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

Statement of T-2 toxin (T2), HT-2 toxin (HT2) and neosolaniol (NEO) in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Introduction

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that will inform the Government dietary recommendations for infants and young children. The SACN is examining the nutritional basis for the advice. The COT was asked to review the risk of toxicity of chemicals in the diets of infants and young children. The reviews will identify new evidence that has emerged since the Government recommendations were formulated and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years.

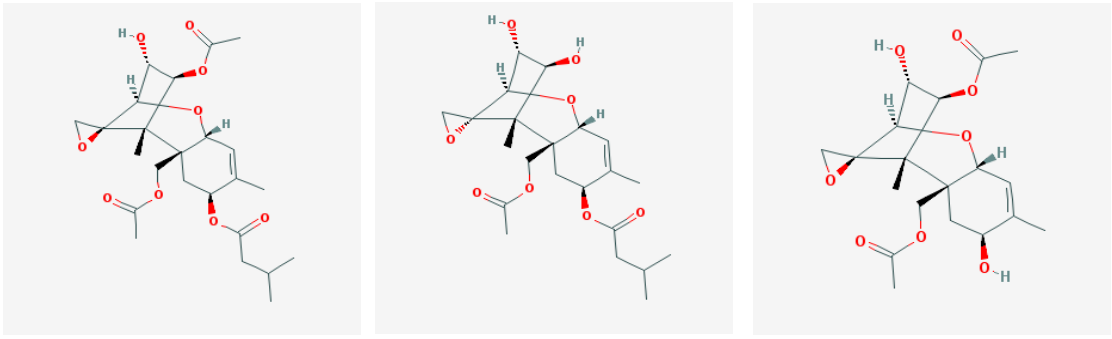
2. The Food Standards Agency (FSA) has completed a survey of 36 mycotoxins in the 2014 Total Diet Study (TDS) – mycotoxins analysis (FSA, to be published). The results of the survey provide information on the concentrations of aflatoxins (B1, B2, G1, G2 and M1), ochratoxin A, zearalenone, fumonisins (B1, B2 and B3), 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, deoxynivalenol, diacetoxyscirpenol, fusarenon-X, T-2 toxin (T2), HT-2 toxin (HT2), neosolaniol (NEO), nivalenol, sterigmatocystin, citrinin, cyclopiazonic acid, moniliformin, patulin and ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine) in relevant foods. Estimates of dietary exposures have been calculated for each mycotoxin for UK infants and young children aged 4 to 60 months using food consumption data taken from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the national diet and nutrition survey (NDNS).

3. This statement gives an overview of 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. It draws on the EFSA opinion (2011) and update (2017a). None of the Government's current dietary recommendations for infants and young children relate to these toxins.

Background

4. T2 and HT2 are type A trichothecenes and are produced by a variety of *Fusarium* species (*F. sporotrichoides, F. poae. F. equiseti, F. acumninatum*). They may also be produced by other crop invasive species of *Myrothecium, Cephalosporium, Verticimonosporum, Trichoderma, Trichothecium* and *Stachybotrys*. The chemical structures of T2 and HT2 are shown in Figure 1. *Fusarium* species grow and invade crops and produce the T2 and HT2 under cool, moist conditions prior to harvest. T2 and HT2 are found predominantly in cereal grains (particularly oats) and their products. Neosolaniol (NEO) (Figure 1.) is a hydrolytic phase I metabolite of T2 and may be formed in fungi and mammals. NEO has been found in some brewed coffee samples, in a sample of cereal-containing baby food and at trace levels in some barley field malt samples. (EFSA, 2017a).

5. T2 and HT2 have been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001, the Scientific Committee on Food (SCF) in 2002 and the European Food Safety Authority (EFSA) in 2011 and 2017. NEO was included in the EFSA 2017 evaluation of T2 and HT2.



T-2 toxin HT-2 toxin Neosolaniol

Figure 1. Chemical structures of T-2 toxin[1], HT-2 toxin[2] and neosolaniol[3]. Taken from the PubChem Open Chemistry Database.

Toxicokinetics

6. The toxicokinetics of T2 and HT2 have been reviewed previously by JECFA (2001) and EFSA (2011).

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[1]National Center for Biotechnology Information. PubChem Compound Database; CID=5284461, https://pubchem.ncbi.nlm.nih.gov/compound/5284461 (accessed Apr. 25, 2018).

[2] National Center for Biotechnology Information. PubChem Compound Database; CID=10093830, https://pubchem.ncbi.nlm.nih.gov/compound/10093830 (accessed Apr. 25, 2018).

[3] National Center for Biotechnology Information. PubChem Compound Database; CID=13818797, https://pubchem.ncbi.nlm.nih.gov/compound/13818797 (accessed Apr. 25, 2018).

