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

Additional data regarding Fusarenon-X in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

1. A discussion paper was presented to the COT in the May 2019 meeting, which reviewed the potential risks from Fusarenon-X (Fus-X) in the diet of infants aged 0 to 12 months and children aged 1 to 5 years[1]. Following that, the Committee wished the Secretariat to obtain additional information, to address the following topics; appropriateness of utilising mink emesis data, comparative toxicity of Fus-X to other trichothecenes, and combined exposures with other mycotoxins.

Appropriateness of utilising mink emesis data

2. The COT previously provided a statement on the potential risks from T-2 toxin (T2), HT-2 toxin (HT2) and neosolaniol (NEO) in the diets of infants and young children in the UK aged 0 to 12 months and 1 to 5 years, respectively[2]. T2 and HT-2 are type A trichothecenes, with NEO being a hydrolytic phase I metabolite of T2 and may be formed in fungi and mammals. The statement details how the European Food Safety Authority (EFSA) utilised a mink emesis study as the basis for the benchmark dose (BMD) calculation to provide the acute reference dose (ARfD) (EFSA, 2017a).

3. The EFSA CONTAM Panel considered the animal model appropriate to investigate vomiting in humans since the dose range for causing emesis in the mink was the same as in humans. The effective dose (ED_{50}) of ipecacuanha alkaloid emetine in the mink was 1.03 mg/kg bw. Due to this similarity, an interspecies factor was not applied in the calculation of the BMD, as it was assumed that humans are not more sensitive than mink towards the emesis effect.

4. However, Percie du Sert et al., (2012) whom performed a literature review on predicting the emetic liability of novel chemical entities concluded that no animal species is a universal indicator of emetic liability and the choice of species should be based on the type of compound of interest. Furthermore, studies conducted lack comparable measures between human and animal data.

5. Type A trichothecene compounds have a hydroxyl, an ester function or a no oxygen substitution at C-8. Whilst the majority of type B trichothecenes have a keto (carbonyl) function at the C-8 position. In Fusarium species, type B trichothecenes typically have a C-7 hydroxyl group, a structural feature that is not present in other genera. However, all Fusarium trichothecenes including (type A and type B) have an oxygen function at C-3 (McCormick et al., 2011).

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[1] https://cot.food.gov.uk/sites/default/files/tox2019-17.pdf

[2] https://cot.food.gov.uk/sites/default/files/cotstatement-t2ht2andneosolaniol.pdf

6. Wu et al., (2013) performed a comparative study of the emetic potency of trichothecenes in mink, as further detailed in paragraph 8. In brief, Fus-X was observed to elicit a greater emetic potency than other type B trichothecenes (deoxynivalenol; DON, 3-acetyldeoxynivalenol; 3-ADON, 15-acetyldeoxynivalenol; 15-ADON and nivalenol; NIV).

Comparative toxicity of Fus-X to other Type B trichothecenes

Acute exposures

7. Wu et al., (2012) compared the anorectic responses to 3-ADON, 15-ADON, Fus-X and NIV in mice models. Food refusal following oral and intraperitoneal (i.p.) exposures to Fus-X, persisted from 48 to 96 hours in mouse (n=8 per dose). The no observed adverse effect level (NOAEL) for Fus-X was 0.025 mg/kg bw and the lowest observed adverse effect level (LOAEL) was 0.25 mg/kg bw, however, NIV had greater anorectic potential following i.p. exposure with a NOAEL of 0.01 and LOAEL of 0.1 mg/kg bw. For NIV oral exposure, the NOAEL was 0.1 mg/kg and the LOAEL 1 mg/kg. The trichothecenes were therefore ranked in order of toxicity for i.p. and oral exposures. I.P.; NIV > Fus-X > DON ≈ 3-ADON ≈ 15-ADON and oral; Fus-X > NIV > DON ≈ 3-ADON ≈ 15-ADON.

8. Fus-X was observed to elicit greater emetic potency than other type B trichothecenes (DON, 15-ADON, 3-DON, and NIV) in a comparative study in female mink (n=60) by Wu et al., (2013). The ED_{50} resulting in emetic events in 50% of animals following i.p. injection was 70 µg Fus-X/kg bw (NIV at 60 µg/kg bw) and oral administration at 30 µg Fus-X/kg bw (NIV at 250 µg/kg bw). The i.p. NOAEL for Fus-X and NIV was 50 µg/kg bw, whilst for oral exposure the NOAEL for Fus-X is 10 µg/kg bw and for NIV 100 µg/kg bw. LOAEL values were; 100 µg/kg bw for both Fus-X and NIV for i.p. exposure, and 50 µg/kg bw for Fus-X and 250 µg/kg bw for NIV for oral exposure.

