WHO FOOD ADDITIVES SERIES: 84

Prepared by the ninety-third meeting of the
Joint FAO/WHO Expert Committee
on Food Additives (JECFA)

Safety evaluation of certain food contaminants

Food and Agriculture Organization of the United Nations

World Health Organization

WHO FOOD ADDITIVES SERIES: 84

Prepared by the ninety-third meeting of the
Joint FAO/WHO Expert Committee
on Food Additives (JECFA)

Safety evaluation of certain food contaminants

The summaries and evaluations contained in this book are, in most cases, based on unpublished proprietary data submitted for the purpose of the JECFA assessment. A registration authority should not grant a registration on the basis of an evaluation unless it has first received authorization for such use from the owner who submitted the data for JECFA review or has received the data on which the summaries are based, either from the owner of the data or from a second party that has obtained permission from the owner of the data for this purpose.

World Health Organization, Geneva, 2024

Food and Agriculture Organization of the United Nations

World Health Organization

Safety evaluation of certain food contaminants: prepared by the ninety-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

(WHO Food Additives Series, No. 84)

ISBN (WHO) 978-92-4-009252-5 (electronic version)
ISBN (WHO) 978-92-4-009253-2 (print version)
ISBN (FAO) 978-92-5-138760-3
ISSN 0300-0923

Suggested citation. Safety evaluation of certain food contaminants: prepared by the ninety-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2024 (WHO Food Additives Series, No. 84). Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at https://iris.who.int/.

Sales, rights and licensing. To purchase WHO publications, see https://www.who.int/publications/book-orders. To submit requests for commercial use and queries on rights and licensing, see https://www.who.int/about/policies/publishing/copyright.

Contents

PREFACE

The monograph contained in this volume was prepared following the ninety-third meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met virtually online from 24 March–1 April 2022. This monograph summarizes the data on the contaminant group trichothecenes T-2 and HT-2 toxins reviewed by the Committee. A monograph on the other features of this contaminant group, which were discussed at a previous meeting in 2001, are published in WHO Food Additives Series 47.

The report of the ninety-third meeting of JECFA has been published by WHO as WHO Technical Report No. 1040. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and dietary exposure and toxicological monographs, such as that contained in this volume, on substances that they have considered.

The monograph contained in this volume is based on a working paper that was prepared by JECFA experts. A special acknowledgement is given at the beginning of the monograph to those who prepared this working paper. The monograph was edited by S. Kaplan, Bern, Switzerland.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned. All experts participating in the ninety-third meeting completed declaration of interest forms.

Any comments or new information on the biological or toxicological properties of or dietary exposure to the compounds evaluated in this publication should be addressed to: WHO Joint Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

Trichothecenes T-2 and HT-2 toxins (addendum)

First draft prepared by
Abdul Afghan,[1] Yiannis Kiparissis,[1] Antonio Agudo,[2] Ellen F. Kirrane[3] and Matt Wheeler[4]

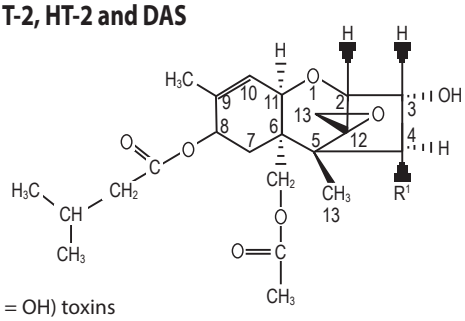
[1] Food Contaminant Toxicology Assessment Section, Bureau of Chemical Safety, Health Products and Food Branch Health Canada/Government of Canada, Canada
[2] Unit of Nutrition and Cancer, Catalan Institute of Oncology, Barcelona, Spain
[3] US Environmental Protection Agency's Center for Public Health and Environmental Assessment, Research Triangle Park, NC, United States of America
[4] NIH/NIEHS Biostatistics and Computational Biology Branch, Research Triangle Park, NC, United States of America

1. Explanation

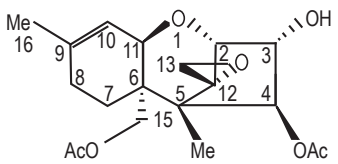
T-2 toxin (T-2) and HT-2 toxin (HT-2) are type A trichothecene mycotoxins, which are structurally related epoxy sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 in a wide range of foodstuffs but they are primarily contaminants of cereals and cereal-based products. T-2 and HT-2 have been reported to be produced by *Fusarium acuminatum*, *F. equiseti*, *F. langsethiae*, *F. poae*, *F. sibiricum* and *F. sporotrichioides*.

T-2 is the trivial name for 4β,15-diacetoxy-3α,dihydroxy-8α-[3-methylbutyryl-oxy]-12,13-epoxytrichothec-9-ene (CAS number 26934-87-2). HT-2 is the trivial name for 15-acetoxy-3α,4β-dihydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytrichothec-9-ene (CAS number 21259-20-1). The structures of T-2 and its metabolite HT-2 differ only in the functional group at the C-4 position (Fig. 1). HT-2 is formed from the deacetylation of T-2, which can occur as a result of metabolism in the fungus, the infected plant or in animals after ingestion. These toxins co-occur with several other type A trichothecenes (for example, 4,15-diacetoxyscirpenol (DAS) and neosolaniol) and modified mycotoxins – phase I and II metabolites formed in the fungus or the infected plant (for example, T-2 triol and T-2-3-glucoside).

Fig. 1
Chemical structures of T-2, HT-2 and DAS



T-2 (R_1 = OAc) and HT-2 (R_1 = OH) toxins



4,15-DAS

T-2 and HT-2 were previously evaluated by the Committee at its fifty-sixth meeting (Annex 1, reference *152*). The Committee concluded at that meeting that there was substantial evidence for the immunotoxicity and haematotoxicity of T-2 in several species, and that these are critical effects after short-term intake. The Committee further concluded that the safety of food contaminated with T-2 could be evaluated from the lowest observed adverse effect level (LOAEL)[1] of 29 µg/kg bw per day for changes in white and red blood cell counts identified in the 3-week dietary study in pigs. The Committee used this LOAEL and a safety factor of 500 to derive a provisional maximum tolerable daily intake (PMTDI)[2] for T-2 of 60 ng/kg bw. The Committee further concluded that the toxic effects of T-2 and its metabolite HT-2 could not be differentiated, and hence HT-2 was included in the PMTDI, resulting in a group PMTDI of 60 ng/kg bw per day for combined concentration of T-2 and HT-2. At its eighty-third meeting in 2016, the Committee included DAS in the group PMTDI of 60 ng/kg bw per day for T-2 and HT-2 (Annex 1, reference *233*).

In response to a request from the Codex Committee on Contaminants in Foods (CCCF) for an updated evaluation, including an exposure assessment on T-2 and HT-2, these compounds were evaluated by the present Committee. At the ninetieth JECFA meeting (Annex 1, reference *251*), information published since 2001 on T-2 and HT-2 concerning analytical methods, sampling, effect of processing, prevention and control, occurrence in food commodities and dietary exposure was reviewed.

For this evaluation, previous assessments (monographs) completed by JECFA, the Scientific Committee on Food (SCF) and the European Food Safety Authority (EFSA), and national and regional governmental authorities were identified. This was followed by a comprehensive literature search to identify any critical new data for the assessment of human health risk. The cut-off dates for inclusion in this report were 1 January 2000 to 24 December 2021.

Numerous publications on the toxicity of T-2 and HT-2 in poultry have become available since the Committee's previous evaluation in 2001 (Annex 1, reference *152*). Considering the acknowledged physiological differences between poultry and humans (Wigley, 2017; WHO/IPCS, 2020) and the overall availability

[1] Prior to the sixty-eighth meeting of the Committee (Annex 1, reference *187*), a NOAEL would have been termed a no-observed-effect level (NOEL) and a LOAEL would have been termed a lowest-observed-effect level (LOEL).

[2] "Historically, JECFA has used the term 'provisional', as there is often a paucity of reliable data on the consequences of human exposure at low levels, and new data may result in a change to the tolerable level. However, as any HBGV [health-based guidance value] would be revisited if new data indicated the need for a change, and as the word maximum is redundant, it is recommended that the terms 'provisional' and 'maximum' no longer be used – that is, using only the terms TDI, tolerable weekly intake (TWI) and tolerable monthly intake (TMI), as appropriate. Tolerable intake values are expressed as an amount (often in micrograms) per kilogram of body weight, as a single value and not a range, and normally using only one significant figure" (WHO/IPCS, 2020).

of information on other more relevant experimental models (for example, mice, rats, rabbits and pigs), the following summaries of biochemical and toxicological aspects of T-2 and HT-2 do not include information from experiments in poultry.

2. Biological data

2.1 Biochemical aspects

In 2001, the Committee concluded that T-2 is readily metabolized by mammalian gut microflora to several metabolites (Annex 1, reference *153*). HT-2 is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2. Metabolism continues in the liver (with biliary excretion), resulting in a substantial, combined first-pass effect. Metabolites of T-2 include HT-2, 3′-hydroxy-HT-2, 3′-hydroxy-T-2, T-2 tetraol, de-epoxy 3′-hydroxy-T-2 triol, de-epoxy 3′-hydroxy-HT-2 and 3′-hydroxy-T-2 triol. Glucuronide conjugates are also formed extensively in most species (with the exception of cats). T-2 and its metabolites are eliminated rapidly. In rats, more than 95% of a radioactively labelled oral T-2 dose of 0.15 mg/kg bw per day was excreted within 72 hours. In the same study, a dose of 0.6 mg/kg bw per day was eliminated more slowly, suggesting potentially saturable metabolism or elimination pathways at doses that are relevant to the studies of toxicity considered in this evaluation. The predominant route of excretion following oral exposure was the faeces followed by the urine (80:20, faeces:urine).

2.1.1 Absorption, distribution and excretion

Various in vitro experiments investigating the gastrointestinal digestion (pre-absorption) of T-2 and HT-2 have been conducted. The results of these studies are summarized below, followed by a summary of the literature specific to the absorption, distribution and excretion of T-2 and HT-2 in experimental animals.

(a) Gastrointestinal metabolism

In an in vitro human digestion model, De Angelis et al. (2014) investigated the stability and bio-accessibility of T-2 and HT-2 using naturally and artificially contaminated bread samples. Concentrations of T-2 and HT-2 in the naturally contaminated bread samples were 47 and 893 µg/kg, respectively. After baking, concentrations of T-2 and HT-2 in the artificially contaminated bread samples were 104 and 629 µg/kg, respectively. Notably the T-2 and HT-2 concentrations in the artificially contaminated bread samples were significantly different after baking, from the concentration used for spiking (for example, 522 µg/kg of T-2

and HT-2 were added during dough mixing), likely due to the conversion of T-2 to HT-2 by the carboxylesterases present in the yeast during breadmaking. The in vitro digestion protocol used in this assay mimics the biochemical conditions in the upper gastrointestinal tract of human adults including, (a) chewing (37 °C for 15 minutes), (b) gastric digestion (37 °C for 120 minutes) and (c) duodenal digestion (37 °C for 180 minutes). The bread samples undergoing simulated digestion were analysed using liquid chromatography–mass spectrometry (LC-MS) at various time points during the procedure. The HT-2 concentration just before gastric digestion was not significantly different from that recorded at the end of the duodenal phase. In contrast, T-2 concentrations decreased by approximately 54 to 70% during gastric digestion but did not change significantly during the duodenal phase. The authors also noted that the amount of HT-2 mono-glucoside in the naturally contaminated bread decreased significantly from the beginning of the gastric stage to the end of the duodenal stage, suggesting hydrolysis of HT-2 mono-glucoside.

Using a similar protocol to that described by De Angelis et al. (2014), Monaci et al. (2015) also investigated the in vitro digestion of bread naturally contaminated with low, medium and high concentrations of T-2 and HT-2. T-2 and HT-2 were quantified using high-performance liquid chromatography–mass spectrometry (HPLC-MS) at 0, 120 (gastric phase) and 180 (duodenal phase) minutes after the respective incubations. At time 0, T-2/HT-2 concentrations in the bread were approximately 31/0, 33/291 and 52/811 ng/mL in the samples with low, medium and high concentrations, respectively. Following the gastric phase (120 minutes), the concentrations were 31/0, 33/176 and 50/654 ng/mL in the samples of bread with low, medium and high concentrations, respectively. Following the duodenal phase (180 minutes), T-2 concentrations were significantly lower in all samples: i.e., 0, 0 and 11 in the samples with low, medium and high contaminant concentrations respectively. Conversely, the HT-2 concentrations increased from 176 ng/mL to 273 ng/mL following the gastric phase in the samples with the medium level of contamination. The concentrations of HT-2 in the samples with low and high T-2 and HT-2 concentrations did not appear to change significantly.

McCormick et al. (2015) investigated the digestion of T-2, T-2 α-glucoside and T-2 β-glucoside in the upper gastrointestinal tract using artificial saliva, gastric juices and duodenal juices and bile. Following stepwise incubation (5 minutes with saliva, 2 hours with gastric juices, and 2 hours with duodenal juices and bile), the digest was analysed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). A colonic fermentation assay was also performed by incubating 500 µg/L of T-2, T-2 α-glucoside or T-2 β-glucoside with microbiota from cultured faeces of healthy and non-smoker donors for 30 minutes and 24 hours. T-2 α- and β-glucosides were reported to be unchanged after incubation with artificial human saliva (results for T-2 and other incubation

intervals were not reported). Following 30 minutes of incubation with microbiota from the faeces of healthy donors, T-2 α- and β-glucosides were unchanged, and T-2 was minimally converted to HT-2; however, after 24 hours, approximately 80% of the T-2 had been converted to HT-2 and less than 30% of the T-2 α- and β-glucosides remained. T-2 β-glucoside was mainly transformed into T-2 (58%) by the microbiota whereas most of the T-2 α-glucoside was transformed into HT-2 (30%), and other metabolites of unknown structure (33%). Similarly, Kasimir et al. (2020) showed in an ex vivo pig caecum model that T-2 and HT-2 glucosides, incubated in active caecal suspensions for 10 to 1440 minutes, are hydrolysed to T-2 and HT-2, and subsequently metabolized to HT-2 and T-2 triol, respectively. Samples were analysed using LC-MS/MS.

In a similar experiment by Gratz et al. (2017), T-2-3-α,D-glucoside, T-2 and HT-2 were individually incubated in triplicate in a shaking water bath in the following order: 1) 5 minutes with artificial saliva; 2) 2 hours in artificial gastric juice; and 3) 2 hours in sodium bicarbonate ($NaHCO_3$), duodenal juice and bile juice. Samples were analysed using LC-MS/MS. The results showed that T-2-3-α,D-glucoside and HT-2 were stable following incubation with artificial digestive juices from the upper gastrointestinal tract, whereas T-2 was partially transformed into HT-2. Gratz et al. (2017) also investigated the fate of T-2-3-α,D-glucoside following incubation with human microbiota from the faeces of five donors, and found that T-2-3-α,D-glucoside was gradually transformed into T-2 and HT-2. Hydrolysis of 2-3-α,D-glucoside to T-2 by human microbiota reached approximately 30 to 50% of the total glucoside added before deacetylation of T-2 to HT-2 was observed. After 6 hours of incubation, only a small proportion of the 2-3-α,D-glucoside was metabolized; however, by the end of the 72-hour incubation period, approximately 90% of the 2-3-α,D-glucoside was converted to T-2 and HT-2. Gratz et al. (2017) also suggested that T-2-3-α,D-glucoside is not transported through a Caco-2/TC7 cell epithelial monolayer, whereas T-2 and HT-2 are efficiently transported through the monolayer. Daud et al. (2020a) reported that HT-2-β-glucoside (HT-2-Glc) was stable in conditions mimicking those of the upper gastrointestinal tract whereas in faecal cultures, HT-2-Glc was hydrolysed efficiently, and no further microbial biotransformation of HT-2 was observed. Specifically, Daud et al. (2020b) demonstrated that HT-2-Glc is hydrolysed by *Butyrivibrio fibrisolvens*, *Roseburia intestinalis* and *Eubacterium rectale* (human gut bacteria) following 48 hours of incubation.

Wu Q et al. (2012) showed that T-2 is transformed into HT-2 following incubation with the caecal chyme of pigs. The caecal samples were isolated from freshly slaughtered pigs and incubated with T-2 for 20 minutes to 24 hours. T-2 did not appear to affect the viability of the caecal bacteria, based on the bacterial degradation of a positive control substance (quercetin). Caecal samples were analysed using high-performance liquid chromatography/electrospray

ionization tandem mass spectrometry (HPLC-ESI-MS/MS). Following 72 hours of incubation of T-2 with microbial isolates from the small and large intestines of chickens, Young et al. (2007) reported that monodeacetylation to form HT-2 was the only reaction observed. By contrast, incubation of HT-2 and T-2 triol with the microbial isolates from chicken intestines resulted primarily in de-epoxidation.

(b) Mice

Tanaka et al. (2016) reported lactational transfer in a preliminary study in mice. Pregnant mice were exposed to dietary concentrations of 0 or 12 mg/kg diet T-2 during gestational day (GD) 6 to postnatal day (PND) 21 (for further study details see section 2.2.5(a)). The concentration of T-2 in milk recovered from the stomachs of the offspring on PND 14 (three animals per group) were determined by HPLC-MS. T-2 concentrations in the milk from the stomachs of the offspring were below the detection limit (0.05 mg/L) in controls and 0.08 mg/L in milk from the stomachs of the offspring of treated dams (raw data not reported).

(c) Rat

Male and female Sprague-Dawley rats were exposed to 1 mg/kg bw HT-2 intravenously (one female and two males) or via gavage (two females and one male). Following a 7-day washout period, the route of exposure in the rats was switched (the rats initially receiving HT-2 via gavage were subsequently administered HT-2 via intravenous injection and vice versa). Blood samples were analysed using ultra-high-performance liquid chromatography–mass spectrometry (UPLC-MS) and samples were taken 5, 10, 15, 30, 45, 60, 90, 120, 240 and 360 minutes after dosing. Following intravenous administration, the maximum concentration (C_{max}) of HT-2 was 80.55 ng/mL, the elimination half-life of HT-2 was 10.5 ± 0.6 minutes, the area under the plasma concentration–time curve ($AUC_{0 \text{ to } \infty}$) was 1077.2 ± 195.1 min·ng/mL, and total body clearance was 943.8 ± 141.0 mL/min per kg. Following administration by gavage, HT-2 was not detected in plasma; however, a hydroxylated metabolite (3′-OH-HT-2) appeared at 5 minutes and gradually decreased over the remaining time (Yang S et al., 2018).

Male Wistar rats (7–9 weeks of age; six per group) were exposed to normal feed or feed containing 20 mg/kg diet T-2 for 14 days. At the end of the exposure period, the rats were killed, and portions of the liver and kidney of each animal were collected and analysed using high-performance thin-layer chromatography. T-2 was not detected in any of the liver and kidney samples. The limit of quantification in the liver and kidney was 100 µg/kg (Chandratre et al., 2014).

Male and female Sprague-Dawley rats (three per sex per group), aged 3 weeks, were administered single bolus doses of either 0 or 1 mg/kg bw T-2 via gavage and killed 8 hours later. The thighbone, knee joints, costal cartilage, skeletal muscle, heart, liver and kidneys were analysed for T-2, HT-2, neosolaniol (NEO), and T-2 triol using HPLC-MS/MS (Yu et al., 2017). T-2 and HT-2 were detected in various tissues with concentrations of HT-2 detected being higher than those of T-2. T-2 concentrations were highest in the thighbone and lowest in the liver (thighbone > costal cartilage > skeletal muscle > knee joints > heart > kidney > liver). HT-2 concentrations were also highest in the thighbone but lowest in the kidney (thighbone > knee joints > costal cartilage > liver > skeletal muscle > heart > kidney). NEO and T-2 triol were not detected in any of the tissues sampled. Yu et al. (2017) estimated that 68.2 to 90.7% of T-2 was converted into HT-2 in the various tissues analysed.

(d) Pig

Seven cross-bred pigs (Duroc × Landrace × Yorkshire), weighing 6.8 ± 0.5 kg, were exposed to a single bolus dose of 0.5 mg/kg bw T-2 via intravenous injection. Blood samples were taken 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120, 240 and 360 minutes after administration and T-2, HT-2, T-2 triol and T-2 tetraol concentrations in the blood were measured using LC-MS/MS. The concentrations of T-2, HT-2 and T-2 triol decreased rapidly in a biphasic manner. No T-2 tetraol was detected. The C_{max} values for T-2, HT-2 and T-2 triol were 1558 ± 298.4, 76.51 ± 11.43, 771.1 ± 182.4 ng/mL, respectively; and elimination half-life values of 11.7 ± 0.79, 37.7 ± 2.44, 25.6 ± 2.9 minutes, respectively, were calculated (Sun et al., 2012).

In a follow-up study, groups of pigs were exposed to single bolus doses of 0.5 or 1 mg/kg bw T-2 via intravenous injection, and the concentrations of T-2, HT-2 and T-2 triol were quantified in plasma (n = 6), tissue (n = 12), urine and faeces (n = 7) using LC-MS/MS methods. To analyse plasma and tissue distribution, groups of pigs were exposed to 1 mg/kg bw T-2; whereas for analysis of urine and faeces, pigs were exposed to 0.5 mg/kg bw T-2. For the plasma study, samples of blood were taken 0, 3, 6, 10, 15, 20, 30 and 45 minutes as well as 1, 1.5, 2 and 3 hours after dosing. For the tissue distribution study, pigs were killed (three per time interval) 0.5, 1, 3 and 6 hours after dosing and samples were collected from the heart, liver, spleen, lungs, kidneys, stomach, small intestines, muscles, brain and fat. For the excretion study, pigs were placed in metabolic cages and separate samples of urine and faeces were collected after 0–4, 4–8, 8–12, 12–24 and 24–36 hours, as well as 1.5–2, 2–3, 3–4 and 4–6 days after dosing. According to the plasma study, the C_{max} for T-2, HT-2 and T-2 triol were 2736 ± 236.3, 208.1 ± 25.3 and 116.3 ± 14.5 ng/mL, respectively. The terminal elimination half-

lives of T-2, HT-2 and T-2 triol were 34.63 ± 2.95, 73.79 ± 5.53 and 159 ± 13.4 minutes, respectively. The reason for the longer terminal elimination half-life in pigs that received 1 mg/kg bw T-2 compared to those previously reported at a dose of 0.5 mg/kg bw T-2 (Sun et al., 2012) is unknown. Tissue concentrations of T-2, HT-2 and T-2 triol were all measured at the 0.5 hours interval, suggesting that T-2 is rapidly metabolized to HT-2 and T-2 triol, and widely distributed. T-2 concentrations rapidly decreased, and 6 hours after dosing T-2 was not detected in any organ. The highest T-2 concentration was measured in the fat tissue, followed by the lung. T-2 concentrations in the brain were the lowest. The highest HT-2 concentrations were observed in the liver followed by the kidney and small intestines with relatively minimal amounts in the brain. T-2 triol was similarly distributed but at lower concentrations. Although T-2, HT-2 and T-2 triol were detectable in the urine, the authors estimated that less than 7% of the administered dose was excreted as T-2, HT-2 or T-2 triol in the urine. Relatively low HT-2 concentrations were measured in the faeces, and T-2 and T-2 triol concentrations in the faeces were below the limit of detection (Sun et al., 2014).

2.1.2 Biotransformation

Several reviews concerning the metabolism of T-2 have been published since the evaluation by the Committee in 2001 (Annex 1 reference *153*).

(a) In vitro

Cultured hepatocytes, and subcellular fractions from the livers of rats, chickens, pigs and fish were incubated with T-2 and analysed using liquid chromatography coupled with time-of-flight and ion trap mass spectrometry. Additionally, T-2 and HT-2 were incubated with recombinant pig cytochrome P450 (CYP) 3A29. Following incubation of T-2 with the liver microsomes, HT-2, NEO, 3′-OH-T-2, and 3′-OH-HT-2 were detected in rats, pigs and chickens, whereas T-2 triol was only detected in rats. Much lower levels of T-2 metabolism were seen in the cultured hepatocytes than in the liver microsomes. Recombinant pig CYP3A29 converted T-2 and HT-2 predominantly to 3′-OH-T-2 and 3′-OH-HT-2, respectively (Wu Q et al., 2011).

The liver microsomes of rats, chickens, pigs, goats, cows and humans were incubated with HT-2 (concentrations not reported) and analysed using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF). The metabolism of HT-2 was also investigated in recombinant human cytochrome enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) and carboxylesterases (CES I and CES II). In total, eight phase I (i.e. 4-de-Ac neosolaniol, 4-acetoxy T-2 tetraol, 3′-OH-HT-2, 4′-OH-HT-2, 4′-OH-HT-2, 7-OH-HT-2, 7-OH-HT-2 and 10-OH-HT-2) and three phase II (i.e. 3-GlcA

HT-2, 4-GlcA HT-2 and 3-GlcA-4-de-Ac NEO) metabolites were identified. The authors reported that hydroxylation, hydrolysis and glucuronidation are the major routes for HT-2 metabolism. Only slight differences in the phase I and phase II metabolism between species were noted (for example the position of hydrolysis, hydroxylation and glucuronidation). Although CYP3A4 appears to be the predominant enzyme for phase I reactions, carboxylesterase metabolism of HT-2 is relatively minor (Yang S et al., 2018).

In an effort to establish T-2 and HT-2 glucuronide standards, Welsch & Humpf (2012) incubated T-2 in pig liver microsomes, in the presence of 5′-diphosphoglucuronic acid, for 2 hours at 37 °C. The glucuronides were directly quantified using LC-MS/MS. T-2 was rapidly hydrolysed to HT-2 and both T-2 and HT-2 were glucuronidated. To investigate interspecies differences in glucuronidation, Welsch & Humpf (2012) incubated T-2 and HT-2 with microsomes from rat, mouse, pig and human livers. T-2 was rapidly hydrolysed by all liver microsomes except for those of mice, where hydrolysis was less pronounced; however, glucuronidation in mice microsomes was significantly greater than in other species. Comparable results were obtained when the microsomes were incubated with HT-2.

Pooled human liver microsomes were incubated with T-2 or HT-2 for 1 hour at 37 °C and analysed using liquid chromatography with high-resolution accurate mass spectrometry. Hydrolysis and oxidation were identified as the main phase I metabolic pathways for T-2 and HT-2. Both T-2 and HT-2 underwent phase II conjugation via glucuronidation (Slobodchikova et al., 2019).

