large group of compounds produced by fungi that attack a wide variety of grass species, including small grains, during the growing season. These compounds are chemically divided into the clavine alkaloids, lysergic acids, simple lysergic acid amides and peptide alkaloids. Two common alkaloids examined for in ergot are ergotamine and ergovaline.

Toxicity

105. Ergot poisoning is termed ergotism. Acute effects include vomiting, dizziness, confusion, drowsiness and convulsions. Chronic ergotism symptoms include intense vasoconstriction and muscular pain, once called St Anthony’s fire. Ischemic extremities can become gangrenous. (Bowman & Rand 1980)

106. EFSA (2012) reviewed the effects of ergot alkaloids on pregnant rats and reported a number of adverse effects including inhibition of implantation, embryotoxicity, and inhibition of lactation. The latter effect has been correlated with activation by EA of dopamine receptors in the hypothalamus, causing inhibition of prolactin release.

107. Other effects such as inhibition of ovulation, termination of pregnancy and occurrence of proestrus and oestrus have been observed in experimental animals (rats, mice and hamsters) injected with various EAs at dose from 0.175 – 3 mg/animal, and it has been suggested that implantation failure was a result of inhibiting release of prolactin from the pituitary.

108. Administration of 1 mg ergocornine methanesulfonate s.c. to pregnant rats on gestational day 7, i.e. post-implantation, resulted in a high incidence of resorptions and visceral malformations. Injection of progesterone reduced these effects, suggesting that they were mediated by progesterone deficiency There was no evidence of teratogenicity.

109. Oral dosing of pregnant rats with 10 mg/kg b.w. ergotamine tartrate on single days between gestational days 4 – 19 led to shortening or absence of nails, phalanges and digits, resembling some of the effects seen following interruption of uterine blood flow.

110. A $BMDL_{10}$ of 0.33 mg/kg bw per day was calculated by EFSA for the incidence of tail muscular atrophy in a 13-week rat feeding study of ergotamine. This effect was considered representative of the vasoconstrictive effects of EAs and provided a suitable reference point for establishment of a group acute reference dose of 1 µg/kg bw and a group tolerable daily intake of 0.6 µg/kg bw per day.

Exposure

111. Estimation of human dietary exposure to EAs was highly influenced by the facts that most of the consumption data in the EFSA's Comprehensive European Food Consumption Database refers to processed food, and that a limited amount of occurrence data on these types of food was available. The chronic dietary exposure in the adult population varied between 0.007 and 0.08 µg/kg body weight (b.w.) per day for average consumers and 0.014 and 0.19 µg/kg b.w. per day for high consumers. The acute dietary exposure in the adult population ranged between 0.02 and 0.23 µg/kg bw per day for average consumers, and between 0.06 and 0.73 µg/kg bw per day for high consumers. Thus, although intakes are generally within the TDI, there is the potential for exceedance at the highest intakes.

112. The Total Diet Study for the UK (FSA, 2014) conducted by Fera determined that the daily dietary intake of ergot alkaloids for adults (aged 19+) was a mean of 0.033– 0.036 µg/kg bw per day (LB–UB) and a 97.5th percentile of 0.079 – 0.083 µg/kg bw per day (LB–UB).

Phytoestrogens

113. Phytoestrogens are plant-derived dietary compounds with structural similarity to 17-β-oestradiol (E2), the primary female sex hormone. This structural similarity to E2 enables phytoestrogens to cause (anti)oestrogenic effects by binding to the oestrogen receptors. The major groups of phytoestrogens present in our diet are isoflavones, prenylflavonoids, coumestans and lignans.

114. Isoflavones are the most studied phytoestrogens. The main isoflavones are genistein, daidzein, glycitein, formononetin and biochanin A, which are mainly found

in soy, soy-based food and legumes usually in their conjugated forms like genistein, daidzin, puerarin, glycitin, ononin and sissotrin (Rietjens *et al* 2017).

115. Prenylflavonoids are found in beverages and supplements that contain extracts of hops. Coumestans are found in soy but also in alfalfa plants, and other food crops such as peas, green beans and brussels sprouts. (Verdeal & Dales 1979). The lignans are a large group of low molecular weight polyphenols found in plants, particularly seeds, whole grains, and vegetables.