9. Yang et al., (2017) assessed the individual or combined toxicological effects of multiple deoxynivalenol-family mycotoxins including; DON, 3-ADON, 15-ADON, Fus-X and NIV on human gastric epithelial cells (hGES). NIV was observed to be the most toxic out of the tested mycotoxins followed by Fus-X, for individual toxic effects at 0 to 3 ppm (cell viability decreased in a dose-dependent manner). For combined toxicological effects, Fus-X + NIV at 2 ppm each decreased cell viability by up to 30%, whilst Fus-X + 15-ADON (2 ppm and 6 ppm, respectively) exhibited the least toxic effect. The former mycotoxin combination resulted in a complete synergistic cytotoxicity and the latter almost entirely antagonistic cytotoxic effects in hGES.

10. Male et al., (2016a) (abstract only) modelled the anorectic potencies of foodborne trichothecenes in a mouse model (3-ADON, 15-ADON, DON, Fus-X, NIV, T2 and HT-2) by the US EPA BMD methodology to calculate the BMD relative to DON 2 hours post-administration. The order of potency based on BMD values was: DON (1) ≈ 3-ADON (1) ≈ 15-ADON (1) < NIV (3) < HT-2 (5) < FUS-X (9) << T-2 (124).

11. Male et al., (2016b) further modelled the emetic potencies of the same trichothecenes as above by BMD methodology utilising the BMDS EPA software (v 2.60.1). Relative potencies were calculated as the ratios of their benchmark doses to that of DON for both oral and i.p. dosing in mink. The authors concluded that mink is more sensitive to oral exposures than to i.p. exposures. The order of emetic potency for the i.p. route was: HT-2 ≈ T-2 > Fus-X > NIV > DON > 15-ADON > 3-ADON. The oral route was: HT-2 ≈ T-2 > Fus-X > DON > 15-ADON > NIV > 3-ADON. The doses and relative potencies are provided in Table. 1. The oral emetic potency of Fus-X relative to DON is 1.04, but 6 times higher than that of NIV.

Table. 1 Emetic benchmark doses and relative potencies of type A and type B trichothecenes in mink via oral and intraperitoneal administration (reproduced from Male et al., 2016b).

Type	Trichothecene	IP BMD (µg/kg bw)	Relative potency	Oral BMD (µg/kg bw)	Relative potency
A	HT-2	31	2.38	14	1.73
A	T2	31	2.38	14	1.73
B	3-ADON	141	0.52	198	0.12
B	15-ADON	108	0.67	40	0.60
B	DON	73	1.00	24	1.00
B	Fus-X	60	1.22	23	1.04
B	NIV	63	1.16	141	0.17

Abbreviations: HT-2 = HT-2 toxin, T2 = T2 toxin, 3-ADON = 13-acetyldeoxynivalenol, 15-ADON = 15-acetyldeoxynivalenol, DON= Deoxynivalenol, Fus-X= Fusarenon-X, NIV = Nivalenol, IP = intraperitoneal, BMD = benchmark dose.
Relative emetic potencies: ratio of the BMD of DON to the BMD of each of the other trichothecenes studied.

12. Alassane-Kpembi et al., (2017a) utilised whole transcriptome profiling (WTP) and histopathological techniques to observe the gastrointestinal effects of Fus-X exposure, utilising DON as a benchmark. WTP analysis was carried out via porcine pan-genomic array with Fus-X concentrations at 2 and 10 µM and DON at 10 µM. Analysis revealed that both mycotoxins down-regulate PPAR[3] and LXR/RXR[4] signalling pathways that control lipid metabolism, however, VDR/RXR[5] activation,

[3] PPAR: peroxisome proliferator-activated receptor. A ligand-activated transcription factor that plays a major regulatory role in energy homeostasis and metabolic function.
[4] LXR/RXR: liver X receptor/retinoid X receptor heterodimer. Acts as sensors of lipid that are derived from both the diet and intracellular metabolism, and therefore has a role in regulating cholesterol and fatty acid homeostasis.
[5] VDR/RXR: vitamin D receptor/retinoid X receptor heterodimer. Acts to regulate the expression of vitamin D target genes.

ephrin receptor[6], GNRH[7], integrin[8] and ceramide[9] signalling pathways were specific to observations for intestinal exposure to Fus-X.

13. Histopathological results showed that Fus-X induced more severe histological alterations than DON to jejunal explants from male piglets (n=6); histo-morphological scores were reduced by 8, 25 and 45% treated with 0.3, 1 and 3 µM of Fus-X, compared to ~30% reduction in the histo-morphological score induced by 10 µM of DON. Additionally, 0.3 – 3 µM Fus-X induced severe lesions on the intestinal mucosa. Observations at the highest dose (10 µM) included; villi with an absence of epithelia, severe atrophy, diffuse cellular debris along the intestinal mucosa and a reduction of villi numbers. The authors concluded that toxicity pathways of Fus-X and DON deviate from one another, and therefore evaluations should be conducted separately.