7. There is very little information on the *in vivo* absorption of T2 and HT2 in animals after oral administration. However, 40 to 57 % of radioactivity was found in bile and blood in studies in which tritiated T2 was administered directly into the small intestine. Only low amounts of T2 were observed in these studies, suggesting extensive hydrolysis to HT2 and other metabolites during the rapid intestinal absorption of T2. Rapid absorption has been confirmed by the excretion of total radioactivity in rats within 48 hours after oral gavage. T2 radioactivity was rapidly distributed to the liver, kidney and other organs without accumulation in any organ in orally dosed rats and mice. (EFSA, 2017a). The metabolism of T2 and HT2 in humans and other species is complex and was reviewed by EFSA (2011). Phase I metabolites arise from either hydrolysis of ester groups; hydroxylation; or de-epoxidation. These reactions may also occur in combination. Glucuronides are the most prevalent mammalian phase II metabolites of T2 and HT2. (EFSA, 2017a).

8. No data have been identified for the toxicokinetics of NEO.

Toxicity

Summary from previous evaluations

9. The toxicity of T2 and HT2 has been reviewed previously by EFSA, JECFA and the SCF. The EFSA 2011 evaluation, concluded that T2 induces haemato- and myelotoxicity and that these effects occurred at lower doses than other toxic effects such as dermal toxicity, developmental and reproductive toxicity, and neurotoxicity. Clastogenicity was observed in some in vitro and in vivo genotoxicity tests. However, this was mainly at concentrations which also inhibited protein and DNA synthesis and caused cytotoxicity. EFSA concluded that T2 inhibited protein-, DNA-, and RNA synthesis and that there were studies indicating that T2 causes apoptosis, necrosis and lipid peroxidation. The pig was identified as one of the most sensitive species. (EFSA, 2011).

Summary of the in vivo toxicity studies published since the 2011 EFSA Opinion and reviewed by EFSA (2017a)

10. A number of acute and subacute toxicity studies had been published since the EFSA 2011 evaluation. These consisted of studies on the anorectic effects (feed refusal, reduced body weight gain, vomiting and retching) of T2 and HT2 at low doses and predominantly in 3 species (mink, pig and mouse). On the basis of these studies, EFSA concluded that it was necessary to establish an acute reference dose (ARfD) for the toxins (EFSA, 2017a). It was also noted that there have been reports of nausea and emesis in humans consuming mouldy grain contaminated with T2-producing strains of *Fusarium poae* and *Fusarium sporotrichioides* (EFSA, 2011).

11. Subchronic toxicity studies published since 2011 had investigated similar endpoints to those used by EFSA in its 2011 evaluation for establishment of an HBGV. They tended to be of longer duration than the pig

studies used but confirmed the immunotoxicity and haematotoxicity of T2 and HT2.

12. EFSA (2017a) concluded that there were only a few *in vivo* studies which compared the acute toxicity of metabolites of T2 and HT2 (phase I metabolites only) with that of T2 and HT2. The endpoints which had been investigated were food consumption, weight gain, lethality and induction of apoptosis in vitro. Generally, all the metabolites tested exerted these effects and were equally or less potent than T2 or HT2. (EFSA, 2017a).

13. The COT assessed the *in vivo* studies published since 2011 and reviewed by EFSA (2017a)[4] and agreed with the studies used by EFSA for establishing an ARfD and updating the tolerable daily intake (TDI).

Studies used in the establishment of the ARfD and the TDI by EFSA in 2017

Wu *et al*., 2016

14. In a study by Wu *et al.* (2016) groups of fasted female mink (n = 4) were given 50 g of feed 30 minutes prior to either, i.p. administration of 0, 0.001, 0.01, 0.05 or 0.25 mg/kg bw of T2 or HT2 or 0, 0.5, 1, 2.5 or 5 mg/kg bw emetine, or administration by oral gavage of 0, 0.005, 0.05, 0.25 or 0.5 mg/kg bw T2 or HT2 or 0, 0.5, 1, 2.5 or 5 mg/kg bw emetine. The animals were then monitored for emetic events[5] for 6 hours. In a 2nd study, 3 groups of fasted female mink (n = 4) were given 50 g of feed 30 minutes prior to 0.5 mg/kg bw T2 or HT2 or 5 mg/kg bw emetine by oral gavage. Emetic events were recorded for up to 2 hours and levels of plasma satiety hormone peptide YY_{3-36} (PYY_{3-36}) and 5-hydroxytryptamine (5-HT) (hormones known to be implicated in emesis) measured. The lowest dose at which emetic events were observed after i.p. administration was 0.05 mg/kg bw for T2 and HT2 and 25% of animals were affected for each. After oral exposure the lowest dose at which emetic events occurred was 0.05 mg/kg bw and 75% of animals were affected for both T2 and HT2. At 0.25 mg/kg bw 4 animals (100%) were affected for both T2 and HT2 via i.p. and oral administration. The lowest doses at which emetic events occurred in animals dosed with emetine was 2.5 (50%) and 1 mg/kg bw (50%) for i.p. and oral administration routes, respectively. The latency of emetic events decreased while duration and frequency of emetic events increased with dose. Oral administration of T2 and HT2 caused increases in plasma concentrations of PYY_{3-36} and 5-HT. The authors concluded that via the oral route NOAELs were 5 µg/kg bw, LOAELs were 50 µg/kg bw and ED_{50}s were 20 µg/kg bw for both T2 and HT2.