Toxicity

116. Rietjens *et al* (2017) reviewed the health effects of dietary phytoestrogens and concluded that despite the reported and widely claimed positive effects on menopausal symptoms, cardiovascular disease and type 2 diabetes, negative effects such as endocrine disruption, reduced thyroid function and infertility could not be ruled out. The balance of effects may depend upon other factors such as age and oestrogen status, reflecting the often-observed partial agonist effects of phytoestrogens at oestrogen receptors.

117. Hüser *et al* (2018) considered the safety of isoflavones in the diet. Factors influencing the biological effects of isoflavones, such as bioavailability, plasma and tissue concentrations, metabolism, age at exposure (pre- vs. postmenopausal women), and duration of isoflavone exposure were taken into account.

Exposure

118. The DFG Senate Commission on Food Safety (SKLM) evaluated the safety of phytoestrogenic isoflavones in food supplements and in supplementary balanced diets (2009). A range of food products and their isoflavone content were tabulated and the results of dietary surveys were presented. A number of dietary studies from around the world were reported upon. A study of 1000 people from a cross section of the population of the UK, Netherlands, Italy, Ireland and Finland found a daily isoflavone intake of <1 mg/day, contrasting with a UK survey of 25 vegetarians that found an average intake of 25 mg/day.

Vitamins

Vitamin A

119. The Expert Committee on Vitamins and Minerals (EVM) published a statement in 2003 that included Vitamin A. Vitamin a is a fat-soluble vitamin and thus can accumulate in tissues. They defined vitamin A as: "… a group of lipid soluble compounds related metabolically to all-trans-retinol. In the diet, vitamin A is found in products of animal origin, as retinyl esters, mainly retinyl palmitate." Vitamin A can

be expressed on a weight basis as Retinol Equivalents (1 RE = 1 µg retinol) or in International Unit (IU).

120. The teratogenic effects of retinoic acids, the active oxidized metabolites of vitamin A, have been known for a long time and documented both in animals and in humans. Children exposed *in utero* to isotretinoin (13-cis-retinoic acid – primarily used to treat severe acne) have been found to exhibit congenital malformations, known as “the retinoic acid syndrome”, which includes small or absent external ears and auditory canals, cleft palate, micrognathia and low set ears. Defects of the central nervous system (micro- or anopthalmia, cerebellar or cortical defects, microcephaly), the thymus and the cardiovascular system (transposition of the heart vessels, aortic arch hypoplasia, ventricular septal defects) have been observed

121. EFSA (2006) derived a tolerable upper limit for vitamin A of 3000 µg of RE per day for women of childbearing age. This was based upon a study by Rothman *et al* (1995), which indicated a rise in the ratio of prevalence of birth defects associated to the cranial-neural crest at doses greater than 3000 µg RE/day of vitamin A (food and supplement).

122. Several different adverse effects of hypervitaminosis A have been reported, some of which, for example hepatotoxicity, are regarded as reversible with withdrawal of the vitamin but others, such as deficits in the eyes and bone, are not. The most common acute effects are nausea, vomiting and vision problems.

123. Symptoms of chronic toxicity include dry thickening of the skin, cracking of lips, conjunctivitis, erythematous eruption, alopecia, reduced bone mineral density, bone joint pain, chronic headache, intracranial hypertension and hepatotoxicity. EVM state that chronic toxicity in adults is generally attributed to supplemental doses of > 7500-15,000 µg RE/day, over weeks, months or years. However, there have been cases of toxicity associated with lower doses of ~1500-3,000 µg RE/day.

124. Determination of a threshold dose for chronic toxicity may be confounded by pre-existing disease, alcohol abuse, drug therapy and limited knowledge of dietary intake.

Exposure

125. EFSA (2015) estimated the average dietary intake in adults as being between 816 and 1,498 µg RE/day. Average daily intakes were in most cases slightly higher in males than in females, mainly owing to the larger quantities of food consumed per day.

126. Government dietary advice, as communicated via the NHS.uk website recommends a daily dietary vitamin A intake for adults aged 19 to 64 of 700 µg for men and 600 µg a day for women. Pregnant women are warned about eating liver or

liver products such as pate, or supplements that contain high concentrations of vitamin A to avoid potential harm to the unborn baby.