Co-occurrences of Fus-X with other mycotoxins

14. Juan et al., (2014) tested the presence of mycotoxins in baby foods (n=75), including 13 infant formula milks (infant formula powders, ready-to-use preparation), cereal-based baby foods (n=25), dairy products (n=11), fruit and vegetable compotes (n=16) and fruit and vegetable purees (n=10) that were commercially available in Italy. Mycotoxins were only detected in cereal-based baby foods; 31% tested positive. The frequency in which Fus-X was detected was 24% (limit of detection; (LOD): 5.5; limit of quantification (LOQ): 20). 146.51 µg Fus-X/kg was the mean incidence level, within a range of 73-604 µg/kg (highest level of incidence occurred in cereal-based baby food containing a percentage of wheat >50%). Various mycotoxin co-occurrences were reported as shown in Table. 2.

15. The authors provided their opinions on what caused the co-occurrences of mycotoxins; the differences of composition of grain in the samples, the mycotoxin-producing fungi in each cereal type and considerations for environmental factors (e.g. humidity and temperature which favours the growth of the fungi and the production of the mycotoxins).

16. Alassane-Kpembi et al., (2017b) further suggested their own views for the co-occurrences of mycotoxins; a complete diet consists of various food commodities, these commodities can be simultaneously contaminated with various fungi, and that most fungi can produce several mycotoxins.

17. Rodríguez-Carrasco et al., (2013) carried out exposure estimates to Fusarium mycotoxins, including Fus-X through cereals intake in Spain. Consumption data were

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[6] Ephrin receptor: transduce signals from the extracellular to intracellular environment through ligand-induced activation of their kinase domain, regulates various developmental processes and adult tissue homeostasis.

[7] GNRH: gonadotrophin-releasing hormone. Plays a role in cell signalling mediated by mitogen-activated protein kinase.

[8] Integrin: transmembrane receptors plays a role in cell signalling.

[9] Ceramide: family of lipid molecules composed of sphingosine and a fatty acid. Plays a crucial role in signal transduction in programmed cell death, cell cycle, differentiation and senescence.

derived from surveys published by the Spanish Food Safety and Nutrition Agency, the population groups were; infants (0-3 years), children (5-12 years). Fus-X was not detected in rice and maize-based products (n=40) (LOD: 0.6 – 5 µg/kg; LOQ: 1.25 – 10 µg/kg presented as a range for all mycotoxins tested), however, it was detected in wheat-based products with both mean and maximum values at 10.8 µg/kg (overall incidence of 0.8% (n=119)). A tolerable daily intake (TDI) of 0.1 µg Fus-X/kg bw/day was utilised as a reference value by the authors, they calculated a probable daily intake of 0.035 and 0.034 µg Fus-X/kg bw/day in infants and children, respectively, based on the mean 10.8 µg Fus-X/kg and consumption data of wheat of 32.9 g/day in infants and 25.1 g/day in children. The calculated %TDI were 36 – 34% for infants and children, respectively. The authors presented additional data for co-occurrence of Fus-X with DON and HT-2 mycotoxins in 1% of wheat-based product samples. The total minimum and maximum concentration were 28.2 µg Fus-X/kg and the calculated combined %TDI for infants was 61% and 24 % in children.

Table. 2 Co-occurrence of mycotoxins and incidence numbers in tested cereal-based baby foods (reproduced from Juan et al. 2014).

Co-occurrence of mycotoxins	Incidence (n=25)
DON, Fus-X	2
DON, Fus-X, ENB	1
DON, Fus-X, β-ZOL	1
DON, OTA, Fus-X	2
OTA, Fus-X, HT-2	1
DON, OTA, Fus-X, NIV	3
DON, OTA, Fus-X, β-ZOL	1
DON, OTA, Fus-X, ENB	1
DON, Fus-X, ENB, β-ZOL	1
DON, OTA, Fus-X, ENB, ENA1, ENB4	3
DON, OTA, Fus-X, ENB, HT-2, β-ZOL	1
DON, OTA, Fus-X, ENB, ENA1, ENB1, ENB4	1

Abbreviations: DON= Deoxynivalenol, β-ZOL= β-zearalenol, ENB= Ennatins, Fus-X= Fusarenon-X, HT-2 = HT-2 toxin, OTA= Ochratoxin-A.

18. Alkadri et al., (2014) investigated the natural co-occurrence of mycotoxins in wheat grains from Italy (n=47). In 3/47 samples Fus-X has been detected (LOD: 15 µg/kg; LOQ: 45 µg/kg), two of which showed co-occurrence of DON. The mean value was 8 ng/mL (range of 5-14 ng/mL).

19. Unpublished data from a Food Standards Agency (Patel et al., 2011) mycotoxin surveillance report, observed co-occurrence of Fus-X with not only type B trichothecene mycotoxins but also, type A, zearalenone, aflatoxins, ochratoxin A and some with fumonisin (n=7) in 77 baby-food products with expiry dates ranging from 2011-2013, however, the measured levels for all trichothecenes were below the LOQ of 10 µg/kg (LOD: 5 µg/kg).