Rahman *et al*., 2014

15. In a study by Rahman *et al.* (2014) 192 male Wistar rats were assigned to 4 groups (n = 48) and dosed with 0, 0.5, 0.75 or 1.0 mg T2/kg (ppm)

[4] https://cot.food.gov.uk/sites/default/files/tox2017-47.pdf

[5] An emetic event was classed as either vomiting or retching. According to Wu *et al.* (2016) vomiting is rhythmic abdominal contraction with oral expulsion of either solid or liquid material. Retching is a response which mimics vomiting but without the expulsion of any material.

(equivalent to 0, 45, 68 and 90 µg T2/kg bw/day, respectively) daily via the diet for 12 weeks. Eight animals were killed at each of 2, 4, 6, 8, 10 and 12 weeks. Rats dosed with T2 showed varying degrees of clinical signs, including dullness, weakness, lethargy, growth retardation, reduced feed intake, reluctance to move and rough hair coat, which worsened over time in groups receiving 68 or 90 µg/kg bw/day. After the 8^{th} and 10^{th} week, respectively animals treated with 90 µg/kg bw/day showed gangrenous dermatitis of the tail (15/24) and facial and podal dermatitis. A statistically significant dose-dependent decrease in bodyweights was seen after 90 days of dosing. Mean body weights were 264, 219, 184 and 160 g for rats dosed with 0, 45, 68 and 90 µg/kg bw/day. Significant decreases in haemoglobin (Hb), packed cell volume (PCV), total erythrocyte counts (TEC), total thrombocyte counts (TTC), total leucocyte counts (TLC), mean corpuscular volume (MCV), mean corpuscular Hb (MCHb), and percentages of lymphocytes was observed but the percentage of neutrophils increased. Generally, all of these observations became more pronounced with study length. After 90 days of feeding mean TECs were 8.97, 5.85, 5.77 and 4.65 $x10^6$/µl in rats fed 0, 45, 68 and 90 µg/kg bw/day, respectively; mean TLCs were 14.8, 8.95, 6.92 and 5.20 $x10^3$/µl in animals dosed with 0, 45, 68 and 90 µg/kg bw/day, respectively; mean TTCs were 122.5, 77.7, 56.5 and 38.0 $x10^3$/µl in animals fed 0, 45, 68 and 90 µg/kg bw/day. (Rahman *et al.*, 2014). The authors concluded that T2 induces microcytic hypochromic anaemia, leukocytopaenia (due to lymphocytopaenia) and thrombocytopaenia in rats, which increased with dose and duration of exposure. When EFSA reviewed this study, they did not identify a NOAEL and considered the lowest dose tested (45 µg/kg bw/day) a LOAEL. (Rahman *et al.*, 2014). EFSA noted that the thrombocyte counts were unusually low in this study compared with other studies. (EFSA, 2017a).

HBGV's established by EFSA, JECFA and the SCF

JECFA 2001 provisional maximum tolerable daily intake (PMTDI)

16. The JECFA Committee concluded that immunotoxicity and haematotoxicity are the critical effects of T2 after short-term intake. JECFA used the lowest observed effect level (LOEL) of 29 µg/kg bw/day for changes in red and white blood cell counts identified in the Rafai (1995 a, b) studies. An uncertainty factor of 500 was applied to establish a provisional maximum tolerable daily intake (PMTDI) for T2 of 60 ng/kg bw. HT2 was included in the PMTDI, which resulted in a group PMTDI of 60 ng/kg bw for T2 and HT2, alone or in combination.

SCF 2001 temporary TDI (tTDI)

17. The SCF considered the general toxicity, haematotoxicity and immunotoxicity of T2 to be the critical effects. They used the haematotoxicity and immunotoxicity of T2 in pigs in a short-term study (Rafai *et al.* 1995b) as the basis for the risk assessment. The SCF noted that slight effects were seen on immune parameters and there was approximately a 10% reduction in feed intake at the lowest dose. An uncertainty factor of 500 was applied and a temporary TDI (tTDI) of 0.06 µg/kg bw (60 ng/kg bw) was established. It was

also concluded that because the toxicity of T2 *in vivo* may be partly attributed to HT2, it was appropriate to establish a combined tTDI for the sum of T2 and HT2.

EFSA 2011 (TDI) (EFSA, 2011a)

18. EFSA performed a benchmark dose (BMD) analysis on the specific antibody response (anti-horse globulin) from Rafai *et al.* (1995a, b), using the PROAST software (version 26.0 under R 2.10.2), following EFSA guidance (2011b). EFSA used the $BMDL_{05}$[6] of 10 µg/kg bw/day for T2 toxin as a reference point to establish a TDI. Due to the rapid metabolism of T2 to HT2 and the fact that T2 toxicity may in part be due to HT2, EFSA decided to establish a group TDI for the sum of T2 and HT2. The default uncertainty factor of 100 was applied to the $BMDL_{05}$ of 10 µg/kg bw/day to establish a TDI of 100 ng/kg bw for the sum of T2 and HT2.