From their study using human liver microsomes, recombinant CYPs (i.e. CYP2C19, CYP3A4, CYP2C9, CYP2A6, CYP2B6, CYP2D6, CYP1A2, CYP2C8 and CYP2E1) and various enzyme inhibitors, Lin et al. (2015) suggested that the metabolism of T-2 is predominantly due to carboxylesterases and that the extent of T-2 metabolism in CYP enzymes occurred in the following order: CYP3A4 > CYP2E1 > CYP1A2 > CYP2B6, CYP2D6 or CYP2C19. T-2 and HT-2 concentrations were analysed using HPLC-TQ MS.

Ge et al. (2010) showed that recombinant pig CYP3A22 (a homologue of the human CYP3A4) converts T-2 into 3′-OH-T-2 and HT-2 into 3′-OH-HT-2. The recombinant CYP3A22 was incubated with T-2 at 37 °C for 3 hours. Samples were analysed using HPLC. Ge et al. (2010) also showed that T-2 exposure induced CYP3A22 activity in primary piglet hepatocytes following incubation at 37 °C for 48 hours.

Yuan Y et al. (2013) demonstrated that T-2 shows a high affinity for recombinant chicken CYP3A37 with a Km of 15.3 μM. Following incubation at 37 °C for 30 minutes, T-2 was metabolized to 3′OH-T-2. Samples were analysed using high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF/MS).

Following incubation of T-2 with human colon carcinoma (HT-29) and human renal proximal tubule epithelial cells (RPTEC) for 48 hours, HT-2, NEO, 3′-hydroxy-T-2, 4-deacetylneosolaniol, T-2 glucuronide and HT-2 glucuronide were identified using HPLC coupled with Fourier transform mass spectrometry. T-2-triol was also identified following incubation with HT-29 cells but not with RPTEC. In both cell lines, HT-2 and NEO were the major metabolites identified (Weidner et al., 2012).

(b) In vivo

Yang S et al. (2013) exposed male and female Wistar rats to a single dose of 0 or 3 mg/kg bw T-2 (3 per sex in the control group and 12 per sex in the treated group) via gavage. Samples of urine and faeces were collected 0 to 24 hours after dosing. Liver S9 was isolated from untreated male and female rats (three per sex) and incubated with 10 mmol/L of T-2 for 2 hours. Samples were analysed using ultraperformance liquid chromatography–quadrupole/time-of-flight tandem mass spectrometry (UPLC-Q/TOF-MS) for the parent compound and metabolites. A total of 19 metabolites were found in vivo and in vitro, and the main metabolic pathways of T-2 were hydrolysis, hydroxylation and de-epoxidation. Higher concentrations of metabolites were found in the urine than in the faeces and 3′-OH-HT-2 was the metabolite with the highest concentration in male and female rats, whereas the metabolites with the lowest concentrations were 5-deacetyl-T-2 and 15-deacetylneosolaniol. Differences in the position of hydroxylation were noted between sexes (for example, hydroxylation at C7 of T-2 was not observed in female rats) and some metabolites produced in vivo were not produced in vitro (for example, 15-deacetyl-T-2 and 3′-OH-15-deacetyl-T-2 were not detected in vitro).

Kravchenko et al. (2001) investigated the effects of an indole-enriched diet on the toxicity of T-2 in male and female Wistar rats. The indole-enriched diets significantly increased phase I (for example, CYP, hydroxylase, hydrolase and carboxyesterase) and phase II (for example, UDP-glucuronyl transferase and glutathione (GSH) transferase) enzyme activities in the liver and small intestinal mucosa of rats. Following exposure to 0.8 mg/kg bw T-2, the indole-enriched diet attenuated T-2-induced effects on body weight (decreased), organ weights (increased relative liver weight), and histopathology (for example, lipid infiltration of the liver and hypoplasia of the lymphoid tissue).

Yang S et al. (2017) investigated the metabolism of T-2 glucuronide in vitro and in vivo. The in vivo metabolism of T-2 glucuronide (following a single gavage dose of 0.5 mg/kg bw) was investigated using fasted male and female Wistar rats. The in vitro investigations included incubation of T-2 glucuronide with pooled rat and human liver microsomes, and with human

and rat faeces. Following exposure to 0.5 mg/kg bw T-2 glucuronide via gavage, T-2 glucuronide, 3-OH-T-2 glucuronide and HT-2 were detected in the urine, whereas T-2 glucuronide and T-2 were detected in the faeces. T-2 glucuronide was predominant in the urine whereas the T-2 concentration was significantly higher in the faeces 0 to 12 hours after exposure. Twelve hours after exposure, T-2 glucuronide and its metabolites were not detected. Following 120 minutes of incubation with human liver microsomes, five T-2 glucuronide metabolites were detected: HT-2 glucuronide, NEO glucuronide, 3´OH-T-2 glucuronide, 4´-OH-T-2 glucuronide and its isomer. A similar pattern was observed in rat liver microsomes; however, NEO glucuronide was not detected. In liver microsomes, most of the T-2 glucuronide remained unchanged; however, the predominant metabolite in the rat and human liver microsomes was 3´OH-T-2 glucuronide. Following incubation (under anaerobic conditions) with human faeces, T-2, HT-2 and NEO were detected, whereas only T-2 and HT-2 were detected in rat faeces. In both the rat and human faeces, the primary T-2 glucuronide metabolite was HT-2. These results suggest that T-2 glucuronide undergoes hydroxylation, hydrolysis and deconjugation reactions and complete excretion within 12 hours of oral exposure.

2.1.3 Effects on enzymes and other biochemical parameters

(a) In vitro

Using the CYP probe substrates midazolam and tolbutamide, T-2 strongly inhibited CYP3A (non-competitive) and moderately inhibited CYP2C (competitive) following incubation with the microsomal proteins isolated from the livers of conventional pigs (hybrid sow × Piétrain boars, 12 weeks of age, eight boars and eight sows). Inhibitor constants (Ki) of 27.0 ± 3.97 μM and 14.8 ± 1.45 μM, were calculated for CYP3A (midazolam) and CYP2C (tolbutamide), respectively (Schelstraete, Devreese & Croubels, 2019).

The effect of T-2 on the expression of CYP1A4, CYP1A5, CYP2C18, CYP2H1, CYP2C45, CYP2D49, CYP3A37 and CYP3A80 mRNA was investigated in chicken embryonic hepatocytes using real-time polymerase chain reaction (qPCR). Shang, Jiang & Deng (2013) found that the expression of CYP1A4 and CYP1A5 mRNA was significantly increased following T-2 exposure. Similarly, the RNA and protein levels of CYP3A22 (a homologue of the human CYP3A4) were measured in Chinese piglet hepatocytes using real-time qPCR and Western blot. Liu et al. (2016) and Ge et al. (2010) found that the expression of CYP3A22 was significantly increased following T-2 exposure. Wang et al. (2011) suggested that T-2 induces CYP3A45 and CYP3A39, carboxylesterases and epoxy hydrolase.

From a study using chicken epithelial cells (LMH cells), Liu et al. (2019) suggested that T-2 induces the expression of CYP1A5 at both the mRNA and

protein levels via the aryl hydrocarbon receptor (AhR), which can be strongly inhibited by both resveratrol and siRNA. Similarly, Jiang et al. (2021) showed that T-2, HT-2, NEO, T-2 triol and T-2 tetraol induce the expression of CYP1A4 in both LMH cells and chicken primary hepatocytes via AhR activation. Alternatively, using human Hep G2 cells, Ye et al. (2019) suggested that CYP1A1 expression is induced via the NRF1 and Sp1 pathways instead of via the AhR. Kruber et al. (2011) found that T-2 exposure induced upregulation of CYP1A1 mRNA expression and led to mitogen-activated protein kinase (MAPK)/p38-dependent interleukin-8 (IL-8) secretion in a human intestinal epithelial cell line (caco-2). Whereas CYP1A1 induction was inhibited by resveratrol (AhR antagonist), secretion of IL-8 was not.

Faisal et al. (2020) investigated the binding affinity of T-2 with human serum albumin (HSA) using circular dichroism and UV spectroscopy. No significant interaction with HSA was observed and, furthermore, T-2 did not affect the interaction of warfarin and naproxen with HSA as demonstrated using ultrafiltration experiments. Consistent with these observations, HSA and fetal bovine serum did not have any effect on the cytotoxicity of T-2 in HepG2 cells.

(b) In vivo

Rabbit

Guerre et al. (2000) investigated the effect of T-2 on total microsomal liver CYP content and the expression of specific CYP in male New Zealand white rabbits following gavage exposure to 0, 0.10, 0.25 or 0.50 mg/kg bw per day T-2 for five consecutive days. Three out of five animals that received 0.50 mg/kg bw per day died, and the effects on the expression of CYP enzymes were limited to the animals dosed with 0.10 and 0.25 mg/kg bw per day. Although no effects on CYP were observed in animals dosed with 0.10 mg/kg bw per day, those dosed with 0.25 mg/kg bw per day showed decreased total CYP content and decreased enzyme activity. Notably, at 0.25 mg/kg bw per day, moderate signs of systemic toxicity were apparent (decreased body weight, reduced spontaneous movements, excessive salivation and peribuccal necrosis). The purity of the T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided.

Pigs

Schelstraete, Devreese & Croubels (2019) investigated the impact of T-2 exposure on the metabolism of midazolam in pigs (hybrid sow × Piétrain boars; 7 weeks of age). The purity of the T-2 used in this study was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Groups of four pigs per sex per group were administered 0

or 500 µg T-2 per day in feed for 2 weeks. Midazolam (0.036 mg/kg bw) was then administered intravenously, and blood samples were taken after 0, 5, 10, 20, 30 and 40 minutes, as well as 1, 1.5, 2, 3, 4, 6, 8 and 10 hours after midazolam injection. After a 48-hour washout period, the animals also received a single oral dose (0.15 mg/kg bw) of midazolam and blood samples were taken at the same sampling intervals. According to the authors, midazolam was chosen because it undergoes substantial first-pass metabolism in humans with equal contributions from the intestine and liver. The kinetic parameters following intravenous exposure to midazolam did not differ significantly between the control and treated groups; however, differences in the kinetic parameters for midazolam were observed between control and treated animals following oral administration. For example, pigs receiving T-2 tended to have a higher average C_{max}, elimination rate constant and absorption rate constant than control values, but similar area under the curve from zero to infinity ($AUC_{(0-\infty)}$) estimates. However, the authors hypothesized that the observed trends are potentially related to the cytotoxic effect of T-2 rather than any specific effect on biotransformation.

Goossens et al. (2012a; 2013a) investigated the effects of T-2 exposure on the absorption of oral antibiotics (i.e. doxycycline and paromomycin, and chlorotetracycline) in pigs. The purity of T-2 was not reported, and the basal feed did not contain any detectable T-2; however, the feed did contain 479 ± 140 µg/kg diet deoxynivalenol (DON) and 44 ± 13 µg/kg diet zearalenone (ZEA). Goossens et al. (2012a) exposed four groups of six animals to either 1) a control feed; 2) T-2-contaminated feed at 99 ± 13 µg/kg diet; 3) T-2-contaminated feed at 111 ± 4 µg/kg diet, supplemented with a mycotoxin binder (glucomannan); or 4) control feed supplemented with a mycotoxin binder, for 7 consecutive days. After 7 days of exposure, doxycycline and paromomycin were administered orally and the plasma concentrations of the antibiotics were determined. No significant difference in the plasma concentrations of paromomycin were observed; however, animals treated with T-2 plus the binder showed significantly higher AUC of doxycycline compared to controls (i.e. 43 961 (±7982) versus 22 653 (±16 275) hour.ng/mL). In a longer term study, Goossens et al. (2013a) investigated the effects of 21 days of T-2 exposure on the absorption of chlorotetracycline in pigs. Similarly, four groups of six pigs were exposed to a control feed (1); feed contaminated with 111 µg/kg diet T-2 (2); feed contaminated with 111 µg/kg diet T-2, supplemented with a yeast-derived feed additive (3); or control feed supplemented with a yeast-derived feed additive (4), for 21 consecutive days. After 21 days of exposure, the chlorotetracycline was administered orally and the plasma concentrations of the antibiotic were determined. Both groups fed T-2 showed higher AUCs (11.18 ± 1.93 (2) and 13.34 ± 2.66 (3) versus 6.0 ± 0.71 hour.µg/mL) and C_{max} (1.52 ± 0.39 (2) and 1.80 ± 0.42 (3) versus 0.74 ± 0.06 µg/mL) values than animals exposed to the blank control feed. The authors

suggested that these effects may be due to decreased integrity of the intestinal epithelial barrier, citing Goossens et al. (2012b) who showed increased passage of doxycycline and paromomycin across a monolayer of intestinal porcine epithelial cells derived from the jejunum (IPEC-J2) cells at cytotoxic concentrations of T-2.

Goossens et al. (2013b) investigated the effects of dietary T-2 exposure on the CYP3A enzymatic activity in the liver of 9-week-old Landrace pigs by monitoring the metabolism of midazolam, a substrate for CYP3A enzymes. The purity of T-2 was not reported, and the basal feed did not contain any detectable T-2; however, the feed did contain 69 ± 20 µg/kg diet DON and 5.8 ± 2.2 µg/kg diet 3-acetyldeoxynivalenol. The pigs were exposed to feed containing approximately 0 or 0.903 mg/kg diet T-2 for 14 days. A significant decrease in hepatic CYP3A activity (as measured by midazolam metabolism) was observed in the treated group. The addition of a glucomannan mycotoxin binder (2000 mg/kg diet) did not appear to attenuate these effects.

Meissonnier et al. (2008) investigated the effects of T-2 exposure on CYP1A-related activities in pigs. Following exposure to feed containing 540, 1324 or 2102 µg/kg diet T-2 for 28 days, reduced CYP1A-related activities and certain *N*-demethylase activities were observed in animals exposed to 2120 µg/kg diet. Further details on this study are discussed in section 2.2.2(d).

2.2 Toxicological studies

The focus for many of the toxicological studies summarized in the following sections was the remedial effects of feed additives on T-2-induced toxicity and on sublethal non-traditional mechanistic-related end-points (for example, measures of apoptosis or oxidative stress in various tissues). This review focuses only on the aspects pertaining to T-2- or HT-2-induced toxicity. For many of the studies, no information on the purity of the test material or the characterization of background concentrations of mycotoxins in basal feed, including T-2 and HT-2, was provided.

2.2.1 Acute toxicity

In 2001, the Committee summarized the available information on the acute toxicity of T-2 in rats, mice, rabbits, guinea-pigs, pigs and chickens, and on the acute toxicity of HT-2 in rats, mice and chickens (Annex 1 reference *153*). Acute oral median lethal dose (LD_{50}) values for T-2 ranged from 1.84 mg/kg bw in day-old chicks to 10 mg/kg bw in mice. Information on only one acute oral toxicity test was available for HT-2 and the LD_{50} was 7.2 mg/kg bw in day-old broiler chicks. The previous Committee (Annex 1 reference *153*) concluded that species, strain and sex-specific differences in toxicity were observed. In particular, they

drew attention to the increased sensitivity of cats, due to their demonstrated deficiencies in conjugation reactions. Clinical signs of toxicity reported included necrosis, scarring and sloughing of the skin following dermal exposure, and necrosis in the gastrointestinal tract following oral and intravenous exposure. Histopathological effects noted following acute exposure include: necrotic lesions in the adrenal glands, spleen, heart, mesenteric lymph nodes (MLN), pancreas, bone marrow, liver, kidneys and meninges. The following is a summary of the results of studies on the acute oral toxicity of T-2 and HT-2 published since the Committee's earlier evaluation (Annex 1 reference *153*). Additional specialized studies investigating the effects of acute exposure to T-2 or HT-2 are included in section 2.2.6.

The acute oral toxicity of T-2 was investigated by McKean et al. (2006) who exposed male Fischer 344 rats (five per group) to 0, 1.0, 2.15, 4.64 or 10 mg/kg bw T-2 via gavage. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. All animals exposed to 4.64 and 10 mg/kg bw died within 24 hours. Animals that died showed feed refusal and diarrhoea shortly after exposure. Surviving animals exposed to 2.15 mg/kg bw showed feed refusal and bloody faeces. Animals exposed to 1 mg/kg bw showed no apparent toxic symptoms. An LD_{50} of 3.71 mg/kg bw was calculated based on these results.

Jacevic et al. (2009) investigated the effects of various adsorbents on the acute toxic effects of T-2 in Wistar rats (sex not specified; eight per group). Exposure to a single oral dose of 1.67 mg/kg bw T-2 induced a significant decrease in body weight, feed and water consumption during the 28-day observation period. No information on the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Administration of polymeric glucomannan adsorbent (Mycosorb®) and modified natural zeolite (Min-a-zelPlus®) immediately after T-2 exposure attenuated its effects.

Wan et al. (2015a) examined the toxic effects of acute oral exposure to T-2 using metabonomic techniques. Female Wistar rats (12 per group) were exposed to 0, 0.5, 2.5 or 4 mg/kg bw T-2 via gavage. No information on the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Seven days after exposure, all rats were killed, and the metabolic profiles of body fluids and multiple organs were determined. During the 7-day post-dosing period, all animals were lethargic and exhibited dry ruffled fur 8 hours post-dosing. Rats given the high dose also exhibited diarrhoea and exudative dermatitis around the mouth and nose; five of these rats died during the 7-day observation period. Body weight gain was significantly diminished in all treated groups in a dose-responsive fashion. Clinical chemistry revealed significantly increased alkaline phosphatase (ALP) in the animals given the high dose. Other significant effects on clinical biochemistry were noted by the authors; however, these effects did not

show a dose-dependent pattern. Histopathological examination revealed basal cell hyperplasia in the gastric mucosa and steatosis of the liver of the rats given the high dose. Metabonomic analysis revealed changes in the urine of the animals in the low- and medium-dose groups, whereas rats in the high-dose group exhibited alterations in urine, liver, spleen, stomach and thymus. Specifically, these changes included alterations in the levels of membrane metabolites, tricarboxylic acid cycle intermediates, a range of amino acids, nucleosides and nucleotides. The authors suggested that, based on a significant depletion of amino acids in the spleen, spleen function was impaired at the high dose.

Atroshi et al. (2000) investigated the effects of antioxidants, tamoxifen, vitamin E and selenium exposure on T-2-induced hepatotoxicity in male rats (strain not specified; 12 per group). Animals were exposed to 0 or 2.8 mg/kg bw T-2 via oral gavage and killed 24 hours after dosing. No information on the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. The livers were removed from the animals and analysed for protein concentration and activities of the following liver enzymes: aspartate aminotransferase (AST), alanine transaminase (ALT), ALP and gamma glutamyl transferase (GGT). Exposure to 2.8 mg/kg bw T-2 significantly increased the concentration of AST, ALT, ALP and GGT and decreased the concentration of protein (24%) in the liver tissue compared to controls. Dietary supplementation with vitamin E and selenium, or tamoxifen 8 weeks before dosing with T-2 attenuated the severity of the effects on liver enzymes.

Jaćević et al. (2020) investigated the effects of exposure to various adsorbents on the acute oral toxicity of T-2 in Wistar rats (sex not specified). Groups (10 per group) of rats were exposed to a single oral dose of 0 or 1.67 mg/kg bw T-2 via gavage and killed 1, 7, 14, 21 or 28 days after exposure. The T-2 used in this experiment was isolated from *F. sporotrichoides* fungi (ITM-391; purity not reported). No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. T-2-treated animals exhibited vomiting, emesis, feed refusal, diarrhoea, decreased body surface temperature, lethargy and weakness 2 to 4 days following exposure. Histopathological examination of the gastric tissue 7 days following exposure revealed segmental degeneration, moderate haemorrhagic foci and collections of neutrophils, macrophages and mast cells in the tunica submucosa. By 28 days postexposure, the intensity of the histopathological lesions was increased, with a prominent lack of epithelial layer, submucosal oedema, reduced gastric pits, cystically altered gastric glands and a large collection of inflammatory cells. The administration of absorbents had variable attenuating effects on the toxicity of T-2.

Lu et al. (2021) investigated the cardiotoxic effects of T-2 in male Wistar rats. Groups of rats (five per group) were exposed to 0 or 2 mg/kg bw T-2 via a single gavage exposure and killed 7 days later. The purity of T-2 used in this

experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Blood samples were taken at the time of sacrifice and several haematological and clinical chemistry parameters were assessed, and various histopathological methods were used to examine the cardiac tissue. A significant decrease in weight gain and a concurrent decrease in plasma growth hormone were observed in treated animals compared to controls. Haematological examination revealed significantly increased thrombocyte counts, whereas clinical chemistry showed increased creatine kinase and lactate dehydrogenase concentrations. Electrocardiography results suggested myocardial damage in treated animals (i.e. exaggerated amplitude of the R wave, appearance of a pathological Q wave, elevated ST segment and a longer QTc interval). Ultrastructural alterations in the hearts of treated animals were also observed, namely, a decreased number of mitochondria and an increased incidence of damaged mitochondria. Furthermore, histopathology of the heart revealed fibrosis, which manifested as a large amount of fibrous tissue hyperplasia and an increased percentage of cardiac collagen in treated animals compared to controls. Immunohistochemical assays on the cardiac tissue suggested that T-2 significantly increased the expression of genes related to fibrosis (for example, TGF-β1, p-smad2/3 and PPAR-γ).

Chattopadhyay et al. (2013) investigated the haematotoxic effects of T-2 on Sprague-Dawley rats (sex not specified). The purity of T-2 used in this experiment was not reported. Groups (six per dose) of rats were injected intraperitoneally with 0.5 mL of sterile water (control) or 0.001 g/L of T-2. Blood was then drawn from a tail vein puncture immediately after exposure as well as at 3, 6 and 24 hours after exposure, and subsequently analysed. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Exposure to T-2 resulted in significant reductions in most of the parameters measured (for example, white blood cell count, red blood cell count (RBC), haemoglobin and haematocrit) compared to concurrent control values 24 hours after exposure.

2.2.2 Short-term studies of toxicity

The following sections summarize the results of studies published since the previous evaluation of T-2 and HT-2 (Annex 1, reference *153*). During the literature search, the results of several short-term toxicity studies investigating the mechanism of Kashin-Beck disease (KBD) were noted. Specifically, in an effort to study the etiology of the disease, an experimental animal model involving a combination of a low-nutrient diet and T-2 exposure has been developed (Kang, Perveen & Li, 2013). The available information indicates that in the presence of selenium deficiency in rats, T-2 is capable of eliciting significant chondronecrosis

at doses as low as 100 μg/kg bw per day (Guan et al., 2013). Due to the nature of the nutrient-deficient feed used in these experiments and the specificity of the effects being measured, these studies were not considered suitable for hazard characterization and are not extensively reviewed here.

(a) Mice

Hou et al. (2021) investigated T-2-induced liver and kidney toxicity in 8-week-old C57BL/6 N male mice following 5 consecutive days of exposure to 0 or 5 mg/kg bw T-2. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. T-2 exposure induced a slight decrease in body weight gain. Serum ALT, AST, creatinine and urea concentrations appeared slightly elevated but were statistically comparable to control concentrations. Histopathological examination revealed slight hepatocellular oedema and mild steatosis in the kidneys of treated animals.

Zhang X et al. (2021) investigated the renal effects of T-2 exposure in male Kunming mice (12 per group), which were exposed to 0, 500, 1000 or 2000 μg/kg bw per day T-2, via gavage, for 28 consecutive days. The test material had a purity of ≥99.8%. The animals were 6 weeks old at the start of the study. At the end of the 28-day treatment period, the mice were sacrificed, and blood samples collected. Gross and histopathological examination focused on the kidney and various indicators of oxidative stress (for example, superoxide dismutase (SOD), CAT, malondialdehyde (MDA) and GSH) and apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining and gene expression). Clinical signs and feed consumption were not reported; however, statistically significant reductions in body weight were noted in animals in the mid- and high-dose groups (no numerical values cited). No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Biochemical analysis revealed significantly increased blood urea nitrogen (BUN) and serum β2-microglobulin in all treated groups. Serum cystatin C and creatinine were also increased in animals in the mid- and high-dose group. Relative kidney weights were significantly increased in all treated groups (no numerical values cited). Compared to controls, histopathological examination of the kidneys from treated animals revealed degeneration of the renal tubule epithelium in all dose groups. The severity of renal tubule epithelium degeneration increased with dose and was accompanied by renal tubulointerstitial fibrosis, reduced glomerular volume and dilation of the Bowman's space in animals in the medium and high-dose groups. Indicators of apoptosis (for example, an increased percentage of TUNEL-positive nuclei in animals in the medium and high-dose groups; increased caspase-3 and -9 activities in all treated mice; and increased Bax and Bcl-2 mRNA and protein expression in the medium and high-dose groups) suggests that the mechanism for renal tubular degeneration in T-2-treated mice

is apoptosis. Indicators of oxidative stress (i.e. increased MDA, decreased GSH content, decreased SOD activity, decreased catalase (CAT) activity, and the mRNA expression of Nrf2, and its downstream target genes (HO-1, SOD1 and CAT)), were also significantly altered in the kidneys of animals in the medium and high-dose groups compared to controls. In a follow-up study, Zhang X et al. (2022) investigated the protective effects of selenium supplementation on T-2-induced renal effects. Following 28 days of exposure to 1000 µg/kg bw per day T-2 via gavage, selenium supplementation attenuated T-2-induced decreased body weight and increased relative kidney weights. Additionally, selenium supplementation significantly attenuated T-2-induced renal histopathology (i.e. destruction of renal tubular structure, detachment of renal tubular nuclei and increased incidence of apoptosis), effects on biochemistry (i.e. BUN and serum β2-microglobulin) and reactive oxygen species (ROS) production. Furthermore, Zhang X et al. (2022) suggested that selenium supplementation attenuated renal cell apoptosis by blocking the mitochondrial pathway in T-2-treated mice (for example, decreasing the protein expression of cytochrome C and the activities of caspase-3/9, as well as regulating the protein and mRNA expressions of Bax and Bcl-2). No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided.