20. As of current, International Committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are considering the co-occurrence of

mycotoxins (e.g. aflatoxins with fumonisins) in their risk assessment processes, however, there is often data gaps and or limitations that prevents the establishment of a combined health-based guidance value (HBGV).

Effects of combined mycotoxin co-exposure on cellular and animal models

21. Aupanun et al., (2019a) investigated the individual and combined toxicity of type B trichothecenes (DON, NIV and Fus-X) on Jurkat human T cells. In both scenarios (alone or in combination) mycotoxins were shown to have a dose dependent effect on proliferating lymphocytes. Based on inhibition concentration (IC_{50}) values (µM), the trichothecenes were ranked in decreasing order of toxicity: Fus-X (1.90 µM) > NIV (3.35 µM) > DON (4.70 µM) > NIV + Fus-X (5.28 µM) > DON + Fus-X (5.56 µM) > DON + NIV (6.98 µM) > DON + NIV + Fus-X (24.19 µM).

22. Mycotoxin interactions were assessed by calculating combination index (CI; CI values >1.1 = antagonism, <0.9 = synergism, 0.9-1.1 = additive)[10] and dose reduction index (DRI)[11]. Calculated values are shown in Table. 3. In brief, DON and Fus-X mixtures generated moderate antagonism, whilst NIV and Fus-X combinations resulted in an additive effect. All three trichothecenes were observed to cause complete antagonistic effects. The authors concluded that immunotoxicity resulting from co-exposure to the tested type B trichothecenes cannot be predicted based on individual effects but depends on the mixtures, relevant concentrations/ratio combinations, as well as the type of target cells.

Table. 3 Combination index (CI) isobologram and dose reduction index (DRI) values for mixtures of Fusarenon-X and other mycotoxins (reproduced from Aupanun et al., 2019a).

Mycotoxin	Combination ratio	10% cytotoxicity CI	10% cytotoxicity DRI	30% cytotoxicity CI	30% cytotoxicity DRI
DON Fus-X	1:0.2	1.65	0.83 0.97	1.38	0.87 1.95
NIV Fus-X	3:1	0.46	6.17 12.28	0.79	1.82 3.29
DON NIV Fus-X	1:0.6:0.2	2.29	0.62 0.81 3.69	2.19	0.74 0.95 2.94

Abbreviations: DON= Deoxynivalenol, Fus-X= Fusarenon-X, NIV = Nivalenol.
Combination Index (CI) values: >1.1 = antagonism, <1.1 = synergism, 0.9-1.1 = additive.
Dose response Index (DRI) values: >1 and <1 indicate supportable and not supportable dose-reduction, respectively; =1 represents no dose-reduction.
Concentration range for each mycotoxin were: DON; 0-4 µM, Fus-X; 0-0.8 nM; NIV; 0-2.4 µM.

[10] Combination Index (CI): parameter to quantify the degree of mycotoxin interaction.
[11] Dose reduction Index (DRI): parameter to quantify how many-fold of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug in isolation.

23. A summary of similar studies utilising CI values to quantify the degree of mycotoxin interaction is presented in Table 4.

24. Aupanun et al., (2019b) recently compared the toxicity of DON alone or mixed with NIV or Fus-X to lymphoid tissues post single oral administration to mice. Male ICR mice (n=5 per group) were administered the following doses: 10% DMSO in water served as the vehicle control, 2.5 mg/kg bw Fus-X, 2 mg/kg bw DON with 2.5 mg/kg Fus-X, 5 mg/kg bw NIV with 2.5 mg/kg bw Fus-X, and 2 mg/kg bw DON with 5 mg/kg bw NIV and 2.5 mg/kg bw Fus-X.

25. From histopathological analysis, the thymuses of treated mice were observed to have decreased numbers of lymphocytes when compared to the control. Lymphocytes were shown to have nuclear condensation or fragmentation throughout the cortex and the cortico-medullary junction. These lesions were more apparent in animals exposed to Fus-X or NIV alone when compared to those treated with DON and all combined mycotoxin-treated groups, however, nuclear condensation or fragmentation were seen in the Peyer's patches of all treated animals. Marked splenic lesions (depletion of lymphocytes and nuclear condensation in the germinal centres of white pulp regions) were seen in mice exposed to Fus-X alone and were less evident in other mycotoxin treated groups and control animals.

26. The expression of apoptosis-related genes after individual and combined mycotoxin exposure were quantified in the thymus and Peyer's patches by quantitative polymerase-chain reaction assay. The expression of Bax and Trp53 were significantly up-regulated in the thymus of animals treated with Fus-X alone, when compared to the control group. No differences were observed in the other treated groups. In the Peyer's patches, Bax and Trp53 were also significantly up-regulated when compared to the control and tertiary combination of mycotoxins. An up-regulation of Caspase-3 was observed in both the thymus and Peyer's patches of mice exposed to Fus-X alone compared to animals receiving DON alone, the binary combination of DON and NIV, or the vehicle control. Caspase-9 was additionally up-regulated in the thymus and Peyer's patches of Fus-X treated animals.