EFSA 2017 ARfD and TDI

ARfD

19. Recent studies have reported anorectic effects at low doses of T2 and HT2 in mice, mink and pig. The lowest does at which acute effects were seen was in mink in a study by Wu *et al.* (2016). Emetine (an *ipecacuanha* alkaloid) was used as a positive control in this study and the ED_{50} obtained was 1030 µg/kg bw via the oral route. Emetine has been used to induce vomiting in humans and the effective dose is in the same range as that given to mink. The mink, *in lieu* of the ferret (which is more expensive and difficult to raise), has been suggested as the model species for emesis in drug testing (Gordon, 1985; Zhang *et al.*, 2006; Percie du Sert *et al.*, 2012). EFSA (2017a) therefore concluded that the mink was an appropriate animal model for vomiting in humans (EFSA, 2017a).

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

21. The BMD analysis was performed using the EFSA guidance on the use of the BMD (EFSA, 2017b). The data used in the BMD analysis are shown in Table 1. *"For quantal response data observed in experimental animals, BMR values of 1, 5 and 10 % (extra or additional risk) were initially proposed. Various studies estimated that the median of the upper bounds of extra risk at the NOAEL was close to 10%, suggesting that a* $BMDL_{10}$ *may be an appropriate default. Also, a benchmark response (BMR) of 10% appears preferable for quantal data because the BMDL can become substantially dependent on the choice of dose-response models at lower BMRs"* (EFSA, 2017a). EFSA (2017a) selected a benchmark response of 10% and used

[6] The default value for continuous data recommended by EFSA is a benchmark response of 5%. The $BMDL_{05}$ is the 95% lower confidence limit for the benchmark dose response of 5% ($BMDL_{05}$)

PROAST software version 38.9. One additional assumption was noted, in that the results from 2 independent experiments on T2 and HT2 were combined and the experiment considered as a covariate. The results from the BMD analysis are shown in Table 2.

Table 1. Data used in the EFSA BMD analysis to establish an ARfD

Substance	Dose (µg/kg bw)	Animals showing emesis	Number of animals (N)	Sex
T2	0	0	4	F
	5	0	4	F
	50	3	4	F
	250	4	4	F
	500	4	4	F
HT2	0	0	4	F
	5	0	4	F
	50	3	4	F
	250	4	4	F
	500	4	4	F

bw: bodyweight

Table 2. Results of the EFSA BMD analysis to establish an ARfD

Models	Number of parameters	Log likelihood	AIC[(a)]	$BMDL_{10}$ (µg/kg bw)[(b)]	BMD_{10} (µg/kg bw)[(b)]	$BMDU_{10}$ (µg/kg bw)[(b)]
Full	8	-4.50[(e)]	25.00	-	-	-
Null	2	-27.73	59.05	-	-	-
Gamma	3	-4.50	15.00	2.97	28.3	44.3
Logistic	2	-4.50	13.00	12.30	42.7	49.8
LogLogistic	3	-4.50	15.00	4.29	37.1	47.1
LogProbit	3	-4.50	15.00	4.02	26.8	49.7
Two-stage[(c)]	3	-4.61	15.22[(b)]	NR[(d)]	NR[(d)]	NR[(d)]
Probit[(e)]	2	-4.50	13.00	11.0	36.1	NR[(d)]
Weibull	3	-4.50	15.00	3.02	29.9	47.9

(a): AIC: Akaike's information criterion
(b): BMD: benchmark dose calculated at 10 % extra risk. $BMDL_{10}$: 95th lower confidence limit (one-sided) of BMD;$BMDU_{10}$: 95th upper confidence limit (one-sided) of BMD.
(c): Model not fulfilling the criterion ($AIC \leq AIC_{min} + 2$)
(d): NR: Not reported
(e): Calculated using BMDS v2.6086, pooling data from the 2 experiments.

22. It was not possible, until very recently, to perform model averaging in this instance using the PROAST software. The overall BMDL - BMDU range

was 2.97 – 49.8 µg/kg bw (when considering all models with AIC ≤ AIC_{min} +2). Following the EFSA guidance (EFSA, 2017b), EFSA selected a $BMDL_{10}$ of 2.97 µg/kg bw for further consideration, as this was the lowest valid $BMDL_{10}$.

23. An uncertainty factor of 10 for intraspecies variability was applied to the $BMDL_{10}$ of 2.97 µg/kg bw derived for emetic response in mink. However, no interspecies variability factor was applied because humans were not considered more sensitive than mink to acute emetic effects. This was based on observations with emetine, and it was assumed that this would also be the case for T2 and HT2. An ARfD of 0.3 µg T2 or HT2/kg bw was established. NEO was equipotent to T2 and HT2 when tested for vomiting in ducklings (Ueno *et al.*, 1974) and was therefore included together with T2 and HT2 in a group ARfD. (EFSA, 2017a).

24. The ARfD, established by EFSA, was accepted by the COT with the following caveats:

i. The AIC values for all the models, except the Two-stage model, fell within the EFSA acceptance criterion (AIC ≤ AIC_{min} +2), however, the BMDU/BMDL ratio is quite large, generally >10-fold.

ii. The COT considered that the lack of an interspecies uncertainty factor might be justifiable for the toxicodynamic component (similar sensitivity to emetine) but there was some concern as to whether the toxicokinetic differences would be accounted for (potential differences in the toxicokinetics of the toxins as compared with emetine).

iii. The Wu et al. (2016) study used only female minks and there did not appear to be any consideration by EFSA as to how suitable this was as a model.