(b) Rats

Chandratre et al. (2014) exposed male Wistar rats to T-2 in an effort to quantify the distribution of T-2 to various organs. Groups of six male rats (aged approximately 7–9 weeks) were exposed to feed containing 0 or 20 mg/kg diet T-2 for 14 consecutive days. The T-2 used in this experiment was manufactured from cultured wheat or maize powder. The basal feed did not contain detectable concentrations of aflatoxin, ochratoxin or T-2. Feed consumption was measured daily, clinical observations of general health were conducted twice daily, and body weights were measured weekly. At the end of the 14-day observation period, animals were killed, blood samples taken, organs weighed, and tissue samples preserved for histopathology. Additionally, indicators of oxidative stress (SOD, CAT and MDA) were measured in the serum and liver. Treated animals exhibited anorexia, lethargy, hunched posture and diarrhoea from day 7 onwards. Following 14 days of exposure, the authors reported a dramatic decrease in body weight (>70%) in treated animals. The decreases in body weights were accompanied by significantly decreased feed consumption (~46% after 14 days). According to the body weight and feed intake values reported, the animals were exposed to approximately 1.4 mg/kg bw per day during the first week of the study followed by a dramatic drop-off during the second week. No deaths were reported. Haematological examination revealed significant decreases

in total leukocyte count, lymphocytes, haemoglobin, total erythrocyte count, packed cell volume and total thrombocyte count. Clinical biochemistry revealed significantly decreased total protein, albumin and glucose whereas creatinine, AST and ALT were significantly increased. Histopathological examination of the spleen and thymus of treated animals showed decreased lymphocytes compared to controls. Increased proliferation of reticuloendothelial cells in the red pulp of the spleen and haemorrhages and oedema in the cortical area of the thymus were also observed. The kidneys of treated animals showed diffuse haemorrhages, glomerular atrophy and moderate to severe degeneration of the epithelium in the proximal convoluted tubules. The livers from treated animals showed hepatocyte degeneration, mild bile duct hyperplasia and portal oedema. The testes of treated animals showed degeneration of spermatogonial cells, severely decreased numbers of spermatids and decreased spermatogenic activity in the seminiferous tubules. An associated decrease in the numbers of spermatozoa was observed in the epididymal ductules. Histopathological lesions were also apparent in the gastrointestinal tract of treated animals (lesions in the forestomach, glandular stomach and small intestine) including increased presence of apoptotic cells in the Peyer's patches in the proximal portion of the ileum. Indicators of oxidative stress were apparent in the serum and liver: for example, significant increases were seen in the concentrations of MDA and decreases in the activity of CAT and SOD in treated animals compared with controls. T-2 was not detected in the liver or kidneys of treated animals.

Yao et al. (2010) suggested that a low-nutrition diet aggravates the adverse effects on bone elicited by exposure to a high dose of T-2. Male and female Wistar rats (15 per sex per group) were exposed to 0 or 1000 μg/kg bw per day T-2 via gavage (5 days per week) either with or without a low-nutrition diet (low in protein, lipids, multivitamins and selenium) for 4 weeks. The purity of T-2 used in this experiment was 99%. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Clinical observations were restricted to hair lustre, activity, size and weight of the rats. Histopathological observations were focused on the effect on the bones of the distal femur and the proximal tibia. Rats exposed to T-2 alone appeared similar to controls until week 4 when lacklustre hair, lower activity levels, loose stools and decreased body weight were reported. Similar effects were reported in the group that received T-2 plus the low-nutrition diet; however, the severity of reaction and incidence of effect increased, while the latency decreased. By the fourth week of exposure, the treated animals showed a 16% decrease in body weight compared to controls whereas the group that received T-2 plus the low-nutrition diet showed a 27% decrease in body weight compared to controls. Two deaths were reported in each of the treated and T-2 plus low-nutrition diet groups. Histopathological examination of bone from the treated group revealed focal necrosis and hypertrophy in 2/10 animals after 2 weeks of exposure, which increased to 5/8 after 4 weeks. The arrangement of proliferative cell columns in the bone marrow of affected animals was irregular, i.e. cell columns appeared shorter and sparser than

in controls. Masson staining of collagen in the bone marrow was pale, relatively sparse and disappeared in the necrotic zones of treated animals. Administration of a low-nutrition diet aggravated the histopathological lesions induced by T-2 exposure.

Rahman et al. (2014, 2016, 2021) investigated the effects of dietary T-2 exposure on the blood, renal and immune systems of male Wistar rats. Groups (48 per dose) of 4-week-old rats were exposed to 0, 0.5, 0.75 or 1.0 mg/kg diet T-2 for up to 12 weeks. Eight animals per dose group were killed following 2, 4, 6, 8, 10 and 12 weeks of exposure. The T-2 used in this experiment was produced on sterile maize and wheat. T-2 concentrations in the feed were quantified using thin-layer chromatography and spectrophotometry, and confirmed using enzyme-linked immunosorbent assay (ELISA). Rahman et al. (2014, 2016, 2021) reported that the basal feed did not contain detectable concentrations of aflatoxin B1, ochratoxin A, citrinin or T-2. Clinical observations of general health were conducted twice daily, and body weights were measured weekly. At terminal sacrifice, blood samples were collected and analysed for various haematology parameters. These included haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), total thrombocyte count (TTC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (Rahman et al., 2014). Subsequent histopathological examination of the kidneys was also conducted and additional analyses of the blood samples, including BUN, creatinine and oxidative stress parameters such as MDA and GSH content, as well as the activities of CAT and SOD were reported (Rahman et al., 2016). In a very similar study by the same authors, which used the same experimental design, the humoral immune response was evaluated using the haemagglutination test and the periodic (weeks 4, 8 and 12) measurement of serum immunoglobulins IgG, IgM and IgA (Rahman et al., 2021). Cell-mediated immune responses were also evaluated by using real-time PCR to detect mRNA expression levels of selected cytokines such as interleukin-2 (IL-2), interferon gamma (IFN-γ), interleukin 4 (IL-4) and interleukin-10 (IL-10), and by the delayed-type hypersensitivity (DTH) test and the lymphocyte stimulation tests (Rahman et al., 2021). Table 1 summarizes selected statistically significant dose-responsive effects that were observed in the studies by Rahman et al. (2014, 2016, 2021) following 12 weeks of exposure to T-2.

Thirteen treated animals died during the study and a dose-responsive increase in mortality was observed: 6.25, 8.33 and 12.00% in the low- medium- and high-dose groups, respectively. A dose-responsive incidence of clinical signs of toxicity was also observed in the treated animals and included dullness, weakness, lethargy and rough coat. Animals in the high-dose group also showed gangrenous dermatitis of the tail and facial podal dermatitis near the end of the study. The appearance of clinical signs started in the sixth, seventh and eighth

Table 1
Summary of selected effects of T-2 exposure in rats reported by Rahman et al. (2014, 2016, 2021) at terminal sacrifice (12 weeks)

	Dietary concentrations of T-2 (mg/kg diet)			
Effect	**0**	**0.50**	**0.75**	**1.00**
Terminal body weight (g)	264.6 ± 9.31	219.0 ± 11.77*	183.85 ± 12.23*	159.95 ± 12.44*
Total erythrocyte count ($\times 10^6$/µL)	8.97 ± 0.06	5.85 ± 0.06*	5.77 ± 0.015*	4.65 ± 0.21*
Total leukocyte count ($\times 10^3$/µL)	14.83 ± 0.73	8.95 ± 0.36*	6.92 ± 0.83*	5.20 ± 0.73*
Total thrombocyte count ($\times 10^3$/µL)	122.50 ± 7.52	77.67 ± 1.80*	56.50 ± 8.79*	38.00 ± 4.07*
Creatinine (mg/dL)	0.45 ± 0.05	0.67 ± 0.08*	1.05 ± 0.06*	1.33 ± 0.07*
Blood urea nitrogen (mg/dL)	11.51 ± 0.56	20.58 ± 1.92*	23.46 ± 2.37*	25.59 ± 3.37*
Relative kidney (% bw)	0.795 ± 0.104	1.057 ± 0.051*	1.241 ± 0.127*	1.759 ± 0.142*
Relative thymus	0.260±0.058	0.115 ± 0.005*	0.089 ± 0.020*	0.053 ± 0.034*
Relative spleen	0.502 ± 0.087	0.233 ± 0.076*	0.183 ± 0.044*	0.107 ± 0.004*
Kidney malondialdehyde (nM MDA/g)	33.49 ± 6.67	68.3 ± 6.50*	102.40 ± 6.37*	129.67 ± 18.81*
Anti-sheep red blood cell antibody titre (at day 70; $\log_2$/0.05 mL)	4.75 ± 0.25	3.75 ± 0.25*	2.25 ± 0.25*	1.50 ± 0.29*
Delayed-type hypersensitivity test skin thickness (mm) at 48 hours	0.973 ± 0.008	0.773 ± 0.021*	0.613 ± 0.025*	0.437 ± 0.031*
Concanavalin A lymphocyte stimulation test (stimulation index; %)	1.044 ± 0.017	0.698 ± 0.003*	0.452 ± 0.069*	0.310 ± 0.004*
IgG (µg/mL)	1033.0 ± 41.3	956.3 ± 20.0*	876.0 ± 27.7*	831.7 ± 22.4*
IgM (µg/mL)	312.2 ± 6.7	252.1 ± 3.9*	236.788 ± 1.2*	206.2 ± 4.0*
IgA (µg/mL)	14.4 ± 0.6	10.2 ± 0.3*	8.975 ± 0.1*	7.7 ± 0.3*

Means presented ± standard error; *$P<0.05$.

weeks for the high-, medium- and low-dose groups, respectively. From week 2 onwards, treated animals showed a dose-responsive reduction in body weights. By week 4 the mean body weights of all treated groups were significantly lower than those of controls. At terminal sacrifice, the percentage reductions in body weight compared to controls were 17, 31 and 40% in the low-, medium- and high-dose group animals, respectively (Rahman et al., 2014). Rahman et al. (2014) also reported that a dose-responsive decrease in feed intake was observed but the data were not presented in the manuscript. In the absence of feed intake values, the doses achieved in this study are uncertain and approximating the doses based on default conversion factors[1] is likely to overestimate the doses actually received by the treated animals.

Dose-responsive decreases in Hb, PCV, TEC and MCV were observed at terminal sacrifice. Additionally, TLC and TTC showed a dose-responsive decrease

[1] Using the JECFA conversion factor of 0.1 mg/kg bw per day per ppm in the diet for young rats, doses of 0, 50, 75 and 100 µg/kg bw per day could be estimated but would be associated with a high degree of uncertainty.

and were significantly reduced in all treated groups compared to controls. An associated dose-responsive decrease in the percentage of lymphocytes was also observed. All these effects appeared to become more pronounced over time and showed no evidence of plateauing by the end of the 90 days of exposure. No significant changes in basophils, eosinophils or monocytes were reported (Rahman et al., 2014). Although historical control values for animals from this laboratory were not available, the Committee noted that the TLC in the control animals ($14.83 \pm 0.73 \times 10^3/\mu L$) appeared very high compared to the "acceptable range" reported by de Kort et al. (2020) for male Wistar rats of similar age (RccHan™:WIST; 3.2 to $10.5 \times 10^3/\mu l$). The high concurrent control TLC values reported by Rahman et al. (2014) call into question the toxicological significance of the effects on TLC observed in the treated groups. For example, the TLC values observed in all of the treated groups (mean values between 5.2 and $8.95 \times 10^3/\mu L$) are within the "acceptable range" reported by de Kort et al. (2020) for Wistar rats (3.2 to $10.5 \times 10^3/\mu L$). Additionally, the TTC values of all animals (means between 38.0 and $122.5 \times 10^3/\mu L$) appear low compared to the acceptable range reported by de Kort et al. (2020; RccHan™:WIST; 702 to $1534 \times 10^3/\mu L$).

Biochemical changes reported in the treated animals involved time- and dose-responsive increases in creatinine (starting at week 4) and BUN (no other biochemical parameters were investigated). Indicators of oxidative stress (i.e. increased concentration of MDA, decreased concentration of GSH, and decreased activities of SOD and CAT) in the kidneys were also reported. These effects were accompanied by dose-responsive increases in relative kidney weight and histopathological lesions. Specifically, animals from all treated groups showed severe degeneration of the proximal convoluted tubule epithelium following 12 weeks of exposure (Rahman et al., 2016). Similar to the haematological effects noted by Rahman et al. (2014), the renal effects also appeared to become more pronounced over time and showed no evidence of plateauing by the end of the 90 days of exposure.

Following 8 weeks of exposure, the relative spleen and thymus weights of animals in all treated groups were significantly decreased in a dose-responsive manner. Following 12 weeks of exposure, the spleen of treated rats showed time- and dose-dependent depletion of lymphocytes in the follicles. Time- and dose-dependent lymphocytic depletion was also observed in the thymus of all treated groups by week 12. Lesions observed in the Peyer's patches were similar, but only occurred in animals in the medium- and high-dose groups, beginning in the fourth week in the high-dose group and at week 12 for rats in the medium-dose group. Results from the haemagglutination test showed a dose-responsive decrease in response to sheep red blood cells (SRBC). Additionally, the concentrations of serum immunoglobulins IgA, IgG and IgM were significantly decreased in a dose-responsive manner, achieving statistical significance at weeks 8 and 12. In

terms of the cell-mediated immune response, the DTH test was positive, and the lymphocyte stimulation test showed a significant decrease in stimulation index in all treated groups. Furthermore, the percentage of CD4+ and CD8+ lymphocytes was significantly decreased and the expression of cytokines (IL-2, IL-4, IL-10 and IFN-γ) was decreased.

In the WHO/IPCS (2012) guidance for immunotoxicity risk assessment, a "necessary criterion" for demonstrating immunosuppression is a dose-responsive effect "in the absence of generalized overt toxicity". Based on the temporality of effects observed by Rahman et al. (2014, 2016, 2021) in the low-dose group, statistically significant immunological effects were accompanied by increased mortality, decreased body weights and evidence of renal toxicity. For example, statistically significant decreases in body weights were observed in rats in the low-dose group beginning at week 4, accompanied by increased creatinine and mild to moderate histopathological changes in the proximal convoluted tubules of the kidneys. Furthermore, Raut et al. (2013) and Fadhil, Alkutbi & Nassir (2021) reported histopathological lesions in the livers of Wistar rats exposed to dietary concentrations of 0.25 mg/kg diet and 0.47 mg/kg diet, respectively for 90 days (see full study summaries below). Rahman et al. (2021) reported that functional effects on the immune system (for example, con-A-induced stimulation of lymphocytes) were evident by week 4; however, altered histopathology of the spleen and thymus in animals given the low dose did not appear until week 6. A no-observed-adverse-effect level (NOAEL) was not achieved in this study since effects (for example, mortality, decreased body weight, renal toxicity and histopathological lesions in the lymphoid tissues) were observed at the lowest dietary concentration tested.

Raut et al. (2013) examined the subchronic oral toxicity of T-2 by exposing male Wistar rats (10 per group) to T-2 at 0, 0.25, 0.5 or 0.75 mg/kg diet for 90 days. The T-2 used in this experiment was from cultured maize and wheat powder. T-2 concentrations in the cultured maize/wheat powder were quantified using thin-layer chromatography and spectrophotometry. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. The animals were approximately 4 to 5 weeks of age at the start of treatment. Clinical observations of general health were conducted daily, and body weights were measured weekly. At the end of the 90-day treatment period, the rats were killed, and blood samples were collected. Organ weights were recorded, and histopathological examination of the testes, epididymis, liver, kidneys, adrenal glands, spleen, thymus, brain and intestines was conducted. Additionally, measurements of oxidative stress-related biochemical parameters in the testes, liver and kidney were made. Table 2 summarizes selected dose-responsive effects observed in the study by Raut et al. (2013) following 90 days of exposure.

Table 2
Summary of selected effects from a study of subchronic oral toxicity of T-2 in male Wistar rats reported by Raut et al. (2013)

	Dietary concentration of T-2 (mg/kg diet)			
Effect	**0**	**0.25**	**0.50**	**0.75**
Terminal body weight (g)	266.7 ± 7.55	249.3 ± 5.55*	230.10 ± 8.52*	215.16 ± 5.81*
Total leukocyte count (uncertain units)	9.22 ± 0.56	8.86 ± 1.01	8.42 ± 1.16	8.34 ± 1.25
TTC (uncertain units)	817.33 ± 15.31	463.67 ± 29.78*	405.50 ± 24.36*	378.50 ± 13.99*
Total protein (g/dL)	8.87 ± 0.07	8.28 ± 0.11*	7.99 ± 0.11*	6.95 ± 0.03*
Albumin (g/dL)	5.07 ± 0.06	4.62 ± 0.12	4.34 ± 0.18*	3.60 ± 0.07*
Alanine aminotransferase (units/mL)	39.69 ± 0.58	45.07 ± 2.03*	51.93 ± 2.55*	61.56 ± 1.08*
Aspartate aminotransferase (units/mL)	41.43 ± 3.99	42.62 ± 3.98*	52.63 ± 3.49*	57.34 ± 6.75*
Alkaline phosphatase (units/mL)	55.56 ± 2.59	53.44 ± 2.71	37.88 ± 4.69*	33.08 ± 6.45*
Creatinine (mg/dL)	0.48 ± 0.01	0.63 ± 0.09	0.66 ± 0.03	0.93 ± 0.15*
Relative thymus weight	0.119 ± 0.012	0.117 ± 0.008	0.113 ± 0.015*	0.076 ± 0.006*
Relative spleen weight	0.242 ± 0.010	0.241 ± 0.011	0.228 ± 0.005	0.212 ± 0.013
Relative liver weight	3.192 ± 0.031	3.272 ± 0.172	3.552 ± 0.090*	3.697 ± 0.057*
Relative kidney weight	0.357 ± 0.005	0.367 ± 0.004	0.414 ± 0.028*	0.439 ± 0.016*
Liver MDA (nM MDA/g)	24.54 ± 1.68	30.01 ± 1.64*	33.65 ± 1.88*	37.87 ± 1.96*
Kidney MDA (nM MDA/g)	31.2 ± 0.55	37.84 ± 1.05*	38.65 ± 1.72*	39.54 ± 1.71*

TTC, total thrombocyte count; MDA, malondialdehyde; means presented ± standard error; * $P \leq 0.05$; relative organ weights are expressed in units per 100 g bw.

No deaths were reported during the treatment period. Treated animals exhibited mild anorexia, weakness and rough coat. Animals given the high dose had significantly lower body weights than controls from day 49 onwards and animals given the medium dose had significantly lower body weights from day 70 onwards. At study termination, animals given the low, medium and high doses had mean body weights that were approximately 6, 13 and 19% lower than the controls, respectively. Since feed intake was not reported, accurate dose estimates are not possible. Using default conversion factors[1] to convert the dietary concentrations to doses is likely to overestimate the doses actually received by the treated animals. According to the results reported by Raut et al. (2013), a dose-responsive decrease in TTC, which was statistically significant at all doses, was observed. Notably, however, statistically significant reductions in RBC count, haemoglobin and leukocyte counts (TLC) were not observed, which is inconsistent with the results of other studies. Additionally, the units for TLC and TTC parameters are poorly described by Raut et al. (2013) and therefore difficult to compare with published historical control values for Wistar rats (de

[1] Using the JECFA conversion factor of 0.1 mg/kg bw per day per ppm in the diet for young rats, doses of 0, 25, 50 and 75 µg/kg bw per day could be estimated but would be associated with a high degree of uncertainty.

Kort et al., 2020). The changes in haematological parameters were accompanied by significant alterations in biochemical parameters, namely, decreased total protein, albumin, globulin and ALP as well as increased ALT, AST and creatinine at 0.50 or 0.75 mg/kg diet. Statistically significant changes in total protein, ALT and AST were also observed at the lowest concentration of 0.25 mg/kg diet (Raut et al., 2013).

Dose-responsive decreases in absolute and relative thymus and spleen weights, and increased relative liver and kidney weights were also noted, reaching statistical significance at the highest dose. Additionally, a dose-related decrease in absolute testes and an increase in absolute and relative brain weights was observed, reaching statistical significance at the highest dose (relative brain weights were also significantly different from controls in the medium-dose group). Histopathological examination showed depletion of the lymphocytes around the splenic arterioles and in the lymphoid follicles with condensed nuclei in animals in the high-dose group. Similarly, moderate to severe lymphocytic depletion in the follicles of the thymus was observed in animals given the high dose. The livers from animals in the high-dose groups showed degeneration of the hepatocytes and hyperplasia of the bile duct epithelium. The kidneys from these animals showed moderate to severe degeneration of the proximal convoluted tubule epithelium and proteinaceous casts in the distal convoluted tubules. Examination of the testes from the animals given the high dose revealed degeneration of the spermatogonial cells, and smaller seminiferous tubules with decreased numbers of spermatozoa in the epididymal ductules. The heart tissue samples from the animals in the high-dose groups had irregularly thickened myocardial fibres with increased intermyofibral spaces and engorgement of blood capillaries. Finally, the brains from these animals showed mild to moderate meningeal congestion and neuropil degeneration, vascular congestion with increased perivascular space in the cerebrum, and mild to moderate degeneration of the Purkinje cells. Examination of the intestines and adrenal glands did not reveal any treatment-related histopathological lesions. Unfortunately, the incidences of histopathological effects were not reported; however, the authors stated that examination of the animals in the low- and medium-dose groups revealed similar changes to those described above for animals in the high-dose group but of lesser intensity. Dose-related increases in the concentration of MDA and decreased CAT were observed in the liver and kidneys of treated animals, whereas the oxidative parameters in the testes appeared similar to those of controls (Raut et al., 2013).

Fadhil, Alkutbi & Nassir (2021) investigated the subchronic oral toxicity of T-2 by exposing groups of male Wistar rats (14 per group) to 0 or 0.47 mg/kg diet T-2 for 90 consecutive days. The concentration of T-2 in the feed was quantified using ELISA; however, no indication of the background concentration of other mycotoxins in the basal feed was provided. Half of the animals in each

group were killed on day 45 and the other half on day 90. Blood and tissue samples were collected at each sacrifice interval. Blood samples were analysed for clinical biochemistry (i.e. serum protein, ALP, AST and ALT) and oxidative stress parameters (i.e. total oxidant capacity (T-AOC), serum MDA, GSH, GSH-Px, CAT and SOD). Histopathological examinations were limited to the liver and small intestine. Clinical observations, feed and water intake, and body weights were not reported, and accurate dose estimates could not be calculated. Clinical biochemistry tests revealed treatment-related increases in serum ALP, AST and ALT concentrations at 45 and 90 days, with higher concentrations at 90 days than at 45 days. Serum MDA concentrations were increased whereas GSH-Px, SOD and CAT activities were significantly decreased at 45 and 90 days. Similar to the results of the clinical biochemistry tests, the effects appeared greater at 90 days than at 45 days. Although no significant change in GSH was observed at 45 days, GSH concentrations were significantly lower in treated animals than in controls on day 90. Histopathological examination of the livers of treated animals revealed moderate to severe degenerative changes and fatty infiltration, predominantly in the hepatic centrilobular area. The small intestines of treated animals showed catarrhal changes characterized by goblet cell hyperplasia and necrosis of the villous epithelial cells, congestion of the blood vessels with infiltration of mononuclear cells in the lamina propria of the mucosal layer of the intestinal wall. Co-administration of an organic acid mixture (acetic acid, propionic acid, formic acid, phosphoric acid and lactic acid) appears to have partially attenuated some of the T-2-induced effects.

(c) Rabbits

Liu et al. (2020, 2021a, 2021b) and Zhang Z et al. (2022) investigated T-2-induced gastrointestinal tract injury, hepatotoxicity, renal toxicity and immunotoxicity in rabbits. Male New Zealand White rabbits (10 per group) were exposed to 0 or 400 μg/kg bw per day T-2 in olive oil for 5 days via gavage. The purity of the T-2 used in these experiments was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Following the 5 days of exposure, rabbits were killed, and the jejunum, liver and kidney were removed and prepared for further analysis. According to Liu et al. (2020), T-2 exposure significantly decreased the height of the intestinal villi, induced oxidative stress (demonstrated by increased MDA concentrations and decreased SOD, GSH-Px and T-AOC activities in the jejunum), increased expression of inflammatory factors (i.e. IL-1β, IL-6 and tumour necrosis factor (TNF-α)) and decreased the integrity of the intestinal barrier. Liu et al. (2021a) reported that T-2 exposure resulted in increased serum levels of AST, ALT and ALP and oxidative stress (for example using DEH staining to detect

ROS and observing increased concentrations of MDA and decreased activities of SOD, GSH-Px and T-AOC) in the liver of treated animals compared to controls. Histopathological examination of the liver revealed unclear outlines, disordered arrangement, severe cytoplasmic vacuolation, fragmented nuclei and significantly reduced glycogen in the liver cells and hepatic sinusoids. Using TUNEL staining, and qPCR and ELISA to detect gene and protein expression of apoptotic related genes (for example, Bax, Bcl-2, Caspase-3 and Caspase-9), the authors concluded that T-2 treatment induces hepatocyte apoptosis. Liu et al. (2021b) reported that, compared to controls, treated animals showed significantly increased serum creatinine and BUN concentrations, and significantly increased protein in the urine. Histopathological examination of the kidneys revealed luminal congestion, cytoplasmic vacuolization, intratubular cast formation and interstitial oedema in the renal tubules. Additionally, treated animals showed increased accumulation of glycogen compared to controls. Indicators of oxidative stress (for example, increased ROS and MDA and decreased GSH-Px, SOD and T-AOC) and inflammation (for example, increased mRNA expression of IL-1β, IL-6, and TNF-α) were also observed in the kidneys of treated animals. Zhang Z et al. (2022) observed that, compared to control animals, treated animals showed significantly lower concentrations of white blood cells, lymphocytes, neutrophils and monocytes. Histopathological examination of the spleen and thymus revealed degenerative changes (bleeding, reduced thymic cells and inflammatory cell infiltration). The levels of ROS in the spleen and thymus of treated animals were elevated compared to controls. Additionally, indicators of oxidative stress (increased MDA and decreased GSH-Px, SOD and T-AOC) and inflammation (increased protein expression of IL-1β, IL-6 and TNF-α) in the spleen and thymus were significantly altered in treated animals compared to controls. Finally, proliferation (as indicated by proliferating cell nuclear antigen (PCNA) immunohistochemistry) in the spleen and thymus of treated animals was reduced compared to controls. Selenium supplementation (at doses as low as 0.2 mg/kg selenomethionine) partially attenuated the effects of T-2 exposure in the jejunum, liver, kidney, spleen and thymus.