HBGV

27. As alluded to in the preliminary discussion paper (TOX/2019/17[12]), Fus-X does not have an established HBGV.

28. JECFA derived a group ARfD of 8 µg/kg bw/day for DON and its acetylated derivatives using the benchmark dose response of 10% ($BMDL_{10}$) of 0.21 mg/kg bw/day for emesis in pigs and an UF of 25 for acute C_{max}-dependent effects. A provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg bw was also established; based on the NOEL of 100 µg/kg bw per day from 2-year feeding study in mice and an UF of 100 which was applied to protect against immunotoxicity, growth reduction, and reproductive effects of DON (FAO/WHO, 2011).

[12] https://cot.food.gov.uk/sites/default/files/tox2019-17.pdf

29. In 2013, EFSA established an ARfD of 14 µg/kg bw/day for NIV, using the $BMDL_{10}$ of 0.14 mg/kg bw/day for acute emetic events in mink and an uncertainty factor of 10 for intraspecies differences. A TDI for NIV of 1.2 µg/kg bw was also established, based on a $BMDL_{05}$ of 0.35 mg NIV/kg bw/day for the reduction in white blood cell counts in a 90-day rat study. An overall UF of 300 was applied to the $BMDL_{05}$; a factor of 3 for uncertainties in the database and of 100 for inter- and intra-species differences (EFSA, 2013).

30. In 2017, EFSA calculated a group TDI of 1 µg/kg bw/day for DON and its acetylated and modified forms. This was based on a $BMDL_{05}$ of 0.11 mg/kg bw/day for reduced body weight gain in mice in a 2-year feeding study in mice (the same mice study that was reviewed by JECFA) and application of an uncertainty factor of 100 for inter- and intra-species differences (EFSA, 2017b).

31. In the same Opinion, EFSA calculated an ARfD of 8 µg/kg bw based on the same emesis in pigs data that JECFA reviewed.

Table. 4 Combination effects of Fus-X with other type B trichothecenes in different cell model types.

Mycotoxin	Cell model	Mycotoxin concentration (µM)	Combination effects	Reference
DON + Fus-X	Caco-2	DON (0.25-4), Fus-X (0.0075-0.12); Ratio 1:0.3	Synergism	Alassane-Kpembi et al., (2013)
	IPEC-1	DON (0.2-15), Fus-X (0.12-9); Ratio 1:0.8	Antagonism	Alassane-Kpembi et al., (2015)
NIV + Fus-X	Caco-2	NIV (0.2-3.2), Fus-X (0.0075-0.12); Ratio 1:0.04	Synergism to additive	Alassane-Kpembi et al., (2013)
	IPEC-1	NIV (0.2-15), Fus-X (0.16-12); Ratio 1:0.8	Additive	Alassane-Kpembi et al., (2015)
DON + NIV + Fus-X	Caco-2	DON (0.25-4), NIV (0.2-3.2), Fus-X (0.0075-0.12); Ratio 1:0.8:0.03	Antagonism to additive	Alassane-Kpembi et al., (2013)

Abbreviations: DON= Deoxynivalenol, Fus-X= Fusarenon-X, NIV = Nivalenol.

Exposure assessment

32. The TDS data for Fus-X, NIV and DON were as reported by Stratton et al., (2015). Acute and chronic exposures were calculated using consumption data from the Diet and Nutrition Survey in Infants and Young Children (DNSIYC) (Lennox et al., 2013) and the National Diet and Nutrition Survey (NDNS) (Bates et al., 2014, 2016). Chronic exposures are presented in Annex A.

33. The mean and 97.5th percentile acute Fus-X exposures for infants aged 4 to 12 months ranged from 0 – 0.02 and 0.001 – 0.05 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile acute exposures ranged from 0.002 – 0.03 - and 0.009 – 0.072 µg/kg bw/day. The calculated mean and 97.5th percentile acute dietary exposures for young children aged 18 to 60 months ranged from 0.002 – 0.032 and 0.011 – 0.10 µg/kg bw/day. These exposures are shown in Tables 5a-c.

34. The sum of mean and 97.5th percentile acute exposure to Fus-X, NIV and DON (and its acetylated derivatives) are presented in Tables 6a-c. Binary mycotoxin exposures for Fus-X + NIV and Fus-X + DON (and its acetylated derivatives) are presented in Annex B and Annex C, respectively.

35. Further details on the exposure assessment for each mycotoxin can be found in the mycotoxins scoping paper (presented to the COT in 2017)[13] (Annex K for DON and Annex O for NIV, and TOX/2019/17 for Fus-X[14]).