25. Using a very recent update to the PROAST software, it was possible to perform model averaging on the Wu et al (2016) data. This resulted in a model averaged $BMDL_{10}$ of 12.2 µg/kg bw, approximately 4-fold greater than the $BMDL_{10}$ used by EFSA to establish the ARfD. Because the COT was uncertain as to the current validation status of the model averaging function of the PROAST software, the ARfD established by EFSA was used to characterise the acute risk. The EFSA ARfD is also more conservative than the one that would be calculated by model averaging.

TDI for T2, HT2 and their metabolites

26. Since 2011, several subacute and subchronic toxicity studies of T2 have been published. In the 90-day study in rats by Rahman *et al.* (2014), dose-dependent decreases in total erythrocyte, leucocyte and thrombocyte counts, as well as a decrease in the percentage of lymphocytes, were

observed. These effects progressed during the study period, with no signs of reaching a plateau at the end. The exposure duration to T2 in this study was longer (90 days) than in the Rafai *et al*. (1995a, b) studies in pigs, not only in absolute terms, but also as a proportion of species lifetime.

27. EFSA (2017a) noted that the effects observed (i.e. anorectic effects and effects on immune system and blood parameters) in the Rahman et al (2014) rat study were essentially similar to those seen in the pig study, confirming the immune system and blood cell production as targets of T2 across species.

28. Therefore, EFSA (2017a) decided, considering the longer exposure duration of the study from Rahman *et al.* (2014) and its biological relevance, to use the total leucocyte counts reported from this study (Table 3) for calculating a new BMD for T2. EFSA used its own guidance (EFSA, 2017b) to calculate the BMD. EFSA used a BMR of 10%, considering such a response in leucocyte counts to be within the individual physiological variation and negligible, and further noted that the selected BMR is slightly below the control standard deviation of the controls in the Rahman *et al.* study (14%). A series of other potentially relevant effects seen in repeat dose experiments with T2 have been used for alternative calculations of a chronic BMD. EFSA (2017a) concluded that the BMD derived as described below was the most appropriate and has therefore been used for risk characterisation. The data from the Rahman *et al.* (2014) study used to derive the BMD are presented in Table 3 and the results of the BMD analysis are shown in Table 4.

Table 3. Data used in the EFSA BMD analysis to establish a TDI

Dose (µg/kg bw/day)	Mean total leucocyte count (x 10^3/µl)	SE[(a)]	Number of animals (N)	Sex
0	14.83	0.73	8	M
45	8.95	0.36	8	M
68	6.92	0.83	8	M
90	5.2	0.73	8	M

(a): SE: standard error

Table 4. Results of the EFSA BMD analysis to establish a TDI

	Model	Number of parameters	Log Likelihood	AIC	$BMDL_{10}$	BMD_{10}	$BMDU_{10}$
	Null	1	-21.99	45.98			
Exponential	3[(a)]	3	-1.14	8.28	3.30	11.52	23.75
	5	4	-1.14	10.28			
Hill	3[(a)]	3	-1.15	8.30	5.95	15.70	27.60
	5	4	-1.15	10.30			

	Full	4	-1.14	10.28			

29. The overall BMDL - BMDU range is 3.30 - 27.60 µg/kg bw (when considering all models with AIC ≤ AIC_{min} +2). A 95% lower confidence limit for the benchmark dose response ($BMDL_{10}$) of 3.3 µg T2/kg bw was derived. EFSA used this value as a reference point for establishing a chronic TDI for T2 and HT2 as it was the lowest valid $BMDL_{10}$.

30. To this value, an uncertainty factor of 200 was applied: a factor of 10 for interspecies variability, 10 for intraspecies variability and 2 for extrapolation from subchronic to chronic exposure duration and for the progression of the toxic effect through the duration of the study with no signs of reaching a plateau at the end. EFSA thus established a TDI of 0.02 µg T2/kg bw.

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

32. EFSA noted that no *in vivo* studies on the haematotoxicity of modified forms of T2 and HT2 were identified. However, as some phase I metabolites have been shown to cause protein synthesis inhibition, it was assumed that may work via a similar mode of action and as such induce haematotoxicity. EFSA (2017a) therefore considered it appropriate to include such metabolites in a group TDI, assuming dose addition as a model of joint action. Because the potencies of the phase I metabolites differ with respect to the inhibition of protein synthesis and other toxic effects, it was decided to assign relative potency factors (RPFs), on a molar basis.

33. When assigning potency factors to the phase I metabolites EFSA used *in vivo* and *in vitro* studies on comparative toxicity. EFSA noted that none of the phase I metabolites were more potent than T2 or HT2. Only those metabolites that had been assessed either *in vitro* or *in vivo* were considered for establishing relative potencies. Since *in vitro* test systems may have a limited capacity for detoxification, results would in general overestimate the toxicity of T2 compared to that *in vivo*. Therefore *in vivo* data were used preferentially. When there were different values for relative potencies for the same metabolite, EFSA used the highest potency so that relative toxicity was not underestimated. EFSA rounded the RPFs to half orders of magnitude to avoid spurious accuracy whilst retaining a conservative approach. The RPFs for all phase I metabolites are detailed in the EFSA Opinion. The relative potency factors (RPFs) calculated for T2, HT2 and NEO were 1, 1 and 0.3, respectively. (EFSA, 2017a).