Szabó et al. (2014, 2016) and Hafner et al. (2016) investigated the haematotoxic and hepatotoxic effects of dietary exposure to T-2. Weaned 35-day-old male New Zealand White rabbits (10 per group) were exposed to T-2 at concentrations of 0 or 2 mg/kg diet for 4 weeks. T-2 used in these experiments was produced by *F. sporotrichioides* strain NRRL 3299. The fungal cultures were mixed directly into the basal feed. The concentrations in the feed were confirmed by LC-MS. Control diets did not contain any detectable concentrations of T-2 (<10 µg/kg diet) or fumonisin B1 (<3 µg/kg diet). Body weights were measured weekly and blood samples were taken 2 and 4 weeks after the start of the exposure. Following 4 weeks of exposure the animals were killed. Samples of blood were

analysed for changes in clinical chemistry and haematology, oxidative stress parameters (GSH, GSH-Px and MDA), as well as changes in the lipid profile of the RBCs. The lipid profiles of liver and hepatic mitochondria were also obtained. Histopathological examination of the liver, kidneys and spleen was conducted. Following 4 weeks of exposure, T-2-treated animals showed significantly lower body weights (approximately 14% lower than controls) and body weight gains (78% of controls). Although no statistically significant changes in feed intake were measured, treated animals showed numerically lower feed intake compared to controls (Hafner et al., 2016). Using the mean day 28 body weights and week 4 feed intake values reported by Hafner et al. (2016), a dose of approximately 129 μg/kg bw per day for the rabbits exposed to T-2 was estimated. T-2 exposure significantly decreased lymphocytes and increased mean cell volume and mean cell haemoglobin. No significant changes in clinical chemistry parameters were observed. The RBCs of treated animals showed decreased Na+/K+ ATPase activity compared to controls and alterations in fatty acid composition of the RBC membrane (for example, increased DHA after 2 weeks of exposure). In terms of oxidative stress parameters in the blood, T-2 exposure significantly increased the concentration of GSH and the activity of GSH-Px following 2 weeks of exposure; however, following 4 weeks of exposure, the concentration of GSH and the activity of GSH-Px in the blood plasma of treated animals was reduced. No significant changes in plasma MDA content were reported in rabbits exposed to T-2. Following terminal sacrifice, no significant difference from control animals was observed in absolute weights of the heart, kidneys and spleen; however, the absolute weight of the liver of treated animals was significantly higher than that of control animals. T-2 exposure induced slight changes in the total hepatic fatty acid composition in the liver (i.e. a decrease in the proportion of palmitoleic acid) and in the liver mitochondria fraction (the proportion of margaric acid was decreased in T-2-treated animals). Histopathological examination revealed fibrosis and bile duct proliferation in the liver, vacuolization and detachment of the tubular epithelial cells in the kidney and depletion of lymphocytes in the spleen of T-2-treated animals.

Kovács et al. (2013) exposed adult male Pannon White rabbits (10 per dose) to 0, 0.05, 0.1 or 0.2 mg T-2/animal per day (equivalent to 0, 10, 20 or 50 μg/kg bw per day) via gavage for 65 days. The T-2 used in this experiment was produced on corn grits by *F. sporotrichioides* strain NRRL 3299. T-2 was extracted and purified before administration. The basal feed contained no detectable concentration of T-2. The animals were approximately 9 months old at the start of treatment. Clinical observations of general health and measurements of individual feed consumption were made daily, whereas body weights were measured weekly. At the end of the 65-day treatment period, semen was collected. Blood samples were collected on the thirtieth and sixty-fifth day of

exposure. Organ weights (liver, spleen, kidneys and testes) were measured, and a histopathological examination was conducted following terminal necropsy. Semen quality was assessed and the concentration of total protein, albumin, glucose, cholesterol, urea and creatinine, as well as the activity of ALP, ALT, AST and GGT were determined from the blood samples. Blood was also analysed for indications of oxidative stress (i.e., GSH-Px, GSH and MDA) and testosterone (following challenge with gonadotropin-releasing hormone (GnRH)-analogue). No deaths occurred and no significant clinical signs of toxicity were observed in any treated animals. Although no significant effect on final body weight was observed, feed intake was significantly decreased in animals that received 20 and 50 µg/kg bw per day early in the study period (for example, during the first week, feed intake was 63 and 47% of control values, respectively). By week four, the feed intake was similar to that of controls and remained so until the end of the experiment on day 65. On day 30, a temporary statistically significant decrease in albumin and urea nitrogen was observed in the animals in the high-dose group. No other significant effects on clinical biochemistry were observed. Blood and seminal plasma antioxidant parameters were also not significantly different from those in control animals. All sperm quality parameters were within control limits except for the ratio of spermatozoa to cytoplasmic droplets at 50 µg/kg bw per day, which was significantly increased. The authors noted a dose-responsive trend in impaired sperm motility parameters; however, the effects did not achieve statistical significance. A slower increase in the GnRH-induced testosterone synthesis was also reported in animals exposed to 20 and 50 µg/kg bw per day. Although no significant effect on organ weights (testes, liver, spleen and kidneys) were observed, histopathological examination revealed centrilobular infiltrate (type not specified) in the liver and increased proliferative activity of the Leydig cells in animals that received 20 and 50 µg/kg bw per day. Animals exposed to 50 µg/kg bw per day also had slightly hyperaemic testes.

In a separate experiment, Kovács et al. (2013) exposed adult male Pannon White rabbits (10 per dose) to feed containing 0, 0.33 or 0.66 mg/kg T-2 (equivalent to approximately 10 or 20 µg/kg bw per day according to the authors) for 65 days and examined the same parameters as previously described. Control animals received feed containing no detectable concentrations of T-2. No statistically significant effect on feed intake was observed over the course of the 9-week treatment period. No significant changes in body weights were noted and all animals appeared healthy during treatment. No toxicologically significant alterations in sperm quality parameters or testosterone concentrations were noted.

(d) Pigs

Frankič, Salobir & Rezar (2008) investigated lipid peroxidation, lymphocyte DNA fragmentation and immunoglobulin production in young, growing, castrated male cross-bred pigs. The pigs were exposed to 3 mg/kg diet T-2, in the presence and absence of vitamin E supplementation (100 mg/kg diet), for 14 days. The average body weight of the pigs at the start of the study was 11.1 ± 0.48 kg. The T-2 used in this experiment was from a fungal culture containing 4900 mg/kg T-2. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Animals were fed predetermined amounts of feed, which were entirely consumed by all control and treated animals. Although numerical reductions in terminal body weight and daily weight gain were observed, statistically significant reductions in daily weight gain were only observed in treated animals co-exposed to vitamin E. No significant difference in oxidative stress parameters (MDA, total antioxidant status or erythrocyte GSH-Px) were observed in the plasma or urine. T-2-treated animals showed significantly lower ALT and AST activities. The Comet assay showed a significantly increased amount of DNA damage in the lymphocytes of treated animals, which was partially attenuated by vitamin E supplementation. Although circulating IgA concentrations in treated animals were similar to those of controls, circulating IgG concentrations in treated animals were significantly lower.

Rafai, Papp & Jakab (2013) investigated the short-term oral toxicity of T-2 in Dutch Landrace × Hungarian Large White F1 pigs (aged 49–70 days during exposure; n = 10 per group; 5 per pen). The animals were exposed to feed containing 0, 300 or 500 µg/kg diet T-2 (equal to 0, 11.2 and 18.0 µg/kg bw per day[1]) for 21 consecutive days. The T-2 used in this experiment was prepared using *F. tricinctum* (NRLL 3299) and purified by liquid-to-liquid partition and liquid chromatography. These authors (Rafai et al., 1995a), had previously found that this method of production and purification yields a purity greater than 90%. The concentration of T-2 in the feed was determined at the beginning of the experiment and on days 7, 14 and 21 using gas-liquid chromatography. The basal feed did not contain any detectable concentrations of aflatoxin B1, T-2, HT-2, nivalenol (NIV), DON, ZEA, ochratoxin A or DAS. The average body weight of the pigs at the start of the study was 13.6 ± 0.52 kg. On the first and fourth day of the experiment, the pigs were administered purified horse globulin via intramuscular injection. Body weights were measured weekly and blood samples were taken on days 7, 14 and 21. Following 21 days of exposure, the pigs were killed. No histopathological examination was conducted. All treated animals appeared normal during the experiment with no clinical signs of toxicity (no

[1] Calculated based on the reported mean body weight and feed intake values (see Appendix 2).

vomiting, emesis or macroscopic skin lesions). However, treated animals did show significantly decreased feed consumption (22 and 28% less than control animals, in the 0.3 and 0.5 mg/kg diet groups, respectively) and corresponding decreases in body weights (10 and 16% less than control animals, in the 0.3 and 0.5 mg/kg diet groups, respectively), and daily body weight gain (24 and 36% less than control animals, in the 0.3 and 0.5 mg/kg diet groups, respectively). No treatment-related effects on biochemistry (glucose, triglycerides, AST, total protein, albumin and urea) were observed. The authors also found no significant difference in anti-horse globulin (anti-HG) antibody titres following challenge with purified horse globulin. Additionally, the authors reported that lymphocyte proliferation, as induced by purified horse globulin, phytohaemagglutinin or concanavalin A (ConA), and phagocytic activity and phagocytic index of circulating granulocytes were unaffected by treatment (raw data not reported). According to the authors, the microbiological feed additive attenuated the effects of T-2 on feed consumption and growth. Table 3 summarizes selected results from the study by Rafai, Papp & Jakab (2013).

It is worth noting that a statistically significant effect on daily body weight gain was not observed by Rafai et al. (1995a) in animals that received doses of approximately 25 and 52 µg/kg bw per day (see Appendix 2 for detailed dose calculations). However, the authors did report statistically significant reductions in feed intake at all doses when averaged over the 3-week exposure period. Rafai et al. (1995 a,b) investigated the short-term oral toxicity of T-2 in Dutch Landrace × Hungarian Large White F1 pigs (average weight at the start of the experiment was 9 kg; n = 10 per group; type of housing, i.e., group or individual, not specified). The pigs were exposed to feed containing 0, 500, 1000, 2000 or 3000 µg/kg diet T-2 (equal to 0, 25, 52, 103 or 125 µg/kg bw per day; see Appendix 2 for detailed dose calculations) for 21 consecutive days. In animals that received 52 µg/kg bw per day, feed intake was not significantly different from that of controls during weeks 1 and 2. However, in contrast to the other treatment groups, a dramatic reduction in daily feed intake by these animals was observed between weeks 2 and 3 resulting in an overall significant reduction in feed intake over the entire 3-week exposure period. As a consequence, no clear dose–response for feed intake and body weight gain is apparent in the complete dataset of Rafai et al. (1995a) (daily body weight gain and the overall average daily feed intakes during weeks 1 to 3 are reported in Table 4). The feed efficiency calculations for both studies by Rafai and colleagues (Rafai et al., 2013; Rafai, Papp & Jakab, 2013) suggest that, at low doses, reduced daily body weight gain is likely to be due to feed refusal, whereas at higher doses there may be an effect on nutrient absorption. Rafai et al. (1995b) also reported that significant reductions in RBC counts were observed on day 21 in the animals in the two highest dose groups, accompanied by decreased haemoglobin at doses ≥52 µg/kg bw per day.

Table 3
Summary of selected effects of T-2 in pigs reported by Rafai, Papp & Jakab (2013)

	Dose (µg/kg bw per day)		
Effect	**0**	**11.2**	**18**
Terminal body weight (kg)	23.8 ± 1	21.5 ± 1*	20 ± 0.88*
Daily body weight gain (weeks 1–3; g)	497 ± 63	377 ± 67*	317 ± 96*
Feed intake (weeks 1–3; g/day)	889 ± 99	694 ± 136*	644 ± 98*
Feed efficiency (%)[a]	56%	54%	49%
Anti-HG titre (log2) on day 21	4 ± 0.55	4.7 ± 0.35	4.6 ± 0.32

n=10 animals per dose; means presented ± standard deviation; see Appendix 2 for detailed dose calculations.
* $P \leq 0.05$.
[a] Calculated as mean body weight gain divided by food intake.

Table 4
Summary of selected effects of T-2 in juvenile pigs reported by Rafai et al. (1995a,b)

Effect (means are presented ± standard deviation)	**Dose (µg/kg bw per day)**				
	0	**25**	**52**	**103**	**125**
Daily body weight gain (weeks 1–3; g/day)	463.8±57	394.8±106.6	444±78.2	318.4±75.4*	222±67.5*
Feed intake (weeks 1–3; g/day)	817.3±160	714.3±160*	773±149*	659±142*	482.5±76*
Feed efficiency (%)[a]	57%	55%	57%	48%	46%
Red blood cell count (x 10^9/L)	6.64±0.45	6.45±0.62	6.61±0.54	6.06±0.64*	5.76±0.56*
Haemoglobin (mmol/L)	0.64±0.04	0.58±0.09	0.56±0.08*	0.49±0.06*	0.46±0.07*
Leukocyte count (×10^6/L)	16.8±2.63	13.6±0.78*	13.5±2.1*	11.4±2.5*	11.3±2.3*
Anti-horse globulin titre (log_2) on day 21	6.3±0.84	4.5±0.84*	5.15±0.9*	4.3±1.17*	4.1±0.7*
Phytohaemagglutinin lymphocyte stimulation (%) on day 21	44.5±7.7	33.4±8.1*	39.5±10.4	21.5±6.6*	20±7.0*
Concanavalin A lymphocyte stimulation (%) on day 21	32.4±4.7	24.3±6*	20.3±9.5*	12.6±5*	11.4±4.3*

n=10 animals per dose; see Appendix 2 for detailed dose calculations.
* $P \leq 0.05$.
[a] Calculated as mean body weight gain divided by food intake.

Leukocyte counts, anti-HG titres and proliferative response of lymphocytes to ConA were significantly decreased at all doses. However, the Committee noted that the anti-HG titres reported for the control pigs by Rafai et al., (1995b) and Rafai, Papp & Jakab (2013) showed significant heterogeneity. Also, the anti-HG titres of treated animals on day 21 in both the studies by Rafai et al. (1995b) and Rafai, Papp & Jakab (2013) were within the range of the two control group values from the two separate studies: i.e. 4.0 ± 0.55 and 6.3 ± 0.84 log_2. The Committee also noted that the dose-response for reduced anti-HG titres in the study by Rafai et al. (1995b) was not clear across the four-dose interval of 25 to

125 µg/kg bw per day, whereas the anti-HG titres in treated animals in the study by Rafai, Papp & Jakab (2013) were increased in comparison with the concurrent controls at doses of 11.2 and 18 µg/kg bw per day. Additionally, WHO/IPCS (2012) indicated that concordance analysis determined that many observational end-points, for instance, changes in leukocytes, have low predictive power for functional immune changes, such as resistance to infection. Histopathological examination of the pigs from the study by Rafai et al. (1995a) revealed dose-dependent lymphocyte depletion in the thymus and spleen at doses as low as 25 µg/kg bw per day; however, the Committee noted that the details concerning the histopathological observations were extremely limited. Table 4 summarizes selected results reported by Rafai et al. (1995a,b).

Meissonnier et al. (2008) investigated the immunotoxic and metabolic effects of T-2 exposure on pigs in two separate studies. Castrated weaned male Piétrain/Duroc/large white pigs (five per group per study) were exposed to diets containing 0, 540, 1324 or 2102 µg/kg diet T-2 for 28 days. The purity of T-2 used in these experiments was >98%. Basal feed did not contain any detectable concentrations of aflatoxins B1, B2, G1, G2, fumonisin B1, ochratoxin A or T-2. DON and ZEA were present in the basal feed at concentrations of 213 and 38 µg/kg diet, respectively. On days 4 and 15 of the experiment, pigs were injected with 1 and 0.5 mg ovalbumin (OVA), respectively. Body weight and blood samples were taken weekly and various parameters measuring humoral (for example, plasma concentrations of antibodies) and cellular (for example, lymphocyte proliferation) immunity were examined. The average body weights of the pigs at the start of the studies were 11.4 ± 0.3 kg. Reduced mean body weight gain compared to control animals (i.e. 90, 92 and 87% of control animal weight gain in the low-, medium- and high-dose groups, respectively) was observed in all treated groups following 28 days of exposure; however, the difference only achieved statistical significance in the animals in the high-dose group. Since the feed intake values were not reported, the doses received by the animals are uncertain. Clinical biochemistry tests revealed no significant treatment-related effects. Although a statistically significant increase in plasma IgA was observed following 7 days of exposure in the high-dose group, circulating plasma (IgA, IgG and IgM) concentrations in pigs following 28 days of low, medium or high dietary concentrations of T-2 were not significantly altered (Meissonnier et al., 2008). ConA-induced lymphocyte proliferation did not differ significantly between treated animals and controls. T-2 exposure did not result in a statistically significant change in IL-2, IFN-γ and IL-4 cytokine production; however, time-dependent trends in IL-2 (increase) and IFN-γ (decrease) were noted. Following injection with OVA, the anti-OVA IgG titres of animals in the medium- and high-dose groups were significantly decreased on day 21 compared to control animals (data were presented in a figure and exact figures are not available in the published report). At terminal necropsy,

relative liver weights and the concentration of MDA in the liver appeared similar to those in control animals, and there was no significant change in the protein content of microsomal or cytosolic fractions in the liver tissue. Histopathological examination revealed no significant treatment-related abnormalities in the liver, gastrointestinal tract (duodenum, ileum and colon) or spleen. Notably, the Peyer's patches in the ileum section and the spleen showed no cellular depletion compared to controls. Examination of the content and expression of CYP enzymes in the livers of treated animals revealed reduced CYP1A-related activities and certain *N*-demethylase activities, compared to controls, in animals in the high-dose group. Meissonnier et al. (2009) reported very similar effects in a study using the same methods; however, according to the authors (Meissonnier et al. (2009), dietary glucomannan supplementation appears to attenuate some of the effects elicited by T-2 at the medium and high doses.

Although it is difficult to conclusively identify the cause of the inconsistency in antibody response to horse globulin (Rafai et al., 1995a,b; Rafai, Papp & Jakab, 2013), the Committee noted that the authors had used a novel protocol. Validated methods to assess T-cell-dependent antibody responses typically use well-characterized antigens such as sheep RBCs or keyhole limpet haemocyanin, rather than a protein mixture such as horse globulin with its corresponding highly variable antigenicity profile (Peachee et al., 2014). Additionally, there was no information in either the study by Rafai, Papp & Jakab (2013) or the one by Meissonnier et al. (2008) regarding the time interval for a peak antibody (IgM and IgG) response or on the use of a positive control (for example, cyclosporin or cyclophosphamide) to validate the performance of the immune function assay.

(e) Goats

Nayakwadi et al. (2020) investigated the effects of dietary exposure to T-2 on young goats (average body weights of approximately 7 kg). Groups of six juvenile (3–4-month-old) Barbari goats were exposed to feed containing 0, 10 or 20 mg/kg diet T-2. The T-2 used in this experiment was produced using cultures of *F. sporotrichioides* var. sporotrichioides (MTCC-1894) and fermentation with maize and wheat. It was quantified by thin-layer chromatography and spectrophotometry. Basal feed did not contain any detectable concentrations of aflatoxin B1, ochratoxin A, citrinin or T-2. Three animals per group were sacrificed after 15 or 30 days of exposure. Body weight was measured, and the animals were examined for adverse clinical signs and blood samples were taken on days 0, 5, 10, 15, 20, 25 and 30. Following terminal sacrifice, the animals underwent gross and histopathological examination. No deaths occurred during the observation period. Treated animals exhibited weakness, lethargy, growth

retardation, feed refusal, reduced movement and diarrhoea. A dose- and time-dependent decrease in body weight gain was observed in treated animals and statistically significant decreases in body weights were observed by day 25 in the high-dose and by day 30 in the low-dose group. Investigation of haematological parameters showed significantly reduced haemoglobin, total leukocyte count and total thrombocyte count in treated animals. Animals in the high-dose group also showed significantly reduced total platelet count on day 25. Investigation of oxidative stress-related parameters revealed increased SOD, CAT and MDA in the intestines, MLN and livers of treated animals. SOD and CAT were also significantly increased in the kidneys of treated animals; however, no significant change was observed in the MDA content. SOD, CAT and MDA were increased in the spleen and brain of animals in the high-dose group only. At terminal necropsy, the relative organ weights of the liver and MLN of animals in the low-dose group were increased. Gross examination of the animals given the low dose revealed diffuse congestion and haemorrhages with pasty mucoid contents in all segments of the intestine, as well as pale cortex of the kidneys. Similar but more severe gross findings were reported in animals in the high-dose group, with the addition of meningeal vessel engorgement in the brain. Histopathological examination revealed a dose-responsive relationship in the severity of degenerative lesions in the liver, intestines (including the Peyer's patches), MLN, kidneys (tubular epithelial cells), spleen and brain. The numbers of apoptotic cells in the liver, intestines, MLN, kidneys and spleen were significantly increased in both treated groups. No apoptotic changes were noted in the brain. The mRNA expression of heat shock proteins, pro-apoptotic proteins and pro-inflammatory cytokines in treated animals were also significantly upregulated in liver, intestines, MLN, kidneys, spleen and brain.

2.2.3 Long-term studies of toxicity and carcinogenicity

No new long-term studies of toxicity and carcinogenicity were identified. Previously, the Committee reported that only one long-term study was available, and that study alone was not suitable for establishing a tolerable intake (Annex 1, reference *153*).

In that study (Schiefer et al., 1987), groups of 50 male and 50 female weanling CD-I mice were fed a semi-synthetic diet containing T-2 (purity, 99%) at 1.5 or 3 mg/kg diet, for 71 weeks. The survival rate (reported in a graph) was consistently lower in the control groups of both males and females, beginning from about week 35; the survival rate of treated males at 71 weeks was 75% or more, whereas that of the controls was about 62%. No statistically significant differences between the groups were found in feed consumption or body weight gain. A dose-related increase in heart weight was seen in males receiving T-2 at

the high dose, which was statistically significantly different from that of controls. No changes were seen in the heart weights of females, and no treatment-related weight changes were reported for other organs in either males or females. Statistically significant differences were observed in haematological parameters in both males (i.e. ↓PCV, ↓RBC, ↑MCH, ↑MCHC and ↓eosinophils) and females (i.e. ↑PCV, ↑MCV, ↓MCHC and ↓lymphocytes) at high and low doses. Females given the high and low dose showed higher antibody titres in response to sheep RBC challenge following 16 months of treatment. The author suggested that these changes were difficult to interpret because of age-related immunological alterations typically observed in aged mice. A dose-related increase in the frequency of squamous mucosa hyperplasia was found in the forestomach of both male and female mice, with an increased frequency of hyperkeratosis. The incidence of pulmonary adenoma increased in a dose-related manner in males (10, 15 and 23% for controls and animals given the low and high dose, respectively) but not in females. The incidences of pulmonary adenomas and hepatic adenomas in males at the high dose were statistically significantly higher than in controls. The incidence of pulmonary adenocarcinoma was 5% in control males and 6% in males given the high dose. The previous Committee noted the lower survival rate of control rats.

2.2.4 Genotoxicity

The Committee previously concluded that, in cultured mammalian cells, low concentrations of T-2 induced DNA strand breaks, unscheduled DNA synthesis, gene mutation, chromosomal aberrations and inhibition of gap-junctional intercellular communication (Annex 1 reference *153*). There was also evidence that DNA strand breaks and chromosomal aberrations are induced in vivo. However, it was unclear whether these effects are a consequence of the interaction of T-2 with genetic material or are secondary to inhibition of protein synthesis by T-2.

Since the Committee's original evaluation (Annex 1 reference *153*), findings from more recent mutagenicity and genotoxicity studies are in general agreement with the previous conclusion. T-2 was negative in the Ames test using *Salmonella* tester strains TA98 and TA100 (0.1–1.0 µg/plate), in both the presence and absence of metabolic activation (Šmerák et al., 2001). Using the Comet assay to assess DNA damage, positive in vitro responses were seen in porcine lymphocytes (0.1–1.0 µM) and human HeLa cells (10 ng/mL), but not in human THP-1 monocytes (Chaudhari et al., 2009b; Rakkestad et al., 2010; Horvatovich et al., 2013). Comet assays yielded positive responses in rabbit and pig lymphocytes (Frankič, Salobir & Rezar, 2008; Hafner et al., 2012). However, Chaudhari et al.

(2009b) suggested that the single-stranded DNA damage observed in HeLa cells was caused by oxidative stress and apoptosis.

In an in vivo micronucleus test, T-2 failed to increase the frequency of micronuclei in white ICR mice injected intraperitoneally with either 1.0 or 5.0 mg/kg bw of the toxin (Šmerák et al., 2001). In a nonconventional in vitro cell transformation assay, T-2 was active at low concentrations (1–2 ng/mL) towards Bhas 42 cells (BALB/3T3 cells that were transfected with the v-Ha-ras gene and designed to detect tumour-promoters), suggesting that T-2 may be a tumour promoter rather than a tumour initiator (Sakai et al., 2007). Finally, T-2 was negative in a somatic mutation test in *Drosophila melanogaster* at a 40 µM concentration (Gürbüzel, Uysal & Kızılet, 2015).

Based on the induction and/or repression of gene expression in yeast, it was suggested that T-2 may exert a significant effect on membrane integrity, inhibit ribosomal function and protein synthesis, and may arrest the cell cycle. The results of this study suggested that T-2 did not cause DNA damage by alkylation, and it did not induce DNA repair mechanisms such as recombination and excision repair. Thus, it was proposed by the study authors that T-2 did not cause direct DNA damage (Iwahashi, Kitagawa & Iwahashi, 2008).

As concluded by the previous Committee (Annex 1 reference *153*), DNA damage may be secondary to other processes. For instance, in the human THP-1 monocyte model, using the alkaline Comet assay with and without formamidopyrimidine DNA glycosylase, it was demonstrated that T-2 (4 µM for 3 hours) did not cause single-strand DNA breaks or oxidative DNA damage (in the presence of formamidopyrimidine DNA glycosylase). Instead, it was suggested that T-2 induces a DNA damage response that involves activation of the ATM/H2AX/Chk2 pathways, which triggers apoptosis (Rakkestad et al. 2010).

Table 5 summarizes the results of the genotoxicity testing studies published since the Committee's last assessment of T-2.

The Committee concluded that although genotoxicity is possible at high concentrations, the weight of evidence suggests that the likely mode of action for the preneoplastic and neoplastic (benign) effects seen in the chronic carcinogenicity study is not via a direct genotoxic mechanism but rather results from repeated cytotoxicity, apoptosis and regeneration.