36. In brief, the food groups contributing the highest exposures to DON in infants and young children were “wholemeal and granary bread”, “miscellaneous cereals breakfast cereals” and “white sliced bread”. “Fats and oils” were the highest contributing group to 3-Ac-DON. NIV was not detected at a level above the limit of detection (LOD) for any food groups. FUS-X levels in the majority of food samples were below the Level of Quantification (LOQ; 10 µg Fus-X/kg). Food groups that had values between the LOD (5 µg Fus-X/kg) and LOQ for Fus-X were vegetable oils, herbs, and spices.

37. The highest estimated upper-bound (UB) mean combined acute exposure for Fus-X, 15-Ac-DON and NIV is 0.82 µg/kg bw/day in 15 to 18 month old children. The corresponding highest sum of UB 97.5th percentile exposures for this combination of mycotoxins is 1.80 µg/kg bw/day in 12 to 18 month old children.

38. The highest estimated sum of UB mean combined acute exposure for Fus-X, 3-Ac-DON and NIV is 0.61 µg/kg bw/day in 15 to 18 month old children. The corresponding highest UB 97.5th percentile exposure is 1.40 µg/kg bw/day in 12 to 18 month old children.

39. For Fus-X, DON and NIV the highest estimated UB mean combined exposure is 1.20 µg/kg bw/day in 15 to 18 month old children. The corresponding highest sum

[13] https://cot.food.gov.uk/sites/default/files/tox2017-30_0.pdf
[14] https://cot.food.gov.uk/sites/default/files/tox2019-17.pdf

of UB 97.5th percentile exposures is 2.70 µg/kg bw/day in 15 to 18 month old children. DON made the highest contribution to combined mean and 97.5th percentile exposures.

Table 5a. Estimated Fus-X acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day).

4 to <6-month olds (n=116)		**6 to <9-month olds (n=606)**		**9 to <12-month olds (n=686)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
0.000-0.004	0.001-0.021	0.001-0.01	0.007-0.049	0.002-0.02	0.006-0.05

Table 5b. Estimated Fus-X acute exposures from the TDS in young children aged 12 to 18 months (µg/kg bw/day).

12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.002-0.03	0.009-0.062	0.002-0.03	0.011-0.072

Table 5c. Estimated Fus-X acute exposures from the TDS in young children aged 18 to 60 months (µg/kg bw/day).

18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.002-0.032	0.011-0.102	0.002-0.028	0.013-0.058

Table. 6a Estimated Fus-X + DON (and acetylated derivatives) + NIV acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day).

	4 to <6-month olds (n=116)		**6 to <9-month olds (n=606)**		**9 to <12-month olds (n=686)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON + NIV	0.00-0.15	0.001-0.76	0.001-0.41	0.007-1.50	0.002-0.59	0.006-1.50
Fus-X + 3-Ac-DON + NIV	0.00-0.12	0.002-0.64	0.001-0.32	0.009-1.20	0.003-0.45	0.009-1.20
Fus-X + DON + NIV	0.042-0.16	0.23-0.84	0.2-0.50	0.86-1.20	0.35-0.78	0.97-2.10

Table. 6b Estimated Fus-X + DON (and acetylated derivatives) + NIV acute exposures from the TDS in young children aged 12 to 18 months (µg/kg bw/day).

	12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON + NIV	0.002-0.78	0.009-1.80	0.002-0.82	0.011-1.80
Fus-X + 3-Ac-DON + NIV	0.003-0.59	0.012-1.40	0.003-0.61	0.014-1.40
Fus-X + DON + NIV	0.49-1.10	1.20-2.50	0.57-1.20	1.40-2.70

Table. 6c Estimated Fus-X + DON (and acetylated derivatives) + NIV acute exposures from the TDS in young children aged 24 to 60 months (µg/kg bw/day).

	18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON + NIV	0.002-0.78	0.011-1.70	0.002-0.73	0.013-1.50
Fus-X + 3-Ac-DON + NIV	0.003-0.59	0.014-1.30	0.003-0.53	0.016-1.10
Fus-X + DON + NIV	0.55-1.10	1.20-2.30	0.056-1.10	1.30-2.30

Risk assessment

40. Direct comparison of the summed acute mycotoxin exposures (Tables 6a-c) are below the ARfD for DON and NIV (8 µg/kg bw/day and 14 µg/kg bw/day, respectively).

41. In the previous discussion paper (TOX/2019/17[15]), chronic exposures of Fus-X were compared to the NIV TDI of 1.2 µg/kg bw/day. Margin of exposures (MOE) for Fus-X were calculated by dividing the $BMDL_{05}$ of the NIV TDI by the estimated UK dietary exposures. These showed no concern for adverse toxicological effects since all MOE values are above 1,000. Although, it should be recognised that there are uncertainties involved with extrapolating the $BMDL_{05}$ from NIV.

42. As the TDI's for NIV and DON are similar (1.2 and 1 µg/kg bw/day, respectively), the MOE range is not expected to change between Fus-X and DON.

43. In this paper, the MOE of the acute exposures of Fus-X will be compared to the ARfD of DON. The $BMDL_{10}$ value was 0.21 mg/kg bw/day for emesis in pigs. MOE values are shown in Table 7a-c. MOE calculations were performed as the HBGVs used for comparison were not for Fus-X or the combination of chemicals specifically.