Uncertainties

34. EFSA (2017a) identified a number of uncertainties in their evaluation.

35. The test compound in the study used to determine the TDI was purified from fungal culture material and its purity was not specified. It therefore cannot be excluded that minor amounts of other mycotoxins, including modified forms, were present.

36. There is uncertainty associated with using a subchronic study to establish a chronic HBGV. Additionally, there were no repeated dose studies available for HT2 which has been included in the group TDI with T2, based on similar acute toxicity profile and potency, structural similarity and because HT2 is an immediate metabolite of T2.

37. EFSA established an ARfD for T2 and HT2 based on a $BMDL_{10}$ derived from observations of emesis in 2 similar acute studies with T2 and HT2. EFSA noted that there is considerable uncertainty associated with the BMDL calculation due to the large dose spacing at the lower doses and the small number of animals used.

38. Dose additivity of T2 and HT2 and their modified forms was assumed, although EFSA noted that, antagonistic or less likely, synergistic, effects of their co-exposure cannot be excluded in principle.

Exposure Assessment

39. T2, HT2 and NEO were measured in the 2014 TDS – mycotoxins analysis (FSA, to be published).

40. Although T2, HT2 and NEO were analysed in various food groups in the mycotoxins TDS, the data were all markedly left censored. All values were below the limit of quantification (LOQ) and several were below the limit of detection (LOD). While these data could be used as a qualitative indicator of mycotoxins present in various food categories, it is not possible to use them for a quantitative estimation of dietary exposures for the following reasons:

41. Because of the way the TDS is done it can lead to high LOQs which significantly influence the UB values, and consequently the exposure assessment. A multi-mycotoxin method and approach was used in the analysis for the various food groups, which is normally a screening technique rather than a sensitive quantitative analytical method. This is reflected in generally poor recoveries for T2 and HT2. Also, the analysis of the TDS samples involved a wide range of food matrices (some of which have not been routinely examined previously) and so existing validated methods were adapted/extended to some of the new matrices and this may have also impacted on recovery for T2 and HT2. Recoveries ranged from 13 - 140% for T2 and 19 - 100% for HT2. For T2 the LOD ranged from 0.10 – 0.78 µg/kg. The LOQ ranged from 3.58 – 38.9 µg/kg. The LOD for HT2 ranged from 1.00 – 5.39 µg/kg and the LOQ from 4.98 – 26.9 µg/kg. Poor recoveries

and higher LOQs/LODs when these are corrected for recovery, led to artificially inflated occurrence levels in some cases.

42. Upper bound exposure estimates resulted in a considerable overestimation of potential exposure. This is not an unfamiliar situation and is routinely encountered in cases where a majority of the occurrence data are left-censored. Recently EFSA have published their updated exposure assessment for T2 and HT2. The same problem was documented in their analysis and they have reported that UB estimations were on average fourfold higher than lower bound (LB) estimations.

43. For these reasons, it is not possible to use the T2, HT2 and NEO occurrence data from the TDS for a quantitative estimation of dietary exposure. An exposure assessment cannot be based solely on the calculated UB levels from the sum of LOQs and therefore alternative survey data were considered for calculating dietary exposures for infants and young children.

44. In a retail survey of mycotoxins in foods for infants and young children (FSA, 2011), T2 and HT2 were not detected in any of the 77 samples examined. Similarly, in a survey of ethnic foods (FSA, 2013), T2 and HT2 were not detected in any of the samples tested. In another retail survey of oat-based products (FSA, 2015), low levels of T2 and HT2 were detected in various products. The FSA survey was commissioned following initial results from the 2014 harvest that showed high levels of T2/HT2 in oat grains. So, the retail survey was commissioned to estimate exposures in an atypically high exposure scenario. The samples collected as part of the survey were oat based as follows: porridge oats (n=56), oat-based breakfast cereals (n=56), oat biscuit products (n=67), black pudding & oatmeal bread (n=6), and oatmeal (n=15). The samples were obtained from major retailers and some convenience type stores.

45. Since oats and oat-based products are reported to have higher levels of T2 and HT2 (EFSA, 2017c), and since none of the other data showed any detectable levels of these mycotoxins, data from this survey, in which actual levels of the mycotoxins were measured in oat-based foods, were used for acute and chronic exposure assessments for T2 and HT2).

46. Samples were analysed for NEO in 2 retail surveys. NEO was not detected in any of the samples tested (LOD of 5 µg/kg and LOQ of 10 µg/kg) of 210 retail samples of wheat, maize, oat and rye-based products (FSA, 2010). In another survey of food for infants and young children (FSA, 2011), NEO was not detected in any of the 77 samples analysed (LOD of 5 µg/kg and LOQ of 10 µg/kg). As there were no detectable levels of NEO in any of the samples, an exposure assessment for this compound was not performed.