2.2.5 Reproductive and developmental toxicity

In its previous evaluation (Annex 1, reference *153*), the Committee concluded that no embryotoxicity or gross fetal malformations were seen at intraperitoneal doses below 0.5 mg/kg bw per day T-2. Continuous administration in the feed, at concentrations of 1.5 and 3.0 mg/kg diet T-2 (equivalent to approximately 220 and 450 µg/kg bw per day), did not result in reproductive or gross developmental effects in a two-generation study in CD-1 mice, although increased spleen weights

Table 5
Results of assays for genotoxicity with T-2 (2001–2021)

Test system	Test object	Concentration	Results	Reference
In vitro				
Reverse mutations	*Salmonella* Typhimurium, TA100, TA98	1 µg/plate	Negative (+S9) Negative(−S9)	Šmerák et al. (2001)
Cell transformation	BALB/3T3 cells	0.001–0.002 µg/mL	Positive (as tumour promoter)	Sakai et al. (2007)
DNA damage	Porcine lymphocytes	0.1, 0.5, 1.0 µM	Positive (after 24 hours and 48 hours)	Horvatovich et al. (2013)
	Human HeLa cells	10 ng/mL	Positive	Chaudhari et al. (2009b)
	Human THP-1 monocytes	4 µM (3 hours)	Negative	Rakkestad et al. (2010)
DNA microarray	Yeast		Negative	Iwahashi, Kitagawa & Iwahashi (2008)
In vivo				
DNA damage (Comet assay)	Rabbits, blood lymphocytes	2 mg/kg diet for 21 days	Positive *Note*: Co-exposure to mannan oligosaccharides appeared to attenuate the effect of T-2 in the Comet assay	Hafner et al. (2012)
	Pig, lymphocytes	3 mg/kg diet for 14 days	Positive	Frankič, Salobir & Rezar (2008)
Micronucleus induction	Mice, bone marrow	1.0 and 5.0 mg/kg bw via intraperitoneal injection (single dose)	Negative	Šmerák et al. (2001)

were observed in male offspring of exposed dams at both doses (Rousseaux, Schiefer & Hancock, 1986).

The following sections summarize the results of studies concerning the reproductive and developmental toxicity of T-2 or HT-2 published since the Committee's evaluation in 2001 (Annex 1, reference *153*).

(a) Mice

Ishigami et al. (2001) investigated the teratogenicity of T-2 in mice. One hundred and eighty female Crj:CD-1 (ICR) mice (10 per gestation day (GD) per dose) were exposed to 0 or 2 mg/kg bw T-2 via gavage on GD 8, 9, 10, 11, 12, 13, 14, 15 or 16. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Twenty-four hours after dosing, half of the dams (five per group) were sacrificed, and the fetuses examined histopathologically. The remaining five animals per group were sacrificed on GD 17.5 and the fetuses examined for skeletal abnormalities. Statistically significantly lower fetal weights were observed

in treated animals exposed to T-2 on and after GD 10. Additionally, an increased incidence of resorbed and/or dead fetuses was observed in the treated groups. Histopathological examination revealed incidences of exencephaly (brain tissue protruding outside the skull) in mice exposed to T-2 on days 8, 10, 11, 14 and 15. Compared to controls, treated fetuses also showed increased incidence of pyknotic or karyorrhectic cells in various tissues and organs, particularly in the central nervous system, viscera and mesenchyma. TUNEL staining and electron microscopic analysis suggested that the fetuses of animals exposed to T-2 showed greater numbers of apoptotic cells in regions where the control fetuses showed evidence of proliferating cells (PCNA-positive). Evidence of incomplete ossification, wavy ribs and short scapula was also observed in treated animals.

Tanaka et al. (2016) investigated the neurodevelopmental effects (i.e. hippocampal neurogenesis) of T-2 exposure in mice. Pregnant ICR mice (13 per group) were exposed to 0, 1, 3 or 9 mg/kg diet T-2 from GD 6 to PND 21. According to the authors' calculations, dams from the 1, 3 and 9 mg/kg diet groups were exposed to doses of 0.14, 0.4 and 1.18 mg/kg bw per day during gestation (GD 6 to birth) and 0.49, 1.39 and 3.79 mg/kg bw per day during lactation (PND 1 to 21). The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. A preliminary toxicity study revealed that at dietary concentrations of 12 mg/kg diet, significant reductions in body weight (12.64 ± 1.80 versus 16.58 ± 0.68 g on PND 21) and reduced absolute brain weight (0.41 ± 0.02 versus 0.44 ± 0.02 g on PND 21) were observed in offspring. The average number of live offspring ranged from 13.75 ± 2.34 to 14.5 ± 2.07 in treated animals and was 14.36 ± 2.5 in control animals. On PND 4 the litters were culled to 10 pups per litter. All dams and selected offspring (4 to 6 males per dam and 1 to 3 females per dam) were sacrificed on PND 21. The remaining offspring were maintained on the basal diet (no T-2 exposure) until sacrifice on PND 77. Dams from the highest dose group showed transient decreases in body weight, feed consumption and water intake. However, at terminal necropsy on PND 22, the average thymus weights (absolute and relative) were significantly decreased in all treated groups compared to control animals. Additionally, the average absolute and relative liver weights of the animals in the high-dose group were significantly increased compared to control animals. Histopathological examination revealed increased severity of squamous cell hyperplasia in the forestomach of dams in the medium and high-dose groups. Dams given the high dose also showed atrophy of the thymus and extramedullary haematopoiesis in the spleen. The number of implantation sites and the live birth ratio were similar in the control and treated groups. In the offspring, significantly decreased body weights were observed from PND 7 to PND 77 in the animals in the high-dose group, compared to control animals. Offspring from dams in the high-dose and medium-dose groups sacrificed on

PND 21, showed significant decreases in absolute brain, thymus, liver and spleen weights, compared to control animals. However, changes in organ weights in animals sacrificed on PND 77 were limited to lower liver and thymus weights in females in the high-dose group. Incidences of increased relative brain weights were observed in animals given the high dose; however, this is likely to be due to significantly decreased body weight. Histopathological examination of the pups revealed increased severity of apoptosis of lymphocytes of the thymus in males in the medium- and high-dose group sacrificed on PND 21. Histopathological lesions were not observed in other organs. Further examination of the brain revealed differential hippocampal neurogenesis. For example, males the medium- and high-dose groups sacrificed on PND 21 showed decreased incidence of GFAP+ and BLBP+ type-1 stem cells and PAX6+ and TBR2+ type-2 progenitor cells in the subgranular zone of the hippocampal dentate gyrus, which coincided with an increased rate of apoptosis. Evidence of lipid peroxidation and oxidative stress was also reported in the brain tissue. However, at PND 77, evidence of neurogenesis-related changes was absent. A parental NOAEL was not identified in this study based on significantly decreased thymus weights, and the lowest dose tested (0.14 to 0.49 mg/kg bw per day) represents the LOAEL. The authors suggested a developmental toxicity NOAEL of 0.14 to 0.49 mg/kg bw per day based on transient adverse effects on neurogenesis in pups from the medium- and high-dose groups. In a follow-up study, Nakajima et al. (2019) showed that the number of cells (for example, astrocytes and neuronal stem cells) expressing metallothionein (i.e. MT-I/II) in the offspring sacrificed on PND 21 increased in several brain regions. At concentrations ≥ 3 mg/kg diet the number of cells expressing metallothionein in the subgranular zone of the dentate gyrus and cerebral cortex were elevated. Furthermore, at 9 mg/kg diet the numbers of cells expressing metallothionein in the hilus of the dentate gyrus, corpus callosum and cerebellum were increased. The authors suggested that this demonstrates a cytoprotective function in response to oxidative stress. By PND 77 the expression of MT-I/II in cells from all brain regions was less than control levels, suggesting recovery (Nakajima et al., 2019).

Yang X et al. (2019a,b) investigated the impact of T-2 exposure on male reproductive function in mice. Male Kunming mice (20 per dose) were exposed to doses of 0, 0.5, 1 or 2 mg/kg bw per day via gavage for 28 days. The purity of T-2 used in this experiment was >99.8%. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Following 28 days of exposure, 10 males per group were sacrificed and the remaining 10 were housed with two females each to test functional mating and fertility parameters. The males from all treated groups sacrificed following 28 days of exposure showed a statistically significant decrease in body weight compared to control animals. The absolute weights of testis, epididymis and seminal vesicle in the animals in the

high-dose group were also significantly decreased following exposure. Animals that received the medium dose also showed decreases in the absolute weights of seminal vesicles. Correspondingly, significant reductions in the concentration of sperm and an increase in sperm malformation rate were observed in animals in all treated groups. Histopathological examination of the testis revealed dose-related effects (for example, decreased layers of germ cells, germ cells detached from the sarcolemma and irregularly arranged seminiferous epithelium). Transmission electron microscopy of the sperm revealed ultrastructural changes, especially involving the mitochondria (disarrangement, swelling and vacuolization). Evidence of oxidative stress (increased ROS and MDA concentrations and decreased total anti-oxidation capacity and SOD activity) was noted in the testis of animals in the medium- and high-dose groups. Using TUNEL staining and real-time qPCR, increased germ cell apoptosis was evident in the seminiferous epithelium of animals in all treated groups. In the males that were housed with untreated females following exposure, T-2-treatment (all doses) significantly decreased the number of viable fetuses and increased the number of animals with resorptions. Dams mated with males from the high-dose group also showed decreased mating and fertility index and decreased numbers of implantation sites. Dams mated with the males from the medium-dose group showed decreased numbers of implantation sites. The average daily sperm production in animals in the medium- and high-dose groups was significantly decreased and the teratozoospermia index and sperm deformity index were increased in animals in all treated groups. Furthermore, decreased concentrations of testosterone, progesterone, GnRH and follicle-stimulating hormone were observed in animals in all treated groups and this was accompanied by decreased follicle-stimulating hormone receptor, luteinizing hormone receptor and androgen-binding protein expression. The authors also reported decreased mRNA expression of various genes related to steroid hormone production (StAR, P450scc, 3b-HSD, P450c17 and 17b-HSD).

Shen et al. (2020), Kang et al. (2020) and Perveen et al. (2020) investigated the toxicity of maternal T-2 exposure on offspring development. Pregnant ICR mice (10 per dose) were exposed to 0, 0.005 or 0.05 mg/kg bw per day via gavage from GD 14 to PND 21. Offspring were weaned on PND 21 and selected offspring (five or six per dose) were sacrificed at PND 21, PND 28 and PND 56, while a selected group of female offspring (six per group) were observed until sacrifice following the onset of the third estrus cycle (Perveen et al., 2020). Exposure was limited to the dams (GD 14 to PND 21). The offspring were not directly administered T-2. The purity of T-2 used in this experiment was not reported. No indication of the concentration of T-2 in the animal feed was provided; however, Shen et al. (2020) and Perveen et al. (2020) reported that, in China, the detection rate of T-2 in feed samples was as high as 79.5%, and the highest content was

735 µg/kg diet. These statistics are from Shi et al. (2018) who further reported that, in China, the detection rate of fumonisin B1 and ZEA in feed samples was as high as 96.1 and 85.2%, respectively; with concentrations ranging up to 6568 and 1478 µg/kg, respectively; and high detection rates for aflatoxin B1, DON, ZEA and ochratoxin A in animal feed and feed ingredients. Serum concentrations of T-2 in the offspring were elevated in both treated groups on PND 21 and PND 28. By PND 56 the serum concentrations in the low-dose group were not statistically significantly different from control animals; however, the serum concentrations of T-2 in the offspring of the animals in the high-dose group remained elevated. T-2 concentrations in the liver of offspring appeared similar to those of control animals except for elevated concentrations on PND 28 in the animals given the high dose. It is noteworthy that the internal concentrations of T-2 in the control offspring were considered elevated by the Committee. For example, on PND 21, the serum and liver concentrations of T-2 in control animals were ~100 µg/L and ~15 µg/g, respectively. Whereas the liver concentrations in treated and control animals remained relatively stable, the serum concentrations in control animals were significantly lower on PND 56, suggesting that perhaps the pups were exposed to an exogenous source of T-2 during the first 21 days of life (see Fig. 1 in Kang, Perveen & Li, 2020 for reported serum and liver concentrations in treated and control animals on PND 21, 28 and 56). Furthermore, the elevated concentrations of T-2 in treated animals on PND 56 suggest possible background T-2 contamination of the basal feed, considering that the bioavailability of unchanged T-2 may be quite low and the predominant absorbed chemical species may be HT-2 (Annex 1, reference *153*). In contrast to the findings of this study, Chandratre et al. (2014) were not able to detect T-2 in the liver or kidneys of rats following direct dosing with approximately 20 mg/kg diet for 14 days (LOQ = 0.1 µg/g). Since details of the methods for measuring T-2 concentrations in the tissue samples were not provided by Kang, Perveen & Li, (2020), a direct comparison with the methods used by Chandratre et al. (2014) was not possible. Nevertheless, the reason for the relatively high concentrations of T-2 in the livers of mice exposed to doses an order of magnitude lower than reported by Chandratre et al. (2014) is uncertain. Considering these uncertainties, the relevance of the effects reported by Shen et al. (2020), Kang, Perveen & Li (2020) and Perveen et al. (2020) to hazard characterization is considered limited.

(b) Rats

Sehata et al. (2003) investigated the developmental toxicity of T-2 exposure in rats. Pregnant Wistar:Slc rats (six per dose) were exposed to single doses of 0 or 2 mg/kg bw via gavage on GD 13 and sacrificed 24 or 48 hours after exposure. The purity of T-2 used in this experiment was not reported. No indication of the

background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Both the dams and fetuses underwent gross and histopathological examination. Although not statistically significant, the body weight of treated dams was decreased compared to control animals 24 hours post-dosing. By 48 hours post-dosing, the absolute and relative weights of the thymus, spleen, liver and salivary gland of treated dams were decreased. Forty-eight hours post-dosing the absolute pancreas weights were also decreased but the relative liver weights were similar to those of control animals. Histopathological examination revealed single cell necrosis in the thymus, spleen, liver, stomach, intestines, salivary glands and pancreas and fatty changes in the liver of treated dams. In the treated fetuses, an increased incidence of single cell necrosis in the central nervous system 24 hours post-dosing, and an increased incidence of single cell necrosis of haemopoietic cells and of hepatocytes after 48 hours, were observed. Sehata et al. (2004a) reported the results of a very similar test; presumably focusing on the dams sacrificed 24 hours after exposure and involving more detailed histopathological examination and gene expression data. TUNEL staining revealed an increased number of apoptotic cells in the liver and placenta of dams and in the fetal liver 24 hours after exposure. Gene expression in the liver and placenta of dams and in the fetal liver 24 hours after exposure revealed changes in the expression of apoptosis genes, metabolic enzymes (phase I and II) and oxidative stress-related genes.

Sehata et al. (2004b) investigated the effects of maternal T-2 exposure on the fetal brain in rats. Pregnant Wistar:Slc rats (27 per dose) were exposed to single doses of 0 or 2 mg/kg bw T-2 via gavage on GD 13. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Eighteen dams per dose (three per sacrifice interval) were killed 1, 3, 6, 9, 12 or 24 hours after treatment and fetal brains were examined for histopathological changes. An additional nine dams (three per sacrifice interval) per dose were killed 6, 12 or 24 hours after treatment and the fetal brains were examined for changes in gene expression. Histopathological examination of the fetal brains revealed apoptosis in the neuroepithelial cells, mainly in the outer layer of the ventricular zone of the telencephalon, in treated fetuses. TUNEL staining revealed that apoptosis in treated animals was increased 1 hour after dosing, peaked after 12 hours and had decreased slightly by 24 hours post-dosing. Correspondingly, microarray analysis of the fetal brains revealed that the expression of apoptosis-related genes (MAPK-related genes, caspase-2 and insulin-like growth factor binding protein-3) were induced by treatment, and that an increased expression of oxidative stress-related genes (heat shock protein 70 (HSP70) and haem oxygenase (HO)) was observed 12 hours post-dosing. Real-time PCR analysis of the fetal brain confirmed the increased expression of apoptosis and oxidative

stress-related genes (HSP70, HO-1, IGF-BP3 and VEGF-A) at 6, 12 and 24 hours post-dosing. Sehata et al. (2005) examined the time-course of effects of maternal T-2 exposure on the dam liver, placenta and fetal liver 1, 3, 6, 9 or 12 hours after exposure to 0 or 2 mg/kg bw via gavage on GD 13. T-2-treatment of the pregnant dams induced apoptosis in the liver and placenta of dams and in the liver of fetuses. TUNEL staining revealed that apoptosis was increased at 1 hour post-dosing and peaked after 6 hours post-dosing in the livers of pregnant dams. Similarly, apoptosis in the placenta of treated dams was increased at 1 hour post-dosing and remained elevated 12 hours post-dosing. In the fetal liver, apoptosis was increased 1 hour post-dosing and peaked 9 hours after dosing in the treated group. Correspondingly, microarray analysis revealed increased expression of oxidative stress and apoptosis-related genes in the liver and placenta of dams and in the liver of fetuses from the treated group. Microarray analysis also revealed decreased expression of lipid metabolism and drug metabolism-related genes in the tissues of treated animals.

Yang R et al. (2015) investigated the effects of neonatal exposure to T-2 on the timing of puberty in female rats. Young female Sprague-Dawley rat pups (20 per group) were administered 0 or 0.1875 mg/kg bw per day T-2 via gavage during PND 15 to 19. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. The rats were weaned on PND 21 and subsequently observed for vaginal opening and estrous cycle using daily vaginal smears. Half of the animals were maintained on a basal diet until consecutive regular estrous cycles were observed in all rats. The other half of the animals were maintained on a basal diet until vaginal opening was observed in the control animals. Following terminal sacrifice, the animals were subjected to macroscopic and histopathological examination and gene expression analysis was conducted on selected tissues. Blood hormone levels were also measured. No clinical signs of toxicity were evident in treated or control animals during the course of the experiment and all animals gained an appropriate amount of weight. However, days to vaginal opening, first diestrus and first estrus were significantly increased in treated animals. No significant difference between treated and control animals was observed in terms of estrus cyclicity, vaginal smears and cycle length. Serum concentrations of luteinizing hormone and estradiol, as well as the expression of GnRH in the hypothalamus and GnRH receptor in the pituitary were significantly reduced in treated animals compared to controls. At necropsy, no significant difference in histopathological lesions of the thymus, liver, heart, kidney, spleen or bone were observed between treated and control animals. However, the relative weights of the uterus and ovaries were significantly decreased in treated animals compared to controls. In the uterus, histopathological examination revealed decreased thickness of the myometrium

and number of endometrial glands in treated animals compared to controls. In the ovaries, there were more primary follicles and fewer corpora lutea in treated animals than in control animals. Additionally, histopathological examination of the vagina revealed decreased thickness of the vaginal wall, mucosal epithelium and keratinization of the epithelium in treated animals compared to controls.

In a follow-up study, Yang R et al. (2016b) followed the same procedure as described in Yang R et al. (2015); but they administered a dose of 0.375 mg/kg bw per day T-2. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. No clinical signs of toxicity were evident in treated or control animals during the course of the experiment and all animals gained an appropriate amount of weight as measured on PND 15, PND 19 and PND 35. However, in contrast to the results of Yang R et al. (2015), days to vaginal opening, first diestrus and first estrus were significantly decreased in treated animals compared to controls. Similarly, in contrast to the results reported by Yang R et al. (2015), serum concentrations of luteinizing hormone and estradiol as well as the expression of GnRH in the hypothalamus and GnRH receptor in the pituitary were significantly increased in treated animals compared to control animals. No significant difference between treated and control animals was observed in terms of estrus cyclicity, vaginal smears and cycle length. At necropsy, no significant difference in histopathological lesions of the thymus, liver, heart, kidney, spleen or bone were observed between treated and control animals. Although the relative weights of the uterus and ovaries were not significantly different in treated animals from those of controls, the number of corpora lutea was significantly increased. Histopathological examination of the uterus revealed increased thickness of myometrium and an increase in the number of endometrial glands in treated rats compared to controls (opposite to what was reported in Yang R et al., 2015). The ovaries of treated animals had significantly more corpora lutea and mature follicles than in controls. Additionally, histopathological examination of the vagina revealed increased thickness of the vaginal wall and mucosal epithelium, and keratinization of the epithelium in treated compared to control animals (also opposite to what was reported in Yang R et al., 2015).

(c) Rabbits

Kovács et al. (2011) investigated the reproductive toxicity of T-2 following exposure of male rabbits. Male Pannon white rabbits (12 per dose) were exposed to 0 or 4 mg T-2 per animal via gavage for 3 days. According to the authors, the doses of T-2 were between 0.78 and 0.99 mg/kg bw per day in the treated group. T-2 used in this experiment was produced by the *F. sporotrichioides* strain NRRL 3299 on corn grits. The T-2 purified from the cultures was then quantified using

thin-layer chromatography. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Since reduced feed intake was observed in the treated animals, a restricted feeding control group was subsequently added. Following exposure, the animals were observed until day 51 when semen samples were taken and a GnRH challenge test was performed. The animals were then killed, and blood samples were analysed for testosterone. Five treated animals died during the experiment (four before day 4 and one on day 35). Necropsy of the moribund animals revealed alterations in the liver (centrilobular fatty liver degeneration), kidney (pallor), stomach and intestine (haemorrhages, congestion, lesions and ulcers in the mucosa, haemorrhagic digesta), heart (pallor) and lung (hyperaemia). No morbidity was observed in either of the control groups (i.e. restricted and unrestricted feed groups). Feed intake was significantly affected in treated animals on each day of treatment. Feed intake in the treated group increased following the exposure period; however, it remained low compared to the unrestricted control group until the end of the observation period. Correspondingly, the body weights of the treated group were significantly lower than those of animals in the unrestricted control group for the entire observation period (notably, during days 17 to 29 the body weights of treated animals were approximately 13% lower than the body weights of the unrestricted controls). On day 51, analysis of semen samples revealed that treated animals did not show any difference in pH, quantity of semen, concentration of spermatozoa or concentration of fructose and zinc. However, treatment did decrease the ratio of spermatozoa showing progressive forward motility (65% to 53% compared to controls), increase the ratio of spermatozoa with abnormal morphology, and decrease the concentration of citric acid in seminal plasma. Additionally, treated animals showed decreased testosterone concentrations (45%) and decreased "GnRH-induced" testosterone concentrations. In the feed-restricted controls, the number of morphologically abnormal spermatozoa in the semen increased; however, the motility of the spermatozoa, composition of the seminal plasma and testosterone concentrations were similar to those of the controls on unrestricted feed. A follow-up study (Kovács et al., 2013) investigating a longer exposure period and examining the effects of lower doses (~0.01, 0.02 or 0.05 mg/kg bw per day) is summarized in section 2.2.2 (c).

2.2.6 Special studies

Subsections 2.2.6 (a) to (e) briefly summarize the available acute in vivo and in vitro mechanistic data published since the Committee's last evaluation of T-2 and HT-2 (Annex 1, reference *153*). Subsection 2.2.6 (f) provides a very brief summary of information concerning the toxicological interaction(s) between, as

well as the relative potency of, T-2/HT-2 and its metabolites, and other related mycotoxins.

(a) Effects on emesis, feed intake, nutrients and energy balance

The effect of T-2 on the intestinal absorption of monosaccharides and on changes in the serum concentration of glucose and other essential elements was discussed in the previous JECFA evaluation (Annex 1, reference *153*). The Committee noted that interpretation of these observations was difficult since feed refusal and decreased feed intake caused by T-2 may have been confounding factors.

More recently, Gonkowski, Gajęcka & Makowska (2020) noted that T-2 may induce histopathological changes in the intestinal mucosal layer, disturb the functionality of the intestinal barrier, influence the enzymatic activity of enteric cells and inhibit mucin production. In addition, T-2 may influence the expression of a wide range of neuronal factors responsible for regulatory processes in the enteric nervous system (ENS) and interfere with various intestinal activities, such as motility, secretion, conduction of sensory stimuli and regulation of the blood flow in the intestinal wall.

Gaigé et al. (2014) investigated gut-to-brain communication and the central mechanisms underlying the physiological and behavioural alterations following acute oral exposure to T-2. The purity of the T-2 used in this experiment was not reported. Groups of 13 individually housed adult male C57B16 mice were administered 0, 0.5, 2 or 5 mg/kg bw T-2 via gavage. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. Following exposure, feed intake and various aspects of energy balance, inflammation and oxidative stress were measured for up to 48 hours. A dose-dependent decrease in food intake was observed in treated animals. In the low-dose group, a significant reduction in feed intake was only observed 3 to 6 hours after dosing. For the remainder of the observation period (up to 48 hours) feed consumption in the low-dose group appeared similar to that of controls. At doses of 2 and 5 mg/kg bw, reduced feed intake was observed immediately (0–3 hours) and persisted until the end of the 48-hour observation period. Up to 12 hours following administration of 2 and 5 mg/kg bw T-2, treated animals showed 53% and 76% lower feed consumption, respectively, compared to controls. After 12 hours the feed intake in all treated animals increased; however, animals dosed with 2 and 5 mg/kg bw continued to show reduced feed intake relative to that of controls. Decreased feed consumption was associated with an increase in time between meals, decreased water consumption and reduced blood glucose. Reduced blood glucose reached a minimum 6–9 hours post-treatment. Nine hours following treatment, blood glucose in treated animals was 75% lower than in control animals. A slight increase in glycaemia was observed after 12 hours;

however, the blood glucose concentrations were still below those in controls 24 hours after dosing with 2 mg/kg bw. A dose-responsive decrease in locomotor activity was observed in treated animals during the first 24 hours – i.e. 21, 50.7 and 65.8% lower than controls, in the groups treated with 0.5, 2 and 5 mg/kg bw, respectively. During the second day of observation, the locomotor activity returned to baseline levels. A dose-dependent decrease in body temperature was observed during the first 6 hours of observation, which recovered in animals given the low- and medium-dose within 48 hours after exposure. However, animals in the high-dose group continued to show depressed body temperature 48 hours after exposure. A group of mice selected from the control and 2 mg/kg bw dose groups were placed in metabolic chambers for 3 days to measure indirect calorimetry. Although maximum rate of oxygen consumption (VO_2) was not significantly affected by T-2 treatment, rate of elimination of carbon dioxide (VCO_2) and energy expenditure were reduced compared to controls. Twelve hours after treatment VCO_2 and energy expenditure returned to control values. Another group of animals exposed to 2 mg/kg bw T-2 was investigated for treatment-related changes in inflammatory biomarkers and oxidative stress. Three hours following treatment, IL-1β, IL-6, cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) mRNA were upregulated compared to controls in the liver and spleen, with IL-6 mRNA expression remaining elevated up to 24 hours after treatment. Twenty-four hours after treatment, a significant increase in the expression of TNF-α was observed in the liver and hypothalamus. Plasma IL-1β was also increased, with a peak at 6 hours and a return to basal concentrations 24 hours after treatment. Additionally, COX-2 mRNA expression in the hypothalamus and dorsal vagal complex was increased 24 hours after treatment. Expression of Nrf2 and UCP-2 in the liver, hypothalamus and brainstem was also significantly increased as early as 3 hours after treatment. SOD-2 mRNA expression was also significantly elevated in the liver 24 hours following treatment. Gaigé et al. (2014) suggested that in addition to the neuronal pathways, the production of ROS and the upregulation in the expression of peripheral and systemic pro-inflammatory cytokines may contribute to the anorectic effect of T-2.

