HBGV’s established by EFSA - Risk Assessment of T-2 and HT-2 mycotoxins in Food

In this guide

EFSA’s group ARfD

17. The lowest dose at which acute effects were seen was in mink in a study by Wu et al. (2016) with an ED50 of 1030 µg/kg bw after oral exposure. Mink were used, in lieu of ferrets (which are more expensive and difficult to raise), and have been suggested as the model species for emesis in drug testing (Gordon, 1985; Zhang et al., 2006; Percie du Sert et al., 2012) and EFSA therefore concluded that the mink was an appropriate animal model for vomiting in humans (EFSA, 2017a).

18. In the study conducted by Wu *et al.* (2016), groups of fasted female mink (n = 4) were given 50 g of feed 30 minutes prior to either a) 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 (positive control), or b) 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 for six hours (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).

19. In a second study by Wu *et al.* (2016), 3 groups of fasted female mink (n = 4) were given 50 g of feed 30 minutes prior to the administration of 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 the levels of the plasma satiety hormone peptide YY3-36 (PYY3-36) and 5-hydroxytryptamine (5-HT) (hormones known to be implicated in emesis) were measured. The lowest dose at which emetic events were observed after i.p. administration was 0.05 mg/kg bw for T2/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/HT2. At 0.25 mg/kg bw 4 animals (100%) were affected for both T2/HT2 via i.p. and oral administration. The lowest doses at which emetic events occurred in animals treated 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/HT2 caused increases in plasma concentrations of PYY3-36 and 5-HT. The study authors concluded that via the oral route the NOAELs were 5 µg/kg bw, LOAELs were 50 µg/kg bw and ED50s were 20 µg/kg bw for both T2/HT2.

20. EFSA used the Wu *et al.* (2016) study for their benchmark dose (BMD) analysis (using PROAST software version 38.9) as the basis for an ARfD and selected a benchmark response of 10%. EFSA combined the results from 2 independent experiments on T2/HT2 and the experiments were considered as a covariate.

21. Until recently, performing model averaging using the PROAST software was not possible. The overall BMDL - BMDU range therefore was 2.97 – 49.8 µg/kg bw (when considering all models with AIC ≤ AICmin +2) and, following their own guidance (EFSA, 2017b), EFSA selected a BMDL10 of 2.97 µg/kg bw for further consideration, as this was the lowest valid BMDL10. 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.

22. An uncertainty factor of 10 for intraspecies variability was applied to the BMDL10 of 2.97 µg/kg bw derived for emetic response in mink, resulting in a

group ARfD of 0.3 µg/kg bw for T2/HT2. No interspecies uncertainty factor was applied because humans were not considered more sensitive than mink to acute emetic effects. This was based on observations with emetine (an ipecacuanha alkaloid), which induces vomiting in humans and minks at the same effective dose. Hence, it was assumed that this would also be the case for T2/HT2. Dose additivity of T2/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.

23. In 2018, the COT accepted the group ARfD for T2/HT2 established by EFSA, with the following caveats:

i. The AIC values for all the models, except the Two-stage model, fell within the EFSA acceptance criterion (AIC ≤ AICmin +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.

24. 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 BMDL10 of 12.2 µg/kg bw, approximately 4-fold greater than the BMDL10 used by EFSA to establish the ARfD. The COT was uncertain as to the current validation status of the model averaging function of the PROAST software. Hence, the Committee continued to apply the ARfD established by EFSA, acknowledging that given the uncertainties of model averaging the EFSA ARfD was more conservative.

EFSA's group TDI

25. In 2011, EFSA performed a BMD analysis on the specific antibody response (anti-horse globulin) detected in studies conducted by Rafai *et al.* (1995a), and Rafai *et al.* (1995b), using the PROAST software (version 26.0 under R 2.10.2) (EFSA, 2011b). 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/HT2. Details on these studies can be found in TOX/2023/04.