47. The consumption data used for the exposure assessments, were from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (DH, 2013) and the National Diet and Nutrition Survey rolling programme (NDNS)

years 1 – 6 (Bates *et al.*, 2014; Bates *et al.*, 2016). Exposures in 0 to 4-month old infants are negligible as infants in this age range are unlikely to consume solid foods, including oat based products. Exposures were assessed for infants aged 4 - <6, 6 - <9 and 9 - <12 months, and for young children aged 12 - <15, 15 – <18, 18 – <24 and 24 – <60 months. Consumption data from DNSIYC was used for children aged 4 – 18 months and from NDNS for children aged 18 – 60 months. The detailed exposure assessments had been reviewed previously by the Committee[7].

48. T2 and HT2 were detected in 200 samples of oat products from the retail survey that were categorised as follows: biscuits and oatcakes; black pudding; drinking oats; flapjacks and oaty snack bars; muesli oat breakfast cereals and granola; oat bread; oatbran and porridge oats. 78% (155 samples) had concentrations of T2 and HT2 above the LOQ (1 µg/kg). Exposure to the sum of T2 and HT2 has been estimated from the results of the retail survey.

Acute exposure

49. Table 5 shows the calculated acute total mean and 97.5th percentile exposures to the sum of T2 and HT2 for infants and young children as lower-bound (LB) and upper-bound (UB) estimates. Total mean and 97.5th percentile exposures ranged from 0.022 (lowest LB) – 0.032 (highest UB) and 0.056 (lowest LB) – 0.11(highest UB) µg/kg bw, respectively. EFSA (2017) reported acute exposure levels for the sum of T2 and HT2 for the consumption of diverse single commodities, therefore UK data could not be compared to the total exposures in table 5.

Chronic exposure

50. Table 6 shows the calculated chronic total mean and 97.5th percentile exposures to the sum of T2 and HT2 for infants and young children. Total mean and 97.5th percentile exposures ranged from 0.0099 (lowest LB) – 0.014 (highest UB) and 0.029 (lowest LB) – 0.063 (highest UB) µg/kg bw/day, respectively. This is comparable to the range of chronic exposures from oat containing commodities reported by EFSA (2017) for UK infants: mean of 0.016 - 0.039 and 95th percentile of 0.045 - 0.090 µg/kg bw/day. For UK toddlers, EFSA (2017) reported mean and 95th percentile exposures ranging from; 0.021 - 0.057 and 0.047 - 0.11 µg/kg bw/day respectively. The data reported by EFSA is for seven food categories, namely: ‘Grains and grain-based products’ (unspecified), ‘Grains for human consumption’, ‘Breakfast cereals’, ‘Grain milling products’, ‘Fine bakery wares’, ‘Pasta (raw)’ and ‘Bread and rolls’.

[7] https://cot.food.gov.uk/sites/default/files/tox2017-47.pdf

Table 5. Estimated sum of T2 and HT2 acute exposures from the 2015 retail survey in infants and young children aged 4 to 60 months (µg/kg bw)

	4 to <6 month-olds (n = 20)		6 to <9 month-olds (n = 273)		9 to <12 month-olds (n = 386)		12 to <15 month-olds (n = 404)		15 to <18 month-olds (n = 371)		18 to 24 month-olds (n = 63)		24 to 60 month-olds (n = 390)	
	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile
Sum of T2 and HT2	0.022 – 0.023	0.056 – 0.057	0.029 – 0.030	0.097 – 0.099	0.029 – 0.030	0.091 – 0.093	0.029	0.096 – 0.098	0.029 – 0.030	0.10 – 0.11	0.031 – 0.032	0.075 – 0.076	0.022 – 0.023	0.068 – 0.069

Table 6. Estimated sum of T2 and HT2 chronic exposures from the 2015 retail survey in infants and young children aged 4 to 60 months (µg/kg bw/day)

	4 to <6 month-olds (n = 20)		6 to <9 month-olds (n = 273)		9 to <12 month-olds (n = 390)		12 to <15 month-olds (n = 404)		15 to <18 month-olds (n = 371)		18 to 24 month-olds (n = 63)		24 to 60 month-olds (n = 390)	
	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile	Mean	97.5th percentile
Sum of T2 and HT2	0.011	0.029 – 0.030	0.014	0.051 – 0.052	0.014	0.059 – 0.060	0.014	0.050 – 0.051	0.013	0.062 – 0.063	0.012 – 0.013	0.042 – 0.043	0.0099 – 0.010	0.032 – 0.033

Risk characterisation

Acute

51. The sum of T2 and HT2 acute mean and 97.5th percentile exposures are below the EFSA ARfD of 0.3 µg/kg bw and are therefore not a health concern. The margin of exposure would be even higher if the model-averaged BMD had been used to calculate an ARfD.

Chronic

52. All chronic mean exposures are below the EFSA TDI of 0.02 µg/kg bw and are not a health concern.

53. The chronic 97.5th percentile exposures range from 145 – 315% of the EFSA TDI. However, the survey data were taken following a harvest year when levels of T2 and HT2 were reported by industry to be elevated in the oat crop and hence reflecting an atypically high exposure scenario. Levels in previous surveys have been below the limit of detection. Therefore, it is likely that the exposures used in this risk assessment are conservative.