Wu W et al. (2015) investigated the comparative effects of trichothecene-induced feed refusal in female B6C3F1 mice. Groups of six mice per dose were administered single doses of T-2 or HT-2 via gavage or intraperitoneal injection at doses of 0, 0.01, 0.1, 0.5 or 1 mg/kg bw. The T-2 and HT-2 used in this experiment had a purity of ≥98%. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Immediately following exposure, mice were given preweighed feed pellets and feed intake was measured 0.5, 1, 2, 3, 6, 16, 24, 48, 72 (intraperitoneal injection only) and 96 (intraperitoneal injection only) hours after exposure. A statistically significant

decrease in feed consumption was observed after both oral and intraperitoneal exposure at doses ≥ 0.1 mg/kg bw of T-2 or HT-2. Following a request from the Committee, the study authors shared the raw data. Peak anorectic effect was observed 30 minutes following exposure and diminished steadily thereafter. A dose-response, corresponding to both the magnitude (the higher the dose the greater the decrease compared to control animals) and the duration (the higher the dose the longer lasting the effect), was observed with respect to decreased feed intake following both oral and intraperitoneal exposure to T-2 and HT-2. Notably, the anorectic response to intraperitoneal exposure appears to last longer than that following oral exposure.

Following on from the study by Wu W et al. (2015), Zhang J et al. (2018a) used the same study protocol to compare the potencies of T-2, HT-2, DAS and NEO and achieved very similar results: i.e. NOAEL = 0.01 mg/kg bw and LOAEL = 0.1 mg/kg bw for both gavage and intraperitoneal exposure to T-2 and HT-2 (see section 2.2.6(f) for further study details).

Zhang J et al. (2017a,b; and 2018b), Wu W et al. (2018a) and Sheng et al. (2018) also used the same exposure protocol to study the effects of oral or intraperitoneal exposure to T-2 and HT-2 on anorexigenic factors measured up to 24 hours after exposure. In the studies by Zhang J et al. (2017a,b; 2018b), groups of six female B6C3F1 mice per dose were administered single doses of T-2 or HT-2 via oral gavage or intraperitoneal injection at doses of 0 or 1 mg/kg bw. Zhang J et al. (2017a) reported plasma concentrations of gut satiety hormones cholecystokinin (CCK) and glucagon-like peptide-1_{7-36} amide (GLP-1); Zhang J et al. (2017b) reported on the gut satiety hormones peptide YY_{3-36} (PYY_{3-36}) and glucose-dependent insulinotropic polypeptide (GIP); and Zhang J et al. (2018b) reported on neurotransmitters 5-hydroxytryptamine (5-HT) and substance P. As previously summarized, oral and intraperitoneal exposure to 1 mg/kg bw T-2 and HT-2 significantly decreased feed intake. Correspondingly, plasma concentrations of CCK, GLP-1, PYY_{3-36}, GIP, 5-HT and substance P were significantly elevated 24 hours following oral and intraperitoneal exposure.

Sheng et al. (2018) and Wu W et al. (2018a) studied groups of six female B6C3F1 mice, which were administered single doses of T-2 or HT-2 via oral gavage at doses of 0, 0.01, 0.1, 0.5 or 1 mg/kg bw. At doses ≥ 0.1 mg/kg bw, significantly decreased feed consumption compared to that of control animals was observed at the 2-hour observation interval but not at 24 hours (the initial feed refusal observed 2 hours following exposure was not apparent 24 hours after exposure). Plasma GIP and PYY_{3-36} were significantly elevated compared to controls at 2 hours, but returned to control levels within 24 hours. At a dose of 0.01 mg/kg bw no statistically significant effect on feed consumption or plasma GIP and PYY_{3-36} was observed (Sheng et al., 2018). Similarly, plasma GLP-1 was increased 2 hours after exposure and returned to control levels within 24 hours

following administration of ≥ 0.1 mg/kg bw; and CCK plasma concentrations were significantly increased at 2 hours and returned to control levels 24 hours after exposure to 0.5 and 1 mg/kg bw (Wu W et al., 2018a). Pretreatment with GIP, orexigenic molecule neuropeptide Y-2, GLP-1, CCK-1 or CCK-2 receptor antagonists effectively attenuated the T-2-induced anorectic response.

Wu W et al. (2016) investigated the emetic response to T-2, HT-2 and a known emetic agent (emetine) in female Standard dark mink. The purity of the T-2 and HT-2 used in this experiment was ≥98%. Although the ferret is considered the gold standard for the pharmacological evaluation of emetic or anti-emetic drugs due to its highly sensitive vomiting reflex (Goineau & Castagné, 2016), Zhang F et al. (2006) suggested that the mink represents a suitable alternative to the ferret for modelling emesis in humans. Wu W et al. (2016) exposed groups of female mink (1 to 2 years of age; four per dose) to single doses of T-2 or HT-2 via oral gavage or intraperitoneal injection and monitored the animals for 6 hours postexposure for evidence of emesis. The oral gavage doses investigated in this study were 0, 0.005, 0.05, 0.25 and 0.5 mg/kg bw T-2 or HT-2; and the intraperitoneal doses were 0, 0.001, 0.01, 0.05 and 0.25 mg/kg bw T-2 or HT-2. No indication of the background concentration of T-2 or of any other mycotoxin in the animal feed was provided. No evidence of emetic responses (for example, retching or vomiting) was observed following oral doses of T-2 and HT-2 at ≤0.005 mg/kg bw. At doses of 0.05, 0.25 and 0.5 mg/kg bw T-2 and HT-2, 3/4, 4/4 and 4/4 animals in each group showed emesis, respectively. Emesis occurred within 30 minutes for most animals and continued for up to 270 minutes. A decrease in latency and an increase in duration of emesis exhibited a dose–response relationship. No evidence of emetic responses was observed following intraperitoneal doses of T-2 and HT-2 at ≤0.01 mg/kg bw. At doses of 0.05 and 0.25 mg/kg bw T-2 and HT-2, 1/4 and 4/4 animals in each group showed emesis, respectively (see Table 6 for complete results). Emesis occurred within 30 minutes in most animals; however, emesis was evident for up to 270 minutes following exposure to T-2 at 0.25 mg/kg bw. A decrease in latency and an increase in duration of emesis exhibited a dose-responsive relationship. Emetic responses in mink exposed to orally administered T-2 and HT-2 were associated with elevated plasma concentrations of satiety hormone peptide $YY_{3–36}$ ($PYY_{3–36}$) and neurotransmitter 5-HT.

Zhong et al. (2014) suggested that increased 5-HT secretion activates emetic signalling in the brainstem. According to Zhong et al. (2014), $5\text{-}HT_3$ receptors are found throughout the brainstem dorsal vagal complex and gastrointestinal tract. Release of 5-HT from the gastrointestinal enterochromafin cells activates local $5\text{-}HT_3$ receptors present on the gastrointestinal vagal afferents resulting in vagal nerve depolarization, which triggers the brainstem to initiate the vomiting reflex. Similar mechanisms have been proposed in humans

Table 6
Summary of emesis data reported by Wu W et al. (2016)

T-2					HT-2				
Dose (mg/kg bw)	Incidence of emesis	Latency (min) mean ± SEM	Duration (min) mean ± SEM	No. of emetic events mean ± SEM	Dose (mg/kg bw)	Incidence of emesis	Latency (min) mean ± SEM	Duration (min) mean ± SEM	No. of emetic events mean ± SEM
Intraperitoneal exposure									
0	0/4	N/A	N/A	0	0	0/4	N/A	N/A	0
0.001	0/4	N/A	N/A	0	0.001	0/4	N/A	N/A	0
0.01	0/4	N/A	N/A	0	0.01	0/4	N/A	N/A	0
0.05	1/4	30	55	20 ± 20	0.05	1/4	28	71	22 ± 22
0.25	4/4	27 ± 3	207 ± 7	184 ± 17	0.25	4/4	24 ± 3	242 ± 7	238 ± 22
Oral exposure									
0	0/4	N/A	N/A	0	0	0/4	N/A	N/A	0
0.005	0/4	N/A	N/A	0	0.005	0/4	N/A	N/A	0
0.05	3/4	23 ± 3	52 ± 15	74 ± 26	0.05	3/4	27 ± 7	50 ± 11	68 ± 27
0.25	4/4	20 ± 3	108 ± 11	147 ± 18	0.25	4/4	22 ± 40	121 ± 21	175 ± 26
0.5	4/4	13 ± 3	171 ± 22	212 ± 53	0.5	4/4	19 ± 2	201 ± 18	245 ± 31

N/A, not applicable; SEM, standard error of the mean.

for emesis and anorexia in pregnant women who have hyperemesis gravidarum (Köşüş et al., 2015). Based on the evidence for a structurally similar trichothecene (i.e. DON) it is likely that these effects are C_{max}-dependent and are more likely to occur when T-2 and HT-2 are presented as a single bolus dose rather than as small increments over a day (Pestka & Smolinski, 2005).

Rychlik et al. (2020) and Makowska, Obremski & Gonkowski (2018) investigated the effects of T-2 exposure on the ENS of pigs. Female large white Polish pigs (8 weeks old; five per dose) were administered 0 or 12 µg/kg bw per day T-2 via a capsule, for 42 days. The source and purity of the T-2 used in this experiment were not reported; however, the basal feed did not contain any detectable concentrations of T-2, aflatoxin B1, ochratoxin A, ZEA, alpha-zearalenol and DON. Following 42 days of exposure, the animals were sacrificed and tissue fragments from the stomach and duodenum were isolated and examined using immunofluorescence methods. Rychlik et al. (2020) briefly reported that no adverse clinical signs were apparent during the study; however, no other clinically relevant observations were reported by the study authors. T-2 treatment resulted in a significant increase in the number of enteric nervous structures immunoreactive to neuronal isoform nitric oxide synthase in the duodenum (Rychlik et al., 2020). T-2 exposure also significantly increased the percentage of vasoactive intestinal polypeptide-like immunoreactive nerve

structures in the stomach and duodenum (Makowska, Obremski & Gonkowski, 2018). In a previous study using similar methods, Makowska et al. (2017) observed an increase in the percentage of calcitonin gene-related peptide-like immunoreactive neurons in the descending colon of pigs exposed to 12 μg/kg bw per day T-2 via a capsule, for 42 days. Although the toxicological significance of these changes in the neurochemical profile of the enteric neurons is uncertain, the Committee indicated that they may reflect T-2-induced changes related to the gut–brain axis.

(b) Alterations of cellular membranes

T-2 and HT-2 are amphophilic molecules capable of exerting cellular toxicity by disturbing cellular and subcellular, particularly mitochondrial, membranes (Rocha et al., 2005). Compromised integrity of cellular and subcellular membranes is commonly assessed with the MTT, LDH, annexin, caspase-based, neutral red and trypan blue cytotoxicity assays (Jain et al., 2018).

Use of the trypan blue dye exclusion assay showed membrane damage in two human lymphoid cell lines (IM-9 and MOLT-4) at T-2 concentrations of 0.2 and 0.6 ng/mL, respectively, after 24 hours (Minervini et al., 2005). Cytotoxicity was evident in the human epithelial and Hep-2 cell lines at ≥100 ng/mL; conversely, CaCo-2 cells were resistant to T-2 exposure at concentrations between 100 and 1000 ng/mL (Calvert et al., 2005). Treatment of human gastric epithelial GES-1 cells with 5 nM of T-2 for 24, 48 and 72 hours caused 6%, 42% and 63% cell death respectively (assessed with the MTT assay). T-2 strongly reduced the viability of GES-1 cells in a time- and dose-dependent manner within a small range of concentrations. However, when the concentrations of T-2 were >40 nM, there was no concentration dependence, only time dependence. These results indicated that even at a very low concentration, T-2-induced a strong reduction in GES-1 cell viability, especially with prolonged exposure. The same study also showed that T-2 caused accumulation of ROS and decreased the mitochondrial membrane potential (Su et al., 2020). Elsewhere, it was suggested that the oxidative stress plays an important role in the cytotoxic effect of T-2 on Leydig cells, as assessed by measuring cell viability, LDH, malondialdehyde, antioxidant activity (SOD), DNA damage and apoptosis induced by T-2 (Yuan Z et al., 2016). However, presence of antioxidants (vitamin C, vitamin E and selenium) decreased but did not completely inhibit T-2-induced cytotoxicity (neutral red assay) in human gingival fibroblast, human colorectal adenocarcinoma (SW742), and human hepatoma (HepG2) cell lines at concentrations ranging from 0.25 to 5.5 ng/mL. The authors of the study suggested that, besides lipid peroxidation, other mechanisms may be involved in T-2-induced cytotoxicity (Shokri et al., 2000). Earlier cellular events, such as apoptosis, may contribute to cytotoxicity.

For instance, little cytotoxicity (plasma membrane damage) was observed even after exposure to 25–50 ng/mL of T-2 and HT-2, concentrations that induced apoptosis in 60–100% of HL-60 cells (Holme et al., 2003). In human RPTEC and normal human lung fibroblasts, cytotoxicity (IC_{50}) was evident at 200 and 500 nM, whereas apoptosis was evident at 100 nM (Königs et al., 2009). Finally, T-2-reduced cell viability in Vero cell line was correlated to induction of lipid peroxidation impairment at the macromolecule levels (protein, DNA and RNA), DNA fragmentation, depletion of mitochondrial membrane potential, and induction of caspase-3-dependent apoptosis (Bouaziz et al., 2006).

The in vitro effect of T-2 on the integrity of intestinal porcine epithelial cells derived from the jejunum (IPEC-J2) cells was investigated using the transepithelial electrical resistance and passage of the antibiotics doxycycline and paromomycin. T-2 decreased transepithelial electrical resistance and increased cellular permeability to the antibiotics in a time- and concentration-dependent manner, suggesting that T-2 may severely disturb the intestinal epithelial barrier (Goossens et al., 2012b). T-2 also induced a concentration-dependent reduction in claudin-3, claudin-4 and occludin mRNA expression levels in Caco-2 cells treated with 1, 3, 10 and 30 μM T-2 (Romero et al., 2016). It is possible that activation of MAPK may be related to the effects on tight junction structure and function of gut epithelial cells (Pinton et al., 2012). T-2 also caused significant barrier disruption, altered protein and mRNA expression of junctional proteins, and induced irregular cellular distribution in human placental epithelial cells. These changes in placental barrier may disturb the maternal-fetal interaction and adversely affect fetal development (Toutounchi et al., 2019). T-2-induced cytotoxicity in Sertoli (SerW3) cells, at a T-2 concentration range of 12–1200 ng/mL, was found to be associated with impaired cell junctional barrier integrity, as evident with a concentration-dependent decrease in transepithelial/transendothelial electrical resistance, and in the expression of junctional proteins such as occluding, ZO-1, N-cadherin and β-catenin (Karacaoğlu & Selmanoğlu, 2017).

(c) Effects on protein synthesis

T-2 and other trichothecenes have the potential to inhibit protein synthesis at the initiation and/or termination of the chain elongation steps by binding to the 60S ribosomal subunit and inhibiting the peptidyl transferase activity (Arunachalam & Doohan, 2013; EFSA, 2017a). Using an in silico analysis, it was suggested that glycosylation at position 3 of the trichothecene molecule seems to diminish ribotoxicity (Dellafiora, Galaverna & Dall'Asta, 2017).

Proteomic analysis conducted with comparative 2D electrophoresis with matrix-assisted laser desorption ionization–time-of-flight mass spectrometry

identified 53 proteins that were either up- or down-regulated (by at least 1.3-fold) in chicken hepatocytes treated with 50 ng/mL of T-2 for 48 hours. Most of the proteins identified were associated with cell redox homeostasis (15%), cell cycle or cell proliferation regulation (15%), transcriptional or translational (13%), stress response (11%), lipid metabolism (11%), transport (9%), protein degradation (7%), and carbohydrate metabolism (5%). Subcellular localization analysis indicated that the affected proteins were localized in the mitochondria (34%), cytoplasm (24%), nucleus (15%), cytosol (7%), plasma membrane (6%), extracellular space (6%), ribosome (4%), peroxisome (3%) and Golgi apparatus (1%) (Mu et al., 2013).

The metabolic profiles of bodily fluids and multiple organs of rats treated with T-2 (single gavage dosages of 0, 0.5, 2.0 and 4.0 mg/kg bw) showed inhibition of protein and DNA biosynthesis and changes in the levels of membrane metabolites, TCA cycle intermediates, a range of amino acids, nucleosides and nucleotides (Wan et al., 2015b). The same group (Wan et al., 2015a) used an integrated transcriptomics, proteomics and RNA interference approach to investigate mechanistic aspects of T-2-induced cytotoxicity in rat pituitary adenoma GH3 cells treated with 10, 20 or 40 nM of T-2 for 4 to 24 hours. An overlap of 921 upregulated and 666 downregulated genes was seen in the animals treated with 10 and 40 nM, with most being associated with protein synthesis and modification. At the 40 nM dose, after 12 and 24 hours of treatment, approximately 25.8–26.7% of the altered genes were associated with protein transcription, translation, modification and degradation, 8.1–11.0% with signalling transduction, 7.7–17.7% with stress-related proteins, and to a lesser extent with ion channel-related proteins, antioxidants, metabolism-related proteins and cytoskeletal proteins. The authors of the study concluded that T-2 inhibited transcription and translation initiation in the pituitary cells by suppressing transcription of aminoacyl-tRNA synthetases, inducing eukaryotic translation initiation factor 2-alpha kinase 2 and reducing the eukaryotic translation initiation factor 5a. T-2 also inhibited the synthesis of proteins by regulating the expression of protein-folding-related proteins and disturbed protein processing and transportation. For instance, T-2 downregulated the expression of sulfhydryl oxidases and protein disulfide isomerases whose function is to directly target disulfide bond formation and protein folding. In addition, differential expression of heat shock proteins (for example, Hsp 90) may be indicative of the involvement of T-2 in the endoplasmic reticulum stress response and on the unfolded protein response.

(d) Apoptosis and oxidative stress

T-2 has the potential to cause apoptosis in vitro in both normal cells (for example, chondrocytes, ovarian granulosa cells, hepatocytes and CD34+ haemopoietic

cells) and immortalized cell lines (for example, HL-60, HepG2 and 3T3 fibroblasts), or in vivo in various organs and fetal tissues.

In vitro

Key cellular apoptotic events such as DNA degradation or the involvement of various caspases, p53 and BCL-2 family proteins were determined with an array of standard analytical methods including microscopy, DNA gel electrophoresis and flow cytometry (Holme et al., 2003).

T-2 induced concentration-dependent apoptosis in HL-60 cells at concentrations as low as 3.1 ng/mL and 12.5 ng/mL, after 24 hours and 4–6 hours of exposure, respectively, whereas HT-2 caused apoptosis at concentrations ≥6.3 ng/mL in the same cell line (Holme et al., 2003). Incubation of human haemopoietic progenitors ($CD34^+$ cells) with various concentrations of T-2 (10^{-9} to 10^{-7} M), resulted in concentration- and time-dependent increases in apoptotic cells after only 3 hours of exposure and maximum numbers were reached after 12 hours (Le Dréan et al., 2005). Treatment of gastric epithelial (GES-1) cells at T-2 concentrations of 2, 4 or 8 nM for 72 hours resulted in 23.9%, 47.4% and 70.7% increases in the incidence of apoptotic cells, respectively (Su et al., 2020).

T-2 can cause apoptosis at relatively low concentrations via multiple pathways. Most studies that focus on understanding the process of T-2-induced apoptosis suggest that intrinsic (i.e. mitochondrial related mechanisms) rather than extrinsic mechanisms appear to be involved. However, the intrinsic mitochondrial pathway of apoptosis may be triggered by intracellular stimuli such as increased levels of ROS, DNA damage and endoplasmic reticulum (ER) stress, and it is not clear whether increased levels of intracellular ROS are causing mitochondrial dysfunction or vice versa. Evidence of oxidative stress has been reported in many experimental animal studies in various tissues (see sections 2.2.1, 2.2.2 and 2.2.5).

In HeLa and Bel-7402 cells treated with T-2, caspase-8 (implicated mainly in the extrinsic or death receptor pathway) was hydrolysed and activated at 16 and 24 hours, respectively, whereas caspase-9 (implicated mainly via the intrinsic or mitochondrial pathway) was activated earlier at 8 and 16 hours, respectively. Since caspase-9 was activated earlier than caspase-8, the authors of the study suggested that T-2-induced apoptosis is mediated via the intrinsic or mitochondrial pathway (Zhuang et al., 2013). Nagase et al. (2001) showed that internucleosomal DNA fragmentation in human leukaemia HL-60 cells exposed to T-2 was attributable to the release of cytC in the cytoplasm, another key event in the intrinsic pathway for apoptosis. Similarly, increases of the pro-apoptotic Bcl-2 and Bax proteins were seen in apoptotic human chondrocytes

exposed to 1–20 ng/mL T-2 (Chen et al., 2006). Furthermore, Bax relocation into the mitochondrial outer membrane, loss of the mitochondrial transmembrane potential, permeability transition pore complex opening, and cytochrome C release in the cytoplasm were seen in HepG2 cells (Bouaziz et al., 2008, 2009). The mitochondrial apoptotic pathway was also implicated in increases of apoptotic human HL-7702 hepatocytes (Yang J et al., 2020), mouse Leydig cells (Zhang Y et al., 2018; Wu J et al., 2018; Zhang YF et al., 2020) and ovarian granulosa cells (Yang F et al., 2019a). In contrast, Huang et al. (2007) suggested that apoptotic signals were mainly transmitted via caspase-8 (for example, via an extrinsic death receptor pathway) in human myeloid leukaemia (U937) cells. Murshedul et al. (2000) exposed *lpr*, MRL/MpJ- +/+, *gld* and C3H/HeJ mice to a single intraperitoneal dose of 2 mg/kg bw T-2 and measured the levels of thymocyte apoptosis. *Lpr* and *gld* mice are deficient in Fas and FasL genes, respectively, while MRL/MpJ- +/+ and C3H/HeJ mice are the respective wild types. According to Murshedul et al. (2000) the apoptotic responses in the thymocytes of *lpr* and *gld* mice were essentially of similar magnitude to those in the wild-type mice and, consequently, Fas/FasL are probably not involved in T-2-induced apoptosis. The Fas/FasL pathway is involved in extrinsic mechanisms of apoptosis and FAS levels are high in CD4+CD8+ thymocytes and FasL is present on thymic epithelial cells and dendritic cells.

Notably, the intrinsic mitochondrial pathway of apoptosis may be triggered by intracellular stimuli such as increased levels of ROS, DNA damage and ER stress. In epithelial cells of goat endometrium, IRE1-JNK and PERK-ATF4-C/EBP homologous protein (CHOP) signal transduction pathways were both activated, suggesting that ER stress contributes to the T-2-induced apoptosis (Yi et al., 2018). Activation of the above transcription factors as well as responses of the upstream PERK, IRE1 and ATF6 sensors and other signalling nodes such as c-Jun N-terminal kinase 1 (JNK) are central to the unfolded protein response, which is responsible for restoring ER homeostasis (Fribley, Zhang & Kaufman, 2009). During sustained ER stress or irreparable ER damage, phosphorylation of JNK and induction of CHOP disturbs the balance between pro-apoptotic and anti-apoptotic Bcl-2 proteins causing an increase of ROS and Ca^{2+} transfer to the mitochondria, which leads to the release of cytC into the cytoplasm (Iurlaro & Muñoz-Pinedo, 2016). Increases of cytosolic Ca^{2+} concentrations, suggested to be involved in the activation of several caspases, resulting in DNA fragmentation, chromosomal condensation and nuclear fragmentation, were observed in HL-60 cells treated with T-2 and HT-2 (Holme et al., 2003). T-2-induced increases in cellular ROS, lipid and DNA oxidative damage, loss of mitochondrial transmembrane potential and apoptosis were observed in embryonic stem cells and fibroblast 3T3 cells at concentrations >1 ng/mL (Fang et al., 2012). Elevated ROS levels accompanied by losses of activity of antioxidant markers such as SOD

and CAT were seen in rat granulosa cells (Wu J et al., 2013) and bovine granulosa cells (Yang F et al., 2019b) treated with T-2. HT-2 exposure induced oxidative stress and resulted in mouse oocyte apoptosis, as shown by ROS accumulation, increased SOD mRNA level, increased expression of the early apoptosis marker annexin V, and increased caspase-3 and bax mRNA levels (Zhu et al., 2016). Some authors have suggested that increases of cellular ROS levels occurred before any alterations in mitochondrial dynamics or injury were seen in human HepG2 and HeLa cells (Bouaziz et al., 2008; Yang J et al., 2020). Conversely, it was suggested that DNA damage and apoptosis, as well as ROS accumulation in GES-1 cells, starts with T-2 directly perturbing the mitochondria, triggering ROS generation by acting on CypD and VDAC1 (Su et al., 2020).

There is an implication that other intracellular molecular factors may be involved in the intrinsic mitochondrial apoptotic pathway. For instance, T-2 exposure induces apoptosis in podocytes (TM3 cells) by inhibiting mTORC2/AKT (mammalian target of rapamycin) to promote Ca^{2+} production (Wang et al., 2018). Oxidative stress may also promote Fas upregulation, activate p53 and/or several other protein kinases (for example, p38 MAPK), increase the expression of heat shock protein (Hsp70), increase inducible nitric oxide synthase activity and decrease the expression of Nrf2, events that are also linked to mitochondrial damage (Da Silva, Bracarense & Oswald, 2018; Zhang X et al., 2018). Agrawal, Bhaskar & Rao (2015) suggested that T-2 can induce apoptosis in human neuronal cells through multiple signal transduction pathways at relatively low concentrations (nM). Regardless of the exact molecular steps leading to apoptosis, most studies indicate that mitochondria appear to play a central role.

In vivo

Nagata et al. (2001) exposed female BALB/c mice to a single dose of 0 or 10 mg/kg bw T-2 via oral gavage (25 animals per dose). The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Following exposure, five animals per dose were sequentially sacrificed 1, 3, 6, 12 and 24 hours after dose administration, and the thymus, MLN and Peyer's patches from the small intestine were analysed. Body weight loss, diarrhoea and rough coat were observed in treated animals before sacrifice. The viable cell count in the thymus, MLN and Peyer's patches in the small intestine decreased significantly between 1 and 24 hours after dosing. Correspondingly, signs of apoptosis were evident, particularly within the thymus. Based on the time-course of the effects, the authors suggested that T-2 affects the Peyer's patch first, followed by the MLN and finally the thymus. T lymphocyte subsets in the thymus, MLN and the Peyer's patch were decreased. Statistically significant decreases were observed,

specifically of CD4+CD8+ and CD4+CD8– in the thymus, and CD3+ and CD4+ in the MLN. Statistically significant decreases were not observed in the Peyer's patch; however, IgM+ and IgA+ B lymphocytes in the Peyer's patch were significantly decreased.