44. A MOE calculation can be carried out, as below:

$$\text{MOE} = \frac{(\text{BMDL10 (mg per kg bw per day) x 1000})}{\text{Exposure value (µg per kg bw per day)}}$$

Table 7a. Calculated MOE's for estimated Fus-X acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

4 to <6-month olds		**6 to <9-month olds**		**9 to <12-month olds**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
N/A-53,000	210,000-10,000	210,000-21,000	30,000-4,300	105,000-11,000	35,000-4,200

[15] https://cot.food.gov.uk/sites/default/files/tox2019-17.pdf

Table 7b. Calculated MOE's for estimated Fus-X acute exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

4 to <6-month olds		**15 to 18-month olds (n=605)**	
Mean	**97.5th percentile**	Mean	**97.5th percentile**
105,000-7,000	23,000-3,400	105,000-7,000	19,000-2,900

Table 7c. Calculated MOE's for estimated Fus-X acute exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
105,000-6,600	19,000-2,100	105,000-7,500	16,000-3,600

45. The MOE calculation for the summed acute exposures have been compared to the DON ARfD $BMDL_{10}$ value, since it made the highest contribution to combined mean and 97.5th percentile exposures.

46. The calculated MOE values for the sum of Fus-X, DON and NIV are shown in Tables 8a-c, for both mean and 97.5th percentile estimated acute exposure values.

Table 8a. Calculated MOE's for estimated summed Fus-X, DON and NIV acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

4 to <6-month olds		**6 to <9-month olds**		**9 to <12-month olds**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
5,000-1,300	910-250	1,050-420	240-180	600-270	220-100

Table 8b. Calculated MOE's for estimated summed Fus-X, DON and NIV acute exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
430-190	180-80	370-180	150-78

Table 8c. Calculated MOE's for estimated summed Fus-X, DON and NIV acute exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day) against the DON ARfD $BMDL_{10}$ value.

18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
380-190	180-90	3,800-190	160-91

Conclusion

47. Fus-X is a type B trichothecene that can be present alone or in combination with other mycotoxins in cereals such as wheat, barley, oats, rye, rice, sorghum, millet and maize.

48. Mink emesis data has been utilised for the derivatisation of BMD's for other trichothecene families. It is assumed that humans are not more sensitive than mink towards the emesis effect, since the dose range for emesis are similar.

49. Comparative toxicity data for Fus-X suggest that it is more toxic when compared to other type B trichothecenes.

50. Fus-X was observed to elicit a greater emetic potency in oral administration to mink compared to DON, 3-ADON, 15-ADON, and NIV, with an ED_{50} for emetic events at 30 µg Fus-X/kg bw. The oral NOAEL in mink was 10 µg Fus-X/kg bw.

51. For anorectic responses, Fus-X was shown to be more toxic than DON, 3-ADON, 15-ADON and NIV, following oral exposure in mice. The oral NOAEL was 0.025 mg Fus-X/kg bw.

52. However, when compared to other trichothecenes, FUS-X has a lower oral emetic relative potency than HT-2 and T2.

53. Fus-X was shown to have a similar but distinct mode of action to DON, based on WTP analysis. VDR/RXR activation, ephrin receptor, GNRH, integrin and ceramide signalling pathways were specific to observations in jejunal explants from male piglets upon exposure to Fus-X, however, Fus-X was shown to induce more histological alterations than DON.

54. From literature, Fus-X has been co-detected with other mycotoxins in baby foods in other EU Member states. UK surveillance data from 2011 has co-detected Fus-X with other type B mycotoxins, although they were below the limit of quantification.

55. The sum of mean and 97.5^{th} percentile acute exposure to Fus-X, NIV and DON (and its metabolites) revealed than DON made the highest contribution.

56. Direct comparison of the summed acute mycotoxin exposures (Tables 6a-c) are below the ARfD for DON and NIV (8 µg/kg bw/day and 14 µg/kg bw/day, respectively).

57. No HBGV has been established by EFSA, JECFA or any EU Member State, however, MOE's for Fus-X were calculated by dividing the $BMDL_{10}$ of the DON ARfD by the estimated acute UK dietary exposures for Fus-X, as well as the estimated summed UK dietary exposures for Fus-X, DON and NIV.

58. Acute exposures of Fus-X showed no cause for acute adverse toxicological effects against the ARfD value of DON, since all MOE values were above 1,000

(Tables 7a-c), however, it must be noted that there are some uncertainties involved in the extrapolation of this data.

59. An MOE of less than 100 was observed in the UB values of the 97.5th percentile exposures for 12-60 months old aged infants and children for the summed exposures of the type B trichothecenes (Tables 8a-c), however, the ARfD is based on a $BMDL_{10}$ value with an UF of 25. Additionally, the probability of co-occurrence for all mycotoxins are considered low. Furthermore, the ratios of each mycotoxin in the mixture are unlikely to be the same levels at any one time. Considerations of other legitimate factors, as described in paragraphs 15 and 16 also need to be kept in mind by risk managers.