26. EFSA used the BMDL05 of 10 µg/kg bw/day for T2 toxin as a point of departure and applied the default uncertainty factor of 100 to establish a TDI of 100 ng/kg bw for the sum of T2/HT2. (NB. the default value for continuous data recommended by EFSA is a benchmark response of 5%; the BMDL05 is the 95% lower confidence limit for the benchmark dose response of 5%).

27. Since 2011 however, several subacute and subchronic toxicity studies on T2 have been published, including a 90-day rat study conducted by Rahman *et al.* (2014).

28. In the 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) (equivalent to 0, 45, 68 and 90 µg T2/kg bw/day, respectively) daily via the diet for 12 weeks. Eight animals each were sacrificed at 2, 4, 6, 8, 10 and 12 weeks. Rats dosed with T2 toxin showed varying degrees of adverse clinical signs, including dullness, weakness, lethargy, growth retardation, reduced feed intake, reluctance to move and rough hair coat, which worsened over time in groups 68 or 90 µg/kg bw/day. Rats treated with 90 µg/kg bw/day showed gangrenous dermatitis of tail (15/24) at 8^{th} week, and facial and podal dermatitis after the 10^{th} week. 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, respectively. Significant decreases in haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total thrombocytes count (TTC), total leucocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular Hb (MCHb), and percentages of lymphocytes were observed but the percentage of neutrophils increased. Generally, all of these observations became more pronounced with study length, with no sign of reaching a plateau at the end. After 90 days of feeding mean TECs were 8.97, 5.85, 5.77 and 4.65 x106 /µl in rats fed 0, 45, 68 and 90 µg/kg bw/day, respectively; mean TLCs were 14.83, 8.95, 6.92 and 5.20 x103 /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 x103 /µl in animals fed 0, 45, 68 and 90 µg/kg bw/day. The study authors concluded that T2 induces microcytic hypochromic anaemia, leukocytopaenia (due to lymphocytopaenia) and thrombocytopaenia in rats which increased with dose and duration of exposure.

29. 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 similar to those seen in the pig study, confirming the immune system

and blood cell production as targets of T2 across species. EFSA also noted that the exposure duration to T2 in the study of Rahman et al. (90 days) was longer than in the Rafai *et al.* (1995a, b) studies in pigs - not only in absolute terms, but also as a proportion of species lifetime.

30. Therefore, EFSA decided, considering the longer exposure duration in the study by Rahman *et al.* (2014) and its biological relevance, to apply the changes in total leucocyte counts reported by Rahman *et al.* (2014) for the derivation of a new BMD for T2. EFSA did not identify a NOAEL, but considered the lowest dose tested (45 µg T2/kg bw/day) to be a LOAEL (EFSA, 2017a). EFSA used a benchmark response (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%).

31. The overall BMDL - BMDU range was 3.30 - 27.60 µg/kg bw (when considering all models with AIC ≤ AICmin +2). A 95% lower confidence limit for the benchmark dose response (BMDL10) of 3.3 µg T2/kg bw was used as a reference point for establishing a chronic TDI for T2/HT2 as it was the lowest valid BMDL10.

32. 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 by the end. EFSA thus established a TDI of 0.02 µg T2/kg bw.

33. Based on HT2’s similar acute toxicity profile and potency, structural similarity to T2 (and the fact that HT2 is an immediate metabolite of T2), and in agreement with their previous assessment in 2011, EFSA concluded that T2/HT2 should be included in a group TDI with the same potency.

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

35. 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. 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 relative potency factors (RPFs) calculated for T2, HT2 and NEO were 1, 1 and 0.3, respectively (EFSA, 2017a).

36. EFSA noted that the test compound in the study used to determine the group TDI for T2, HT2, and NEO was purified from fungal culture material and its purity was not specified; therefore, EFSA could not exclude the possibility that minor amounts of other mycotoxins (including modified forms) were present. Furthermore, EFSA noted there was 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.