Vitamin E

127. EFSA (2015) published an opinion on dietary reference values for vitamin E.

128. Vitamin E is a fat-soluble vitamin consisting a mixture of tocopherols (α, β,γ,δ etc). Dietary fat is required for absorption and vitamin E enters the blood stream incorporated into chylomicrons. The tocopherols other than α do not bind strongly to α-tocopherol transfer protein (α-TTP) in the liver and are more efficiently metabolised by the hepatic enzyme ω-hydroxylase, leaving the α- isomer to circulate and accumulate in the tissues. Commercially, a racemic mixture of the 8 stereoisomers of α-tocopherol termed all-rac-α-tocopherol is used. Intake is measured as α-tocopherol equivalents/day (α-TE/day).

Toxicity

129. Vitamin E is generally considered to be of low toxicity. For example, the MSD Manual states that “Many adults take relatively large amounts of vitamin E (alpha-tocopherol 400 to 800 mg/day) for months to years without any apparent harm. Occasionally, muscle weakness, fatigue, nausea, and diarrhoea occur. The most significant risk is bleeding. However, bleeding is uncommon unless the dose is > 1000 mg/day or the patient takes oral coumarin or warfarin. Thus, the upper limit for adults aged ≥ 19 years is 1000 mg for any form of tocopherol.”

130. The EVM (2003) state that vitamin E is of low toxicity, but that in some studies, supplementation has been associated with an increased risk of mortality from haemorrhagic stroke, and a small excess of cardiovascular deaths compared with controls. The mechanism of toxicity may be due to inhibition of the metabolism of structurally- similar vitamin K, and thus of blood coagulation.

131. The SCF (2003) set a Tolerable Upper Intake Level (UL) for adults of 270 mg α-TE/day, rounded to 300 mg α-TE/day using an uncertainty factor of 2, based on blood clotting as the critical adverse effect. This was derived from a NOAEL of 540 mg α-TE/day from the study by Meydani et al. (1998) where 88 healthy subjects over 65 years of age received either a placebo, 40, 134 or 537 mg α-TE/day (all-rac-α-tocopherol) for four months.

132. SCF noted that reproductive toxicity studies in rats indicated that vitamin E (as water soluble d-α-tocopherol polyethylene glycol 1000 succinate) did not have adverse effects on reproductive function at doses of up to 2% of the diet and d-α-tocopherol was not teratogenic in mice. Thus, the UL was regarded as also applying

to pregnant and lactating women as there was no indication from animal studies of a specific risk for these population groups.

133. EFSA (2015) concurred with earlier reports that the acute and short term toxicity of α-tocopherol was low and that there was no evidence of genotoxicity or carcinogenicity.

Exposure

134. In adults (≥ 18 years) in EU countries (eight surveys), average α-tocopherol intakes ranged between 7.8 and 12.5 mg/day in women and between 8.2 and 16 mg/day in men, and average α-TE intakes ranged between 8.9 and 13.5 mg/day in women and between 10.1 and 16.0 mg/day in men.

135. The EVM reported mean and 97.5th percentile values of daily intake of vitamin E of 8.5 and 18.5 mg/ day respectively for both men and women.

136. Government dietary advice, as communicated via the NHS.uk website states that the amount of vitamin E needed daily by adults is 4 mg for men and 3 mg a day for women and that this amount should be available from the diet. Although the advice states that taking too much could be harmful, it also says that taking 540mg (800 IU) or less a day of vitamin E supplements is unlikely to cause any harm. There is no specific advice for pregnant women or those considering pregnancy.

Vitamin C

137. Vitamin C or (ascorbic acid, 3-oxo-L-gulofuranolactone or L-threo-hex-2-enonic acid) is a water-soluble enzyme cofactor that is involved in a number of biochemical processes, including synthesis of catecholamines, collagen formation and mixed-function oxidase activity.