54. Whilst an effect on health cannot be entirely excluded it is doubtful that children would be exposed to these levels in normal harvest years. It is therefore unlikely that there will be chronic effects.

Conclusions/Discussion

55. T2 and HT2 are type A trichothecenes and are produced by a variety of *Fusarium* and other fungal species. *Fusarium* species grow and invade crops and produce the T2 and HT2 under cool, moist conditions prior to harvest. T2 and HT2 are found predominantly in cereal grains (particularly oats) and their products. NEO is a hydrolytic phase I metabolite of T2 and may be formed in fungi and mammals. NEO has been found in some brewed coffee samples, in a sample of cereal-containing baby food and at trace levels in some barley field malt samples.

56. There is very little information on the *in vivo* absorption of T2 and HT2 in animals after oral administration. T2 is rapidly absorbed after direct administration into the small intestine and is extensively hydrolysed to HT2 and other metabolites. It is rapidly distributed to the liver, kidney and other organs without accumulation. Excretion is also rapid. The metabolism of T2 and HT2 in humans and animals is complex and phase I and phase II metabolites are produced. No data have been identified for the toxicokinetics of NEO.

57. The toxicity of T2 and HT2 was reviewed by EFSA in 2011. Since the 2011 evaluation, a number of acute and subacute toxicity studies had been published, focussing predominantly on the anorectic effects of the toxins at low doses (mink, pig and mouse). Subchronic toxicity studies published since 2011 had investigated similar endpoints to those used by EFSA in its 2011

evaluation for establishing an HBGV. They tended to be of longer duration than the pig studies used but confirmed the immunotoxicity and haematotoxicity of T2 and HT2.

58. Prior to 2017, HBGVs had been established for T2 and HT2 by JECFA, SCF and EFSA. In their 2017 Opinion, EFSA established a group ARfD of 0.3 µg/kg bw for T2, HT2 and NEO and a group TDI of 0.02 µg/kg bw for T2 (x1), HT2 (x1) and NEO (x0.3).

59. As levels of NEO were below the LOD in all samples of wheat, maize, oat and rye-based products analysed in two UK surveys, no exposure assessment was performed for this metabolite.

60. Acute and chronic exposures were calculated for the sum of T2 and HT2 using occurrence data from a retail survey of oat-based products commissioned by the FSA in 2015 and consumption data from NDNS and DNSIYC. Exposures in 0 to 4-month old infants are negligible as infants in this age range are unlikely to consume solid foods, including oat based products. Mean and 97.5th percentile acute exposures ranged from 0.022 – 0.032 and 0.056 – 0.11 µg/kg bw, respectively. These were all below the ARfD of 0.3 µg/kg bw and are therefore not of toxicological concern.

61. Mean and 97.5th percentile chronic exposures were calculated and ranged from 0.0099 – 0.014 and 0.029 – 0.063 µg/kg bw/day, respectively. All the mean exposures were below the TDI of 0.02 µg/kg bw and are therefore not of toxicological concern. The chronic 97.5th percentile exposures ranged from 145 – 315% of the EFSA TDI. Whilst an effect on health cannot be entirely excluded it is doubtful that children would be regularly exposed to these levels, which were measured in a year in which levels of T2/HT2 in oat grains were particularly high, for a prolonged period. In most years, levels of T2 and HT2 will be much lower than those observed in this harvest. It is therefore unlikely that dietary exposure levels of T2, HT2 or NEO would be of any toxicological concern in infants and young children.

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Abbreviations

5-HT	5-hydroxytryptamine
ARfD	acute reference dose
BMD	benchmark dose
BMDL	95 % lower confidence limit for benchmark dose
BMR	benchmark response
bw	bodyweight
DH	Department of Health
DNA	deoxyribonucleic acid
DNSIYC	Diet and Nutrition Survey in Infants and Young Children
ED_{50}	dose causing emesis in 50 % of animals tested
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
Hb	Haemoglobin
HBGV	health based guidance value
HT2	HT2 toxin
i.p.	intraperitoneal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	kilogram
LB	lower bound
LOAEL	lowest observed adverse effect level
LOEL	lowest observed effect level
LOD	limit of detection
LOQ	limit of quantification
µg	microgram
MCHb	mean corpuscular haemoglobin
MCV	mean corpuscular volume
mg	milligram
NDNS	National Diet and Nutrition Survey
NEO	neosolaniol
NOAEL	no-observed adverse effect level
PCV	packed cell volume
PMTDI	provisional maximum tolerable daily intake
ppm	parts per million
PYY_{3-36}	anorectic peptide pancreatic peptide YY_{3-36}
RNA	ribonucleic acid
RPF	relative potency factor
SCF	Scientific Committee on Food
T2	T2 toxin
TDS	total diet study
TEC	total erythrocyte counts
TLC	total leucocyte counts
TTC	total thrombocyte counts
tTDI	temporary tolerable daily intake
UB	upper bound
UBMD	95 % upper confidence limit for benchmark dose
WHO	World Health Organization

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