Chaudhari et al. (2009a) investigated T-2-induced alterations in oxidative stress parameters in male Swiss albino mice (four per dose) following exposure to 0, 5.61 or 11.2 mg/kg bw T-2 via intraperitoneal injection. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Compared to control mice, exposure to 5.61 or 11.2 mg/kg bw induced a significant increase in ALT and a decrease in AST and LDH, 4 hours after administration. However, no effect on relative liver weight was observed. No significant increase in expression of heat shock protein 70 (HSP-70) in the liver was observed in animals dosed with 5.61 mg/kg bw; however, animals that received 11.2 mg/kg bw T-2 showed a 1.65-fold increase in expression compared to control animals. A slight increase in hepatic glutathione was observed at 5.61 mg/kg bw T-2, but a significant decrease was observed at 11.2 mg/kg bw T-2. A threefold increase in hepatic MDA levels was observed following exposure to 5.61 and 11.2 mg/kg bw T-2 compared to control mice. An increase in the hepatic activity of antioxidant enzymes, glutathione-*S*-transferase (GST), glutathione peroxidase (GSH-Px) and CAT, was observed. In contrast, the activity of SOD was significantly decreased at a dose of 11.2 mg/kg bw T-2. Additionally, gene expression analysis showed downregulation in hepatic GST, GSH-Px and CAT.

Al-Sien & Al-Seni (2014) investigated the effects of goji extract and charcoal on the acute toxicity of T-2 in male white MF1 mice (10 per group) exposed to 0 or 0.2 mg/kg bw T-2 via intraperitoneal injection. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. T-2 exposure significantly increased blood concentrations of ALT, ALP, total lipids, total antioxidants (TAS) and TNF compared to control mice. Goji extract appeared to have significantly attenuated all the elevated blood parameters affected by T-2 exposure. The attenuating effects of charcoal on T-2-elevated blood parameters were limited to TAS and ALT.

Vilà et al. (2002) examined the effects of vitamin E status on T-2-induced toxicity in female CD-1 mice. Groups of mice (four per dose) were administered a single dose of 0, 1, 2, 3 or 4 mg/kg bw T-2 via gavage and fed basal diets containing either normal (60 IU/kg) or high (500 IU/kg) concentrations of vitamin E. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Treatment with 4 mg/kg bw induced a significant decrease in plasma α-tocopherol (vitamin E) and an increase in oxidative lipid injury (as indicated by MDA levels) in the liver. Seventy-two hours after treatment with 4 mg/kg bw

T-2, animals showed significantly increased absolute liver weight and decreased absolute thymus and spleen weights. T-2 had no effect on the liver at doses ≤2 mg/kg bw when dietary vitamin E levels were normal or high. Supplementation with vitamin E appeared to decrease the extent of oxidative lipid injury in the liver caused by T-2 exposure.

Zhu et al. (2020), Kong et al. (2021) and Luo et al. (2020) investigated the effects of betulinic acid exposure on T-2-induced oxidative damage in the thymus, spleen or intestines of male Kunming mice exposed to 4 mg/kg bw T-2 via intraperitoneal injection. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. T-2 exposure alone significantly increased ROS production and MDA content in the thymus, decreased the content of IgG in serum, and decreased the levels of SOD and GSH in the thymus (Zhu et al., 2020). In the spleen, accumulation of ROS, decreased SOD activity, increased MDA content, as well as decreased T-AOC was observed (Kong et al., 2021). Kong et al. (2021) also noted that T-2 alone caused a significant increase in total cholesterol and a decrease in triglycerides and lymphocytes. Luo et al. (2020) reported that T-2 exposure decreased CAT, GSH-Px and GSH, and increased MDA in the intestines. Betulinic acid appears to attenuate all of these effects in male mice exposed to T-2.

Guo et al. (2018) investigated the neurotoxic effects of acute T-2 exposure in the brains of female Wistar rats. Groups of rats (five per group) were exposed to single doses of 0 or 2 mg/kg bw T-2 via gavage and sacrificed either immediately or 1, 3 or 7 days following dosing. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. The authors reported that, following exposure to T-2, the rats exhibited "frightened mental status" which lasted for 3 days. Although only low concentrations of T-2 were detected in the brain (0.012 µg/kg), 3 days following exposure, pathological lesions were observed (bleeding of the cerebral cortex and expression of autophagy-related genes). Seven days following exposure, lesions in the pituitary (congestion of the anterior pituitary and expression of apoptosis-related genes) became apparent.

Evidence of oxidative stress or apoptosis has also been observed in the developing fetus of mice (Ishigami et al., 2001); in the kidney, liver, blood and placenta of rats (Sehata et al., 2003; Sehata et al., 2005; Raut et al., 2013; Chandratre et al., 2014; Rahman et al., 2016; Fadhil, Alkutbi & Nassir, 2021; Zhang X et al., 2021); in the intestines, liver, kidneys, spleen, thymus and blood of rabbits (Kovács et al., 2013; Szabó et al., 2014, 2016; Hafner et al., 2016; Liu et al., 2020, 2021a,b; Zhang Z et al., 2022); in the blood, liver and urine of pigs (Frankič, Salobir & Rezar, 2008; Meissonnier et al., 2008); and in the intestines, MLN and

livers of goats (Nayakwadi et al., 2020). Additional information on these studies can be found in sections 2.2.2 and 2.2.5.

(e) Altered resistance to infection and miscellaneous effects on the immune system

Li, Cuff & Pestka (2006) investigated whether T-2 exposure interferes with the gut mucosal immune response in female Balb/c mice (number not reported) following exposure to 0, 0.05, 0.2, 0.5, 1.0 or 2.0 mg/kg bw T-2 via intraperitoneal injection. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Two hours following T-2 administration, mice were exposed to 10^7 plaque-forming units (PFU) of reovirus, strain Lang (T1/L) or saline vehicle via oral gavage. Reovirus L2 RNA concentrations in the faeces 4 days after infection were significantly elevated in a dose-responsive fashion in mice treated with T-2 at doses ≥0.05 mg/kg bw (data provided in a figure, raw or summary data not published). In a separate experiment using 1.75 mg/kg bw T-2, T-2 exposure induced an increase in intestinal plaque-forming viral titres after 5 days and detection of the virus in the faeces was significantly higher at 1, 3, 5 and 7 days in T-2-treated mice compared to control animals. Exposure to 1.75 mg/kg bw T-2 also suppressed the induction of reovirus-specific IgA in faeces (from the gastrointestinal-associated lymphoid tissues) and IgA and IgG_{2a} in the serum. Correspondingly a decreased secretion of reovirus-specific IgA and IgG_{2a} was also observed in Peyer's patch and lamina propria fragment cultures prepared 5 days after infection.

Li et al. (2006) investigated whether T-2 exposure alters host resistance to lung infection by reovirus in female Balb/c mice (12 per group) following exposure to 0, 0.02, 0.2, 0.5, 1.0 or 2.0 mg/kg bw T-2 via intraperitoneal injection. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Two hours following T-2 administration, mice were intranasally instilled with 10^7 PFU of reovirus, strain Lang (T1/L) or saline vehicle. Reovirus L2 RNA concentrations in the lung 4 days after infection were significantly elevated in mice treated with T-2 at doses ≥0.2 mg/kg bw (data provided in a figure, raw or summary data not published). In a separate experiment using 1.75 mg/kg bw T-2 (n = 6), T-2 exposure increased lung viral burden, bronchopneumonia and pulmonary cellular infiltration in reovirus-infected mice.

Shakhov et al. (2016) examined the immune response of albino outbred rats to inoculation with *Salmonella* following 6 days of exposure to oral doses of T-2. The rats (24 per dose; sex not specified) were exposed to 0, 140 or 560 µg/kg bw per day T-2 for 6 days. Although not explicitly stated, the method of dosing was assumed to be gavage to non-fasted animals. The source and purity of the

T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. One day after the sixth day of exposure, half of the animals in each dose group were inoculated (intra-abdominally) with 1.9 billion cells of *Salmonella* and monitored for clinical signs for 6 days (non-exposure period). On the seventh day of postexposure observation, the animals were killed, and the blood was analysed for various cytokines. T-2 exposure significantly altered cytokine profile following infection with *Salmonella*. For example, T-2-exposed animals showed increased IL-1β and IL-4, and decreased TNF-α, IL-8, IL-2, IFN-γ, IL-10, and IFN-γ/IL-4 ratio. The authors suggested that these changes are indicative of a T-cell deficit and suppression of T-helper 1 lymphocyte function.

Verbrugghe et al. (2012a) investigated the effects of T-2 on *Salmonella* Typhimurium infection in pigs (commercial closed line based on Landrace; ~4 weeks old; five per dose) exposed to feed containing 0, 15 or 83 μg/kg diet T-2 for 23 days. The purity of T-2 used in this experiment was not reported. The basal feed did not contain any detectable concentrations of T-2 or other mycotoxins, as confirmed by multimycotoxin LC-MS/MS. On day 18 of exposure, the pigs were inoculated orally with 2×10^7 CFU of *Salmonella* Typhimurium WT_{nal}. At the end of the exposure period, the pigs were sacrificed and samples of tonsils, ileocaecal lymph nodes, duodenum, jejunum, ileum, caecum, colon, contents of caecum and colon and rectal faeces were analysed. The average daily body weight gain of animals in the high-dose group was significantly lower than that of control animals (for example, 27% of control) prior to inoculation. Since the feed intake values were not reported, the doses received by the animals are unknown. Although no significant difference in mRNA expression of IL-6, IL-8, IL-12, IL-18, TNF-α, IFN-γ and MCP-1 in response to *Salmonella* Typhimurium infection was observed in the intestinal samples of treated animals compared to controls, a statistically significant decrease in mRNA expression of IL-1β in pigs exposed to 15 μg/kg diet compared to controls was observed. The decreased mRNA expression of IL-1β cytokine is of questionable toxicological significance, especially considering that no difference was observed at a concentration of 83 μg/kg diet. Although T-2 treatment significantly reduced the numbers of *Salmonella* Typhimurium organisms per gram in the bowel contents of the treated pigs, Verbrugghe et al. (2012a) suggested that, based on in vitro examination, T-2 promotes the susceptibility of porcine macrophages and intestinal epithelial cells to *Salmonella* Typhimurium invasion and promotes the translocation of *Salmonella* Typhimurium over an intestinal porcine epithelial cell monolayer. Verbrugghe et al. (2012a) also suggested that the in vivo decrease of *Salmonella* Typhimurium in the bowel contents might be due to T-2's direct effect on the motility and pathogenicity of the bacteria. For example, T-2 exposure in vitro significantly decreased the motility and invasiveness of *Salmonella* Typhimurium.

In a separate study investigating dietary exposure of pigs to 83 µg/kg diet T-2, Verbrugghe et al. (2012b) suggested that the addition of dietary glucomannan appears to attenuate T-2-induced toxicity (i.e. reduced body weight gain) in pigs and further reduces caecal colonization by *Salmonella* Typhimurium.

Wojtacha et al. (2021) investigated the effects of T-2 exposure on the gut-associated lymphoid tissue (GALT) of pigs. Polish large white prepubertal gilts aged 8 weeks (15 per group) were administered 0 or 14.5 µg/kg bw per day T-2 via capsule, and sacrificed following 14, 28 or 42 days of exposure (five animals per group per sacrifice). The purity of T-2 used in the experiment was not reported. Basal feed did not contain any detectable concentrations of T-2, aflatoxin B1, ochratoxin A, ZEA, alphazearalenone or DON. Immediately after sacrifice, segments of the ileal wall were sampled approximately 2 cm before the ileocaecal valve. Flow cytometric analysis showed that the average percentage of CD2+ T cells in the treated group was significantly lower than in control animals following the 28-day sacrifice; no significant difference from control animals was observed on days 14 or 42. TCRγδ+ lymphocytes in the treated groups were significantly higher than in the control animals on day 14; no significant difference from control animals was observed on days 28 or 42. The percentages of CD4+ Th helper cells, CD8+ cytotoxic T cells and CD4+CD8+ T cells were not significantly different from those of control animals. Although CD5+ B cells in treated animals did not differ from control animals, the CD21+ B cells were significantly lower in treated animals than in control animals on days 28 and 42. T-2 exposure also altered pro-inflammatory cytokines in the ileal mucosa. For example, on day 42, significant increases in the concentration of IFN-γ, IL-2, IL-12/23p40, IL-17 A and IL-1β were observed in treated animals compared to control animals. Finally, T-2 altered the concentrations of various anti-inflammatory and regulatory cytokines. For example, treated animals showed higher IL-4 and IL-10 concentrations on day 14, and lower concentrations of TGF-β on days 14, 28 and 42, compared to control animals. The authors suggested that these results show the T-2 exposure stimulated the GALT and compromised the integrity of the intestinal mucosal barrier.

Salimian et al. (2014) investigated the effects of selenium and vitamin E on T-2-induced immunotoxicity in male Balb/c mice. Groups of eight mice were exposed to 0 or 2 mg/kg bw T-2 via intraperitoneal injection, and alterations in lymphocyte populations were observed between 12 and 72 hours post-dosing. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. T-2 significantly decreased the percentage of CD8+ lymphocytes in the blood 12 hours after exposure, whereas the percentages of CD3+ and CD4+ lymphocytes were significantly increased 24 hours after exposure. The percentage of CD8+, CD3+ and CD4+ lymphocytes had returned to control levels by 48

hours. Although concurrent and pre-dosing injection of 3 mg/kg bw selenium or 14.3 mg/kg vitamin E attenuated T-2's effect on CD8+ lymphocytes, only concurrent injection with selenium mitigated T-2's effect on CD3+ and CD4+ cells.

Obremski et al. (2013) investigated the effect of T-2 exposure on the cellular immune response in the GALT of 2-month-old female Polish large white pigs (15 per dose) that were exposed to feed containing 0 or 0.2 mg/kg diet T-2. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Following 14, 28 or 42 days of exposure, five animals per group were sacrificed and sections of the ileum were analysed for various cellular immune parameters (for example, lymphocyte subpopulations and gene expression profiles of cytokines). Body weights and feed consumption were not reported. However, alterations in the percentage of various lymphocytes and the expression of certain cytokines were observed. For example, increased percentages of CD8+ (on days 14 and 42; and a decrease on day 28), decreased CD21+ (on days 28 and 42), and decreased CD4+CD8+ (on days 14 and 28) were observed in the Peyer's patches of treated animals compared to control animals. Additionally, a significant decrease in the mRNA concentrations of IL-10 and a gradual, but not statistically significant, decrease in IL-4 and IFN-γ transcripts was also observed in the Peyer's patches of treated animals compared to control animals.

Guo et al. (2020) investigated the inflammatory response induced by acute T-2 exposure in male Kunming mice. Groups of animals (three per group) were administered a single dose of 0 or 2 mg/kg bw T-2 via gavage. The purity of T-2 used in this experiment was not reported. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. All animals were killed 24 hours after exposure and the spleens examined. Compared to control animals, the treated animals showed blurred demarcation between red and white medulla and irregularly shaped splenic nodule lymphocytes. Additionally, a small number of extramedullary haemopoietic cells and an increase of multinucleated giant cells were observed in the red medulla. The expression of miR-155-5p and IL-6 was significantly upregulated in treated animals compared to control animals.

Semenov et al. (2018) investigated the effects of T-2 exposure on the sensitization response of male rats exposed to ovalbumin. Male Wistar rats (20 per group) were exposed to feed containing 0 or 50 mg/kg diet T-2 for 21 days. The T-2 used in this experiment was obtained from "grain substrate inoculated with toxicogenic strains of fungus" and had a purity of 97.8%. No indication of the background concentration of T-2 or any other mycotoxin in the animal feed was provided. Half of the animals in each group acted as non-sensitized controls. The other half underwent sensitization to ovalbumin; induction took place on days 1,

3 and 5 and elicitation occurred on day 21. The severity of systemic sensitization was graded, and blood samples were taken to measure specific antibodies to the allergen. Feed intake and body weights were not reported. Although the clinical observations from control and T-2-treated animals appeared similar following sensitization with ovalbumin, the concentration of circulating IgG antibodies to ovalbumin in T-2-treated animals was significantly higher than in untreated controls (6.4 versus 4.6 mg/mL). T-2 treatment also significantly increased the permeability of the intestinal mucosa compared to control animals (as measured by the increased percentage of PEG 4000 absorbed by the treated animals).

(f) Related contaminants, modified forms and metabolites

Mycotoxins are a diverse set of chemical compounds produced by fungi, which can contaminate multiple components of human food and animal feed and, consequently, co-occurrence of mycotoxins in food and feed is common (Shi et al., 2018). However, most regulatory limits are based on considerations of the toxicity of single exposures (COT, 2021) and it is very rare that co-occurrence of multiple mycotoxins is examined at a single sample level (Battilani et al., 2020). The Committee's previous evaluation of DON (Annex 1, reference *153*) highlighted the need for studies on the combined effects of DON and other trichothecenes that may be present in human food. As the trichothecenes have similar toxic properties, albeit with different potencies, the Committee recommended that toxic equivalency factors be developed, if sufficient data become available. According to more recent reviews, Battilani et al. (2020) concluded that the toxicological information on co-exposure to mycotoxins is scarce. COT (2021) noted that most of the studies available cover only a limited number of mycotoxin combinations and are generally more focused on animal models of agricultural importance. COT (2021) also noted that the toxicity of combinations cannot be predicted based on the toxicity of individual mycotoxins and Grenier & Oswald (2011) suggested that the interactions between mycotoxins are complex and may vary according to animal species, dose, length of exposure and effect measured.

Although a thorough review of the combined effects of all trichothecenes is beyond the scope of this monograph addendum, a summary of the recent (since the Committee's original evaluation; Annex 1 reference *153*) research on T-2 and/or HT-2 and their potency or behaviour in relation to other contaminants is provided below. Studies that exposed experimental animals or cell lines to characterized mixtures of mycotoxins from mouldy feedstuffs without a concurrent comparison of effect with T-2 alone were not considered for this summary.

The combined toxicity of T-2/HT-2 and DAS has been addressed in a previous evaluation by the Committee (Annex 1, reference *235*). According to

the monograph for DAS (Annex 1, reference *235*), the few studies that considered the combined effects of DAS and T-2 observed a consistent additive dose effect for end-points such as in vitro inhibition of protein synthesis and lymphocyte proliferation. Additionally, oral lethal doses following acute exposure and the incidence of oral lesions, feed refusal and decreased egg production following short-term dietary exposure in chickens displayed additive dose effects. However, the previous Committee also noted that although T-2 appears to be more potent than DAS in vitro and in vivo, the available data are insufficient for establishing relative potencies.

In vitro

Tajima et al. (2002) investigated toxicological interactions (inhibition of DNA synthesis after 24 hours of exposure) of T-2, DON, NIV, ZEA and FB1 in mouse fibroblast L-929 cells. At high concentrations, a mixture comprising all five compounds was observed to have a less than additive action on DNA inhibition; however, at lower concentrations, additivity was observed for the five-substance mixture. When examining interactions between specific combinations of mycotoxins, a significant interaction between T-2 and NIV was noted. Specifically, a synergistic interaction between NIV and T-2 on DNA inhibition was observed.

Using the MTT assay, Königs et al. (2009) investigated the cytotoxicity of T-2, HT-2, NEO, T-2-triol and T-2-tetraol in primary human RPTEC and primary human lung fibroblast cells. The cells were exposed to the mycotoxins at concentrations between 0.1 nm and 100 µM for 24 hours. T-2 showed the lowest IC_{50} values in both cell cultures (0.2 and 0.5 µM, respectively) followed closely by HT-2 (0.8 and 0.7 µM, respectively). Higher IC_{50} values (>2 µM) were reported for NEO, T-2-triol and T-2-tetraol.

Ruiz et al. (2011a,b) investigated the toxicological interaction (cytotoxicity following 24–72 hours of exposure) of beauvericin (BEA), DON and T-2 in Chinese hamster ovary-K1 (CHO-K1) and mammalian kidney epithelial (Vero) cells. Based on the results of the individual substances, T-2 appeared to be the most potent, whereas BEA was the least potent in both cell lines. Mixtures of T-2 and BEA appeared more potent in CHO-K1 cells than combinations of BEA and DON, and T-2 and DON. For example, BEA and DON and T-2 and DON showed antagonistic interaction at 24, 48 and 72 hours, whereas the T-2 and BEA combination showed synergism at all times of exposure in CHO-K1 cells. The combination of all three demonstrated synergism after 24 and 48 hours of exposure in CHO-K1 cells; however, a moderate antagonistic effect was observed after 72 hours of exposure at the lower concentrations. In the Vero cells, antagonism was observed in response to all combinations following 24, 48 and

72 hours of exposure, with the greatest degree of antagonism coming from the combination of DON and T-2.

Behm, Föllmann & Degen (2012) used the neutral red assay to investigate the cytotoxicity of BEA, citrinin, enniatin B, moniliformin (MON), ergocornine, ergotamine, FB1, OTA, patulin, DON, T-2, HT-2, ZEA and α-ZEA in Chinese hamster lung fibroblast cells (V79). The cells were exposed to graded concentrations of the mycotoxins for 48 hours. T-2 and HT-2 were the most potent, with IC_{20} values of 2.1 and 9.3 nM, respectively. Starting with DON, all the rest of the mycotoxins were more than two orders of magnitude less potent, with IC_{20} values of ≥500 nM.

Smith et al. (2017, 2018) investigated the toxicological interaction (cytotoxicity following 48 hours of exposure) of DON, NIV, FB1, ZEA, MON and T-2 in human monocytic leukaemia cells (THP-1) and human hepatoma cells (HepaRG). In THP-1 cells, T-2 was the most cytotoxic followed by NIV, DON, MON, FB1 and ZEA. In HepaRG cells, T-2 was the most cytotoxic followed by NIV and DON. ZEA, MON and FB1 were much less toxic, with significantly lower IC_{50} values (>10 μM). In both cell lines, mixture analysis was limited to NIV and T-2. In THP-1 cells, an additive interaction was observed for the NIV and T-2 mixture at low (for example, 0.01 μM NIV + 0.075 nM T-2) concentrations and an antagonistic relationship at higher (for example, 10 μM NIV + 0.075 μM T-2) concentrations. In contrast, in the HepaRG cells, isobologram analysis indicated that the NIV and T-2 mixture led to synergism at low (for example, 0.6 μM NIV + 0.04 μM T-2; CI = 0.48) and high (for example, 10 μM NIV + 0.67 μM T-2; CI = 0.49) concentrations, and antagonism at medium (for example, 3 μM NIV + 0.2 μM T-2; CI = 2.28) concentrations.

Somoskői, Kovács & Cseh (2018) investigated the combined effects of T-2 (0.5 ng/mL) and FB1 (1, 2 and 10 ng/mL) on early embryo development using isolated zygotes from BDF1 mice. Following 96 hours of exposure, the proportion of blastocysts was similar in T-2- and FB1-treated embryos. Conversely, the proportion of blastocysts decreased significantly when T-2 and FB1 were combined (for example, 0.5 ng/mL T-2 + 1, 2 or 10 ng/mL FB1). Individually, both T-2 and FB1 decreased the proportion of late blastocysts; however, combined treatment significantly lowered the proportion of late blastocysts compared to the separate exposures.

Lin et al. (2019) investigated the individual and combined effects of DON (5–10 000 nM) and T-2 (0.1–1000 nM) in human C28/I2 chondrocyte cells and newborn rat primary costal chondrocytes. Following 48 hours of exposure, T-2 was 285-fold more cytotoxic than DON in human chondrocytes and 22-fold more cytotoxic in the rat chondrocytes. Additivity, antagonism and synergism were all observed in the binary mixtures of DON and T-2. Based on the results presented, the cytotoxic effects of the combination of DON and T-2 appear to be

influenced by the proportion of each toxin included in the mixture, the cell line and the concentration.

Pomothy et al. (2020) investigated the effects of DON (0–50 µM), T-2 (0–50 nM) and the mixture of T-2 and DON on porcine intestinal epithelial (IPEC-J2) cells. Following 48 or 72 hours of exposure, DON, T-2 and the mixture induced significant cytotoxicity, reduced the transepithelial/transendothelial electrical resistance of the cell monolayer (except T-2 alone), and induced oxidative stress (for example, increased H_2O_2 production). The DON and T-2 mixture was cytotoxic even with a combination of non-cytotoxic concentrations of DON and T-2 (5+10 µmol/L, respectively) following 72 hours of exposure. Rosmarinic acid was shown to attenuate these effects.

Tran et al. (2020) investigated the cytotoxic effects of T-2, HT-2, DAS, DON, BEA, ZEA, enniatin (ENN)-A, ENN-A1, ENN-B, ENN-B1, alternariol (AOH), alternariol-9-methyl ether (AME), tentoxin (TEN) and mycophenolic acid (MPA) on mouse macrophages (RAW 264.7), human hepatoblastoma (HepG2) and human embryonic kidney (HEK 293 T) cells. DON, BEA, ZEA, ENN-A, ENN-B, ENN-B1, AOH, AME, TEN and MPA were not cytotoxic at concentrations up to 50 nM. The IC_{50} values for T-2, HT-2 and DAS were 3.57–13.37 nM, 5.07–47.44 nM and 3.66–17.74 nM, respectively. The combination of T-2 with DON, ENN-A1 or ENN-B showed antagonistic behaviour, whereas the behaviour of all other combinations was synergistic or additive. Silibinin was shown to attenuate these effects.

Taroncher et al. (2021) investigated the individual and combined effects (cytotoxicity) of T-2, HT-2, NEO, T-2-triol and T-2-tetraol in human hepatocarcinoma (HepG2) cells. Following 24 or 48 hours of exposure, T-2 and HT-2 were the most cytotoxic followed by NEO and T-2 triol; T-2-tetraol was the least potent of the substances tested. For example, using the MTT assay, the following IC_{50} values were recorded after 24 hours: 68.6, 68.2, 95.6, 1120 and 2480 nM for T-2, HT-2, NEO, T-2-triol and T-2-tetraol, respectively. Correspondingly, the T-2 and HT-2 mixture showed the highest toxic potential at 24 hours. The lowest toxicity was observed for all combinations with T-2-tetraol, followed by those containing T-2-triol and NEO. The predominant interaction observed in the binary mixtures was antagonism, except for T-2+HT-2, T-2-triol+HT-2, T-2-tetraol+T-2, T-2-tetraol+HT-2 and T-2-triol+T-2-tetraol, which showed additivity at high concentrations.