Toxicity

138. EFSA (2013) did not set a Tolerable Upper Intake Level (UL) for vitamin C. The limited available data from studies in animals and humans were considered to suggest a low acute toxicity of vitamin C (Johnston, 1999; EFSA, 2004). Relationships between vitamin C intakes and adverse gastrointestinal effects or renal effects in relation to urinary excretion of oxalate were assessed, and reversible acute gastrointestinal intolerance or diarrhoea was regarded as the most clearly defined adverse effect at intakes of 3-4 g/day. However, data on a dose-response relationship for adults (including older adults) or for children were considered to be insufficient (EFSA, 2004). Despite the extensive use of high doses of vitamin C in supplements, there were only a limited number of controlled studies that specifically investigated adverse effects.

139. Government dietary advice, as communicated via the NHS.uk website states that enough vitamin C should be available by eating a varied and balanced diet, and that taking too much could be harmful, but that taking 1,000mg or less of vitamin C supplements a day is unlikely to cause any harm. There is no specific advice for pregnant women or those considering pregnancy.

Exposure

140. The EVM reported mean and 97.5th percentile values of daily intake of vitamin C of 64 and 160 mg/ day respectively for both men and women.

Other biological constituents

Resveratrol

141. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenol stilbene, with two phenol rings linked to each other by an ethylene bridge. It has been found in more than 70 plant species, especially in grapes' skin and seeds, and in small amounts in red wines and various human foods.

Toxicity

142. Salehi *et al* (2018) reviewed the pleiotropic physiological effects that have been reported for resveratrol *in vitro* and *in vitro*. Although this compound is widely regarded as beneficial to health, having, for example, antioxidant, cardioprotective and anti-tumour effects, its purported benefits seem to be dose-dependent, with higher doses appearing to act in the opposite direction to lower doses. Moreover, the parent compound has very low oral bioavailability (F) so that any physiological effects are seen best when derivatives with higher F values are used or are produced by the gut microbiota. The balance of positive and negative effects may be influenced by pre-existing disease states and/ or age.

143. EFSA (2018) published an Opinion on the safety of synthetic *trans*-resveratrol as a novel food. At the proposed intake of 150 mg/day, the NDA Panel found no evidence of genotoxicity, carcinogenicity, renal toxicity, developmental toxicity or oestrogenic activity from *in vitro* or *in vivo* tests.

144. A GLP- and OECD guideline 471-compliant Salmonella typhimurium reverse mutation assay proved negative, as did an *in vitro* chromosome aberration study using human lymphocytes. There were significant increases in structural chromosome aberrations at doses of 10, 20 and 30 µg/ml without activation and at 40 and 50 µg/ml with activation. There was no change in the proportion of immature

erythrocytes in an *in vivo* mammalian erythrocyte micronucleus test using Sprague–Dawley rats given 0, 500, 1,000 or 2,000 mg/kg body weight (bw) per day trans-resveratrol for 2 consecutive days by gavage. The test thus indicated the absence of clastogenic activity in vivo. The Panel therefore considered that this was sufficient to rule out concern over the positive in vitro chromosomal aberration tests.

145. In a 90-day study, Wistar rats received diets containing trans-resveratrol at 0, 120, 300 or 750 mg/kg bw per day. At the highest two doses, animals gained less body weight (~ 10%) than controls throughout the study for males and from week 4 for females. The reduced body weight was associated with reduced food consumption., which returned to control levels during the recovery period. There was no effect on body weight and food consumption in the 120 mg/kg bw per day group.

146. A benchmark dose approach provided a BMDL05 of 344 mg/kg bw per day derived from body weight data of female rats.

147. No chronic toxicity/carcinogenicity studies were carried out. A GLP-compliant embryo–fetal toxicity study found no treatment-related soft tissue or skeletal abnormalities, so the NOAEL for fetal development was at the highest dose (750 mg/kg bw per day) tested. The only maternal effect observed was reduction in body weight, which occurred at all doses. The Panel considerd 120 mg/kg bw for maternal toxicity LOEL considers that the studies provided did not raise concerns of developmental toxicity.