60. It is therefore concluded that the acute co-exposure of Fus-X with DON and NIV are unlikely to result in adverse toxicological effects.

Questions to ask the Committee

61. Members are invited to consider the following questions:

i). Does the additional data presented allow the Members of the Committee to determine a point of departure for benchmark dose modelling?

ii). Do the Committee agree that additive exposures of type B trichothecenes are unlikely to cause adverse toxicological effects?

iii). Does the Committee have any other comments?

Secretariat

June 2019

Abbreviations

15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
ARfD	Acute reference dose
BMD	Benchmark dose
CI	Combination index
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DON	Deoxynivalenol
DRI	Dose reduction index
ED50	Effective Dose 50
EFSA	European Food Safety Authority
EFSA CONTAM	Panel on Contaminants in the Food Chain
EPA	Environmental Protection Agency
Fus-X	Fusarenon-X
GNRH	Gonadotrophin-releasing hormone
HBGV	Health based guidance value
hGES	Human gastric epithelial cells
HT-2	HT-2 toxin
IC_{50}	Inhibition concentration 50
i.p.	Intraperitoneal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
LXR	Liver X receptor
MOE	Margin of exposure
NEO	Neosolaniol
NIV	Nivalenol
NOAEL	No observed adverse effect level
PPAR	Peroxisome proliferator-activated receptor
RXR	Retinoid X receptor
T2	T-2 toxin
TDI	Tolerable daily intake
UB	Upper-bound
VDR	Vitamin D receptor
WTP	Whole transcriptome profiling

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

4 to <6-month olds (n=116)		**6 to <9-month olds (n=606)**		**9 to <12-month olds (n=686)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
0.000-0.002	0.001-0.01	0.000-0.008	0.003-0.03	0.001-0.013	0.003-0.004

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

12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.001-0.002	0.004-0.04	0.001-0.02	0.005-0.04

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

18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.001-0.02	0.006-0.06	0.001-0.002	0.004-0.04

62. The mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.013 and 0.001 – 0.035 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.02

- and 0.004 – 0.04 µg/kg bw/day. The calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.001 – 0.02 and 0.004 – 0.06 µg/kg bw/day.

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

4 to <6-month olds (n=116)		**6 to <9-month olds (n=606)**		**9 to <12-month olds (n=686)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
0.000-0.11	0.001-0.60	0.001-0.29	0.007-1.10	0.002-0.42	0.006-1.10

Table. b Estimated Fus-X + NIV acute exposures from the TDS in young children aged 12 to 18 months old (µg/kg bw/day).

12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.002-0.55	0.009-1.30	0.002-0.57	0.011-1.30

Table. c Estimated Fus-X + NIV acute exposures from the TDS in young children aged 24 to 60 months old (µg/kg bw/day).

18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.002-0.54	0.011-1.20	0.002-0.49	0.013-0.99

Table. a Estimated Fus-X + DON (and acetylated derivatives) acute exposures from the TDS in infants aged 4 to 12 months old (µg/kg bw/day).

	4 to <6-month olds (n=116)		**6 to <9-month olds (n=606)**		**9 to <12-month olds (n=686)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON	0.000-0.042	0.001-0.18	0.001-0.13	0.007-0.44	0.002-0.20	0.006-0.51
Fus-X + 3-Ac-DON	0.000-0.012	0.002-0.058	0.001-0.32	0.009-0.13	0.003-0.052	0.009-0.13
Fus-X+ DON	0.042-0.051	0.23-0.26	0.20-0.22	0.86-0.92	0.35-0.38	0.97-1.00

Table. b Estimated Fus-X + DON (and acetylated derivatives) acute exposures from the TDS in young children aged 12 to 18 months old (µg/kg bw/day).

	12 to <15-month olds (n=670)		**15 to 18-month olds (n=605)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON	0.002-0.26	0.009-0.56	0.002-0.29	0.011-0.65
Fus-X + 3-Ac-DON	0.003-0.072	0.0012-0.16	0.003-0.076	0.014-0.18
Fus-X+ DON	0.49-0.54	1.20-1.30	0.57-0.62	1.40-1.50

Table. c Estimated Fus-X + DON (and acetylated derivatives) acute exposures from the TDS in young children aged 24 to 60 months old (µg/kg bw/day).

	18 to 24 month-olds (n=118)		**24 to 60 month-olds (n=688)**	
	Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
Fus-X + 15-Ac-DON	0.002-0.30	0.011-0.69	0.002-0.27	0.013-0.56
Fus-X + 3-Ac-DON	0.003-0.082	0.014-0.20	0.003-0.069	0.016-0.14
Fus-X+ DON	0.55-0.62	1.20-1.30	0.56-0.62	1.30-1.40