Exposure

148. Weiskirchen and Weiskirchen (2016) reviewed the therapeutic potential of resveratrol. Their literature searches revealed that the consensus “recommended daily allowance” of resveratrol was 1 g per day, but stated: “Typical resveratrol concentrations reported for conventional food products are: peanuts without seed coats, 0.03– 0.14 μg/g (126); red wines, 0.361–1.972 mg/L (127); white wines, 0–1.089 mg/L (128); rosé wines, 0.29 mg/L (129); beers, 1.34–77.0 μg/L (130); skin of tomatoes ~19 μg/g dry weight (131); dark chocolate, 350 μg/kg; milk chocolate, 100 μg/kg (132); Itadori tea, 68 μg/100 mL (37); red grapes, 92–1604 μg/kg fresh weight (133); white grapes, 59–1759 μg/kg fresh weight (133); and apples, 400 μg/kg fresh weight. On the basis of these given concentrations, it is not possible to absorb the recommended dose of resveratrol through uptake of any of these nutrients or combinations thereof”.

149. Commercially available food supplements can contain up to 1000 mg resveratrol.

Caffeine

150. Caffeine (1,3,7-trimethylxanthine) is a widely consumed stimulant that is found in coffee, tea, carbonated beverages (primarily cola-flavoured and “energy” drinks) and has a long history of intake.

Toxicity

151. Caffeine intoxication is characterised by nervousness, irritability, anxiety, and insomnia and, at higher doses, tremor, tachycardia, palpitations and gastrointestinal upset. Reported adverse effects at extreme doses include vomiting and abdominal pain, hypokalaemia, hallucinations, increased intracranial pressure, cerebral oedema, stroke, paralysis, rhabdomyolysis, altered consciousness, rigidity, seizures, arrhythmias, and death. (Reviewed by Seifert *et al* 2013).

152. EFSA (2015) stated that habitual caffeine consumption from all sources up to 400 mg per day (about 5.7 mg/kg bw per day for a 70-kg adult) consumed throughout the day would not give rise to safety concerns for healthy adults. For pregnant women, intakes from all sources up to 200 mg per day consumed throughout the day were not regarded as giving rise to safety concerns for the fetus arising from low birth weight.

153. Current Government advice as stated on the NHS website says (NHS, 2021) that pregnant women should limit their intake of caffeine to 200 mg/day. This was based on COT recommendations from 2008.

154. In their Statement in 2008, COT concluded:

> “The evidence that is now available does not make it possible to identify a threshold level of caffeine intake below which there is no elevation of risk, and it seems likely that risk is increased in association with intakes in the order of 200 mg per day and perhaps even lower. However, if the relation is indeed causal, then the absolute increase in incidence of Fetal Growth Restriction (FGR) from intakes less than 200 mg per day is likely to be less than 2% of infants. The literature suggests a positive association of caffeine intake with miscarriage, but there are uncertainties relating to possible recall bias and residual confounding. Data on maternal caffeine consumption during pregnancy and associations with adverse effects other than FGR and spontaneous miscarriage, such as pre-term birth and congenital malformations, are inconclusive.”

155. However, James (2020) reviewed recent studies on the effects of maternal caffeine consumption and pregnancy outcomes and concluded that there is no level of consumption that is without risk in this context. The author concluded that the cumulative scientific evidence supports pregnant women and women contemplating pregnancy being advised to avoid caffeine. The Committee had a number of reservations about this study and did not consider that it changed current advice.

Exposure

156. EFSA (2015) estimated that the minimum and maximum mean intakes of caffeine for adults aged 18 to < 65 years to be 0.5 and 4.3 mg/kg bw/day. The minimum and maximum 95th percentile intakes were estimated to be 1.5 and 10 mg/kg bw/day.

Summary

157. This paper lists substances highlighted by SACN as being of potential interest in the maternal diet from the point of view of COT. This is the first of two such papers and focuses on substances that are either endogenous to foodstuffs or of biological origin. The COT are asked to triage the substances to prioritise which will need separate papers for full assessment, which can be grouped together and which could be omitted. A second paper on exogenous chemicals will be presented to the Committee at the March meeting.

Questions for the Committee

1) Which of the compounds in the current paper would the Committee like to be prioritised for single papers and which can be grouped together into a combined statement?

2) Are there any substances for which the Committee would like to see Further detail before deciding?

3) Which compounds, if any, to the Committee think need not be on the list for toxicological assessment?

4) Does the Committee have any other comments on this paper?

Secretariat

January 2021

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