In vivo

Mice

Male et al. (2016a) gathered dose–response data on the anorectic effects of DON, 3-acetyldeoxynivalenol (3-ADON), 15-actetyldeoxynivalenol (15-ADON), NIV,

fusarenone-X (FUS-X), T-2 and HT-2 in mice. Using benchmark dose (BMD) modelling, the anorectic potency of each mycotoxin was determined. According to their analysis, DON, 3-ADON and 15-ADON exhibited equal anorectic potencies. In comparison to DON, 3-ADON and 15-ADON, they found that NIV, FUS-X, HT-2 and T-2 are 3-, 9-, 5- and 124-fold more potent, respectively. The BMDs for anorexia for HT-2 and T-2 were reported as 0.3 and 0.01 mg/kg bw, respectively, from the data summarized in Wu W et al. (2015), specifically based on the observations 2 hours following exposure. Details concerning the dose–response analysis were not provided. However, using the data provided to the Committee for Wu W et al. (2015) (see section 2.2.1(a)), inadequate model fit was achieved for T-2 and HT-2 at the 2-hour observation using PROAST (v70.1).

Zhang J et al. (2018a) compared the anorectic potencies of T-2, HT-2, DAS and NEO in mice. Following oral exposure, the NOAELs reported by Zhang J et al. (2018a) were 0.01, 0.01, 0.1 and 0.01 mg/kg bw, for T-2, HT-2, DAS and NEO, respectively. Following intraperitoneal exposure, the NOAELs were 0.01 mg/kg bw, except for DAS, which elicited effects at the lowest dose of 0.01 mg/kg bw. According to Zhang J et al. (2017a), oral and intraperitoneal exposure of mice to T-2, HT-2, DAS or NEO resulted in decreased feed intake that was associated with elevated concentrations of $PYY_{3–36}$ and GIP. Generally, $PYY_{3–36}$ and GIP concentrations peaked 2 hours following exposure to all mycotoxins.

Rats

Semenov et al. (2018) investigated the effects of combined dietary exposure to DON (5 µg/kg diet) and T-2 (50 µg/kg diet) on the sensitization response of male Wistar rats to OVA. Based on these results, a much stronger sensitization reaction was achieved following combined exposure (5 µg/kg diet DON + 50 µg/kg diet T-2) than following separate exposures. For example, the concentrations of OVA antibodies (IgG) following sensitization with DON, T-2 or the combination were 5.8 ± 0.4, 6.4 ± 0.4 or 16.2 ± 0.6 mg/mL, respectively.

Rabbits

Szabó et al. (2014) investigated the combined toxicity (haemopoietic effects) of dietary exposure (up to 4 weeks) to T-2 (2 mg/kg diet), fumonisin B1 (FB1; 10 mg/kg diet) and the combination (2 mg/kg diet T-2 + 10 mg/kg diet FB1) in male Pannon white rabbits. The combination of T-2 and FB1 in feed induced similar effects to those of T-2 alone on body weight and organ weights; however, it also induced antagonistic effects on RBC sodium pump activity. In a follow-up study, Szabó et al. (2016) also reported a synergistic response of the hepatic total lipid profile of rabbits similarly treated.

Mink

Male et al. (2016b) gathered dose–response data on the emetic effects of DON, 3-ADON, 15-ADON, NIV, FUS-X, T-2 and HT-2 in mink. Using BMD modelling, the emetic potency of each mycotoxin was determined following oral and intraperitoneal exposure. According to their analysis, emesis is more readily induced in mink following oral exposure than intraperitoneal exposure. For example, the BMD (modelling details, including the benchmark response (BMR), were not provided) values for HT-2 and T-2 were 31 µg/kg bw for intraperitoneal exposure and 14 µg/kg bw for gavage exposure. Details concerning the dose–response analysis were not provided. The gavage BMD values reported for DON, 3-ADON, 15-ADON, NIV and FUS-X were 24, 198, 40, 141 and 23 µg/kg bw. When considering the emetic response at 0.5 mg/kg bw per day, Male et al. (2016b) proposed the following rank order for relative potency: HT-2~T-2 > DON~FUS-X~NIV > 15-ADON~3-ADON.

2.3 Observations in domestic animals/veterinary toxicology

The previous Committee (Annex 1, reference *153*) noted that cats would be expected to be more susceptible to T-2 toxin than other species in view of the demonstrated deficiencies in conjugation reactions. A study in lambs and a study in calves were also mentioned in the earlier monograph (Annex 1, reference *153*).

More recently, Ferreras et al. (2013) reported clinical and pathological findings following T-2 poisoning of dairy sheep (Awassi) due to the inadvertent consumption of contaminated feed. Clinical signs first appeared in a group of approximately 440 ewes that were being milked, and included anorexia, decreased water consumption, excessive salivation, ruminal atony, soft faeces and apathy. Eighty-four animals died shortly after the delivery of a new batch of cereal grain (mainly maize), and another 106 sheep died subsequently. Sheep that survived showed reduced weight gain, wool loss and a high percentage of abortions. Examination of the feed revealed T-2 concentrations ranging from approximately 15 to 56 µg/kg diet and concentrations of DON, DAS and AFB1 <4 µg/kg diet. The report summarized the examination of six animals (aged 1 to 2.5 years) exposed to the contaminated feed. The animals included two ewes examined following 8 days of exposure; two ewes examined 2 months after the start of the outbreak; and two ewes examined 4 months after the start of the outbreak. The two ewes examined following 8 days of exposure showed gross lesions consisting of rumenitis and ulcerative abomasitis, depletion of the lymphocytes in the lymphoid organs, necrosis of the exocrine pancreas, myocarditis and intense oedema of the skin and brain. Animals that survived the acute stage showed weight loss and reproductive inefficiency accompanied by gastrointestinal inflammation, myocardial fibrosis

and necrotic and suppurative lesions in the oral cavity. The animals examined 2 and 4 months after the outbreak also showed evidence of opportunistic infection (for example, mycotic mastitis and parasitic pneumonia). Serum biochemistry revealed increased concentrations of lactate dehydrogenase and creatine kinase, which are probably related to the lesions that were observed in the heart (i.e. myocarditis and myocardial fibrosis). The Committee noted that the concentrations of T-2 measured in the feed appear lower than would be expected given the acute nature of the exposure and effects. For example, the authors noted that similar effects have been observed in experiments in sheep (Friend et al., 1983), but at much higher doses (for example 600 µg/kg bw per day via gelatin capsule). The authors noted that the presence of other mycotoxins could not be ruled out.

2.4 Observations in humans

T-2 and HT-2 toxins co-occur with DAS, and at its eighty-third meeting (Annex 1, reference *235*), the Committee included DAS in the group PMTDI for T-2 and HT-2. Therefore, studies dealing with observations in humans exposed to T-2, HT-2 and DAS are also considered here.

2.4.1 Biomarkers of exposure

Monitoring the presence of mycotoxins and their metabolites in urine to assess exposure has gained increased acceptance, as available analytical techniques allow the quantification of multiple biomarkers with an easy non-invasive sampling method. Thirteen human biomonitoring studies that measured T-2, HT-2 or DAS in urine were identified in the literature (Table 7). Most of these studies were conducted in European populations: Belgium (Huybrechts et al., 2015; Heyndrickx et al., 2015), Germany (Gerding, Cramer & Humpf, 2014; Gerding et al., 2015), Hungary (Szabó-Fodor et al., 2021), Spain (Rodríguez-Carrasco et al., 2014), Sweden (Warensjö Lemming et al., 2020), the United Kingdom of Great Britain and Northern Ireland (Gratz et al., 2020) and the European food consumption validation (EFCOVAL) project (De Ruyck et al., 2020), which included samples from five countries (Belgium, Czechia, France, Netherlands (Kingdom of the) and Norway). Biomonitoring studies were also available from Bangladesh and Haiti (Gerding et al., 2015), Brazil (Franco et al., 2019), China (Fan et al., 2019), Iran (Islamic Republic of) (Niknejad et al., 2021) and Thailand (Warth et al., 2014). In two studies (De Ruyck et al., 2020; Szabó-Fodor et al., 2021) the participants collected their urine over a 24-hour period, whereas the participants in the other studies provided spot samples of early morning urine. All the studies measured multiple mycotoxins (between 10 and 58 analytes), including parent compounds and metabolites. Most of them used LC-MS/MS,

Table 7
Biomonitoring studies of T-2, HT-2 and DAS in urine

Reference	Country, year	Population	No. of analytes	Analytical method	LOD/LOQ (ng/mL)	Results
Rodríguez-Carrasco et al. 2014	Valencia (Spain), 2013	54 volunteers; 16 aged 8–14 years; 38 aged >18 years	15	GC-MS/MS	0.5/1 T-2 1/2 HT-2, DAS	T-2, DAS not detected; HT-2 4/54 (7.4%) (co-occurrence with deoxynivalenol)
Gerding et al. 2014	Münster (Germany)	101 volunteers (aged 20–30 years)	23	LC-MS/MS with scheduled MRM	0.025/0.05 T-2 0.2/0.4 HT-2	HT-2, HT-2-4-GlcA not detected; T-2 1/101 (1%)
Gerding et al. 2015	Germany, Bangladesh, Haiti, 2012–2014	Volunteers; 50 Germany, 95 Bangladesh, 142 Haiti	23	LC-MS/MS with scheduled MRM	0.01/0.02 T-2 0.45/0.9 HT-2	T-2, HT-2, HT-2-4-GlcA not detected
Warth et al. 2014	Bangkok and provinces (Thailand)	60 volunteers: 6 aged 10–19 years; 54 aged 20–76 years	15	LC-MS/MS	1/1 T-2 12/40 HT-2	T-2, HT-2 not detected
Huybrechts et al. 2015	Belgium	29 volunteers (32 samples)	32	LC-MS/MS	0.005/0.02 DAS 0.01/0.03 T-2 0.2/0.5 HT-2	T-2, HT-2 not detected
Heyndrickx et al. 2015	BIOMYCO study, Belgium, 2013–2014	155 children (3–12 years); 239 adults (19–65 years)	33	LC-MS/MS	0.005/0.02 DAS 0.01/0.03 T-2 0.2/0.5 HT-2	DAS, T-2, HT-2 not detected
Fan et al. 2019	Nanjing (China), 2017	260 adults aged 18–66 years	25	UHPLC-MS/MS	LOD 0.03–0.5 LOQ 0.1–1	HT-2 not detected; T-2 6/260 (2.3%)
Franco et al. 2019	Sao Paulo and Santa Caterina, Brazil, 2016	86 volunteers, mean age 47 years, sampled twice	19	UHPLC-MS/MS	0.013/0.040 T-2 0.024/0.075 HT-2	T-2, HT-2 not detected
Warensjö Lemming et al. 2020	Sweden, 2016–2017	1096 adolescents, aged 11–18 years	35	HPLC-MS/MS	Not specified	1/1096 HT-2-3-glucuronide (0.1%), with value <LOQ
Gratz et al. 2020	United Kingdom	21 children (12 boys, 9 girls), aged 2–6 years	11	LC-MS/MS	0.006/0.013 T-2 0.013/0.031 HT-2	T-2 1/21 (5%); HT-2 1/21 (5%)
De Ruyck et al. 2020	EFCOVAL project, Belgium,Czechia, France, Netherlands (Kingdom of the), Norway, 2006–2010	600 adults (45–65 years); 188 with urine samples	58	UPLC-MS/MS followed by HRMS	0.008/0.013 T-2 0.022/0.036 HT-2 0.009/0.022 DAS	T-2 41/188 (22%); HT-2 12/188 (6.4%); T-2 group (T-2/HT-2/T-2OH3/T-2OH4) 56/188 (30%); DAS 25/188 (13%)
Niknejad et al. 2021	Golestan province, Iran (Islamic Republic of)	17 oesophageal cancer patients (mean age 69 years); 10 controls (mean age 33.5 years)	10	GC-MS/MS	0.1/1 T-2 1/2 HT-2	T-2 1/17 (6%) cases, none detected in controls; HT-2 3/17 (18%) cases, 1/10 (10%) among controls

Reference	Country, year	Population	No. of analytes	Analytical method	LOD/LOQ (ng/mL)	Results
Szabó-Fodor et al. 2021	Somogy, Baranya and Pest counties, Hungary	60 volunteers (41 healthy, 19 coeliac patients)	12	LC-MS/MS	0.25/0.83 T-2 6.25/20.8 HT-2	T-2, HT-2 not detected

Note: If no year is given in the column headed "Country, year", the study authors did not report the date (year) of sample collection.
DAS, diacetoxyscirpenol; GC-MS/MS, gas chromatography tandem mass spectrometry; HRMS, high-resolution mass spectrometry; HT-2-4-GlcA, HT-2-toxin-4-O-glucuronide; LC-MS/MS, liquid chromatography tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MRM, multiple reaction monitoring; T-2OH3, T-2 toxin triol; T-2OH4, T-2 toxin tetraol; UHPLC-MS/MS: ultra-high-performance liquid chromatography tandem mass spectrometry; UPLC-MS/MS, ultraperformance liquid chromatography tandem mass spectrometry.

except those from Spain (Rodríguez-Carrasco et al., 2014) and Iran (Islamic Republic of) (Niknejad et al., 2021), which used GC-MS/MS.

DAS was assessed in only four studies. It was not detected in any of the samples from 54 volunteers in Valencia, Spain (Rodríguez-Carrasco et al., 2014), nor in the 32 samples from volunteers (Huybrechts et al., 2015) or the 394 samples (155 children, 239 adults) in Belgium (Heyndrickx et al., 2015), but it was found in 13% (25 out of 188) of urine samples from adults in the EFCOVAL project (De Ruyck et al., 2020).

Neither T-2 nor HT-2 were detected in the samples from Bangladesh, Germany or Haiti (Gerding et al., 2015), Belgium (Huybrechts et al., 2015; Heyndrickx et al., 2015), Brazil (Franco et al., 2019), Hungary (Szabó-Fodor et al., 2021) or Thailand (Warth et al., 2014). Exposure to T-2 and/or HT-2 was very low in most of the remaining studies: HT-2 was present in 7.4% (four out of 54) of samples in Spain (Rodríguez-Carrasco et al., 2014), and 0.1% (one out of 1096) in Sweden (Warensjö Lemming et al., 2020). T-2 was found in one out of 101 samples in Germany (Gerding, Cramer & Humpf, 2014), and 2.3% (6 out of 260) of samples in China (Fan et al., 2019). Both T-2 and HT-2 were present in one out of 21 samples (5%) in children in the United Kingdom (Gratz et al., 2020), whereas T-2 was present in 1 out of 17 samples (6%), and HT-2 in 4 out of 37 (11%) in Iran (Islamic Republic of) (Niknejad et al., 2021). The occurrence of T-2 and HT-2 was slightly higher in the EFCOVAL study in five European countries (De Ruyck et al., 2020). Urine samples were available from 188 adults: T-2 and HT-2 were found in 41 (22%) and 12 (6.4%) samples, respectively. Furthermore, when T-2 was defined as a group, including the parent compound T-2, HT-2 and T-2 toxin triol and tetraol, the prevalence of detection in urine was almost 30% (56 out of 188 samples). In this study, urine was collected twice, on two days about one month apart; the data shown correspond to the mean of the two measurements.

Six studies also measured mycotoxins in blood samples. Three of them, carried out in Spain, measured 19 mycotoxins in plasma by LC-MS/MS in three different settings: 438 healthy adults (Arce-López et al., 2020); 79 participants aged 2–26 years, including healthy children, and participants with

autism spectrum and hyperactivity disorders (Arce-López et al., 2021a); and 94 adults, including 25 healthy adults, 44 participants with Parkinson's and 24 with Alzheimer's disease (Arce-López et al., 2021b). Neither T-2 nor HT-2 were detected. Mycotoxins in blood were also assessed in three of the studies that had also measured them in urine: neither T-2 nor HT-2 were detected in the plasma samples of 260 adults from China (Fan et al., 2019) and 1096 adolescents in Sweden (Warensjö Lemming et al., 2020). In the EFCOVAL study (De Ruyck et al., 2020) mycotoxins were measured in serum from 268 adults with different levels of exposure to type A trichothecenes: the prevalence of detection was 23% (61 samples) for DAS, 25% for T-2 (68 samples), and 14% for HT-2 (38 samples). The serum measurements were carried out in non-fasted blood samples collected 1 or 2 weeks before the first urine sample collection.

In the EFCOVAL project (De Ruyck et al., 2020) 24-hour dietary recall interviews were carried out twice, 1 month apart, and matched against the EFSA database of mycotoxin contamination of foods (EFSA, 2010) to estimate dietary exposure to mycotoxins, using the average of the two 24-hour dietary recalls. T-2 and HT-2 were present in at least one 24-hour dietary recall for most of the participants (99%), whereas DAS was present only for 1% of them. However, for the 188 participants with data on dietary exposure and urine excretion, the agreement between the two measurements was quite poor: the percentage agreement was only 13% for DAS, 24% for T-2 and 9% for HT-2, with Spearman's correlation coefficients close to zero for all of them. Urine samples were collected during the 24-hour period following the two days on which participants had been asked for a 24-hour dietary recall.

Overall, the data available point to a very low level of exposure to T-2, HT-2 and DAS as measured by excretion in the urine. The rates of detection seem slightly higher in the EFCOVAL study (De Ruyck et al., 2020), but there are two major differences between the EFCOVAL study and the others. First, the EFCOVAL population is composed of adults aged 45–65 years, whereas the other studies include children and young adults. However, other studies on populations with similar age ranges to the EFCOVAL study (Warth et al., 2015; Heyndrickx et al., 2015) showed consistently lower levels of T-2 and HT-2. On the other hand, the analytical technique used in the EFCOVAL study is the one with the lowest values of limit of detection (LOD)/LOQ. Nevertheless, in studies with LOD/LOQ of T-2 close to those in the EFCOVAL study (Garding et al., 2015; Huybrechts et al., 2015; Heyndrickx et al., 2015; Franco et al., 2019; Gratz et al., 2020), the detection rate of T-2 was much lower, or it was not even detected.

Apart from the potential difference between analytical performances, some differences observed between studies are difficult to explain. An additional drawback is the possibility for target components to exist in metabolized or otherwise modified forms, which are not assessed in targeted analysis. Overall,

these findings suggest that biological measurements of type A trichothecenes in the urine or blood are generally not sufficient for describing dietary exposure.

2.4.2 Biomarkers of effect

No studies of biomarkers of effect were identified.

2.4.3 Clinical observations

DAS was investigated under the name of anguidine in the 1970s and early 1980s for its potential as a cancer chemotherapeutic agent (Annex 1, reference *235*). Several phase I (safety) and phase II (efficacy) clinical trials were conducted on cancer patients administered an intravenous infusion of DAS at doses of 0.1–10 mg/m^2 (equivalent to 2.7–270 µg/kg bw). However, the trials were discontinued due to the lack of efficacy and observations of adverse effects. The main adverse effects after acute and repeated exposure included myelosuppression, characterized by decreased levels of lymphocytes and platelets, emesis and hypotension. Mild nausea was reported at doses of 41–65 µg/kg bw; more severe effects (vomiting, hypotension and myelosuppression) were reported at doses of 81 µg/kg bw or above, with a dose-dependent increase in frequency and severity. The reported adverse effects were consistent with the target sites of toxicity observed in the animal studies No clinical studies after oral administration of DAS and no other clinical data in humans have been identified.

2.4.4 Epidemiological studies

The Committee previously summarized the reports of outbreaks or acute poisoning events associated with exposure to trichothecene-contaminated foods due to fungi (Annex 1, reference *153*). The observations of necrotic lesions of the oral cavity, oesophagus and stomach and, in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia, and lethality in the former Soviet Union between 1937 and 1947 has been historically associated with the consumption of mouldy grains containing T-2 toxin. Additionally, acute poisoning events associated with Fusarium-infected grains have been reported in China, India, Japan and the Korean peninsula; these events were associated with nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhoea, bloody stools, dizziness and chills. In each of these events, the concomitant presence of T-2 with DON, acetyldeoxynivalenol, NIV and other trichothecenes cannot be completely ruled out. Consequently, the contribution of other mycotoxins, and potentially other environmental factors, to the effects observed is uncertain. No other reports of human poisoning related to T-2 exposure were found in the latest search of the literature. Nevertheless, the effects noted above are consistent with those observed in experimental animals exposed to dietary T-2.

Most of the recent literature concerning the potential effects of T-2 exposure and human health is predominantly focused on Kaschin–Beck disease (KBD). KBD is a form of chronic degenerative osteoarthropathy endemic to several Chinese provinces, southeast Siberia and the Democratic People's Republic of Korea. It is characterized by focal necrosis of chondrocytes in the hypertrophic zone of growth plate cartilage, which can result in growth retardation, and disability in the advanced stages. The disease usually becomes evident at ages between 5 and 15 years, and clinical features include generalized osteoarthropathy involving the ankles, knees, interphalangeal joints, wrists and elbows with foreshortened phalanges, and more severe involvement of distal and lower limbs (Kraus, 2015).

Several studies in China have measured T-2 in grain samples from KBD-endemic or non-endemic areas (Table 8). In 2010, naturally contaminated grain samples (153 wheat and 181 flour) were randomly collected from three KBD-endemic villages and a non-KBD-endemic village (30 flour samples) in Qinghai Province, China (Sun et al., 2012). The average T-2 contamination levels (μg/kg) of wheat and flour were 78.9 and 47.5, respectively. The average T-2 level in the non-KBD-endemic village was 12.2 μg/kg, significantly lower than that of grain from KBD-endemic areas. The average selenium content was significantly lower in KBD-endemic villages. In the same province (Qinghai, China), in 2010, 133 children aged 7–14 years (including 25 KBD patients) in 11 KBD-endemic villages were surveyed together with 50 healthy children of the same age in a non-KBD-endemic village (Lei et al., 2016). Samples of soil, drinking water and home-grown wheat flour, as well as blood, urine and hair samples from the children were collected in the selected villages. The content of T-2 was below the detection limit (0.2 μg/kg) in all samples assayed (56 from KBD villages, 27 from the non-KBD village). The selenium levels in wheat flour samples and drinking water, as well as in hair samples of children from the KBD-endemic area were significantly lower than those from the non-KBD-endemic area. This study also measured DON in wheat flour; the average level in samples from the KBD-endemic area (289 μg/kg) was significantly higher than in samples from the non-KBD-endemic area (199 μg/kg). A two-stage survey was conducted in Heilongjiang and Gansu provinces of China between 2014 and 2017 (Sun et al., 2019). Cereal (white flour and corn) and soil samples were collected, as well as hair samples from some children aged 7–12 years in the second phase. The average contents of T-2 in flour and corn samples from the non-KBD-endemic area were 9.2 μg/kg and 3.2 μg/kg, respectively, whereas for the KBD-endemic regions, the contents were 5.8 μg/kg and 1.7 μg/kg. There was no significant difference in selenium content in flour between KBD-endemic and non-endemic areas, but the selenium content in children's hair was significantly lower in the non-KBD-endemic area. A systematic review and meta-analysis of seven epidemiological studies (Li et al., 2016) compared the T-2 concentrations in KBD-

Table 8

T-2 concentrations in grains in Kashin-Beck disease (KBD)-endemic and non-KBD-endemic areas in China

Reference	Place and date	Grain samples	T-2 concentration		Other results and observations
			KBD-endemic area	Non-endemic area	
Sun et al. 2012	Quinghai Province, October 2010	Wheat (grain and flour)	Grain: 78.9 µg/kg Flour: 47.5 µg/kg	Flour: 12.2 µg/kg	Significant difference (T-2 in flour); also significantly lower levels of selenium in KBD-endemic areas
Lei et al. 2016	Quinghai Province, May 2010	Wheat flour	All samples from both areas were below the detection limit (LOD 0.2 µg/kg)		Selenium in wheat, drinking water and hair samples significantly lower in KBD-endemic areas. Deoxynivalenol in wheat flour significantly higher in KBD-endemic areas
Sun et al. 2019	Heilongjiang and Gansu Provinces, 2014–2017	Wheat (flour), corn (grain)	Wheat: 5.8 µg/kg Corn: 1.7 µg/kg	Wheat: 9.2 µg/kg Corn: 3.2 µg/kg	Selenium in hair significantly lower in KBD-endemic areas; no differences in selenium in grain
Li et al. 2016	Summary, five studies; several provinces; 1995–2012	Wheat flour and corn flour	Wheat: 89.6 µg/kg Corn: 88.1 µg/kg	Wheat: 54.9 µg/kg Corn: 34.4 µg/kg	Statistically significant differences for both wheat and corn

endemic and non-endemic areas. The mean concentrations of T-2 in wheat flour were 89.6 µg/kg and 54.9 µg/kg for endemic and non-endemic areas, respectively. The corresponding measurements for corn flour were 88.1 µg/kg and 34.4 µg/kg. In both cases the differences were statistically significant.

Community-based trials have also been carried out in China to assess the efficacy of interventions for prevention or treatment of KBD. In the Qinghai Province of China (Sun et al., 2014), 280 children aged 6–11 years from villages in the KBD-endemic area were divided into three groups. The control group received no intervention (n = 64), the second received 150 kg/person of rice from non-KBD areas (n = 103) and the third, 7 kg/family of selenium-iodine salt (n = 113) for 12 months. KBD was assessed by radiological findings in the metaphysis and distal ends of the phalanges of the hands. After 1 year, the proportions of patients newly diagnosed with KBD were 69.2% in the control group, 15.8% in those supplied with rice from non-KBD areas, and 18.8% in the selenium-iodine salt group. The differences between the control group and the two intervention groups were statistically significant. A review synthesized the results of community trials that aimed to assess the efficacy of changing grain in KBD-endemic areas in China (Han et al., 2015). The intervention consisted of exchanging the locally produced grain for grains produced in non-KBD-endemic areas. Overall, there were 1609 participants aged 0–18 years (970 in the intervention group and 639 in the control group). KBD was assessed by clinical and X-ray criteria according to national standards. The pooled odds ratio (OR) of four studies carried out in

healthy children was 0.15 (95% CI 0.03–0.70), reflecting a protective effect of changing the grain (lower incidence in the intervention group). A comprehensive review evaluated several KBD control strategies reported in 22 trials including patients with confirmed KBD resident in endemic areas in China (Yu et al., 2019). Interventions included improvement of water from deep wells and household taps, change of grain (local grain replaced with grain from non-KBD areas) and salt-rich selenium. Primary outcomes were new cases of KBD in healthy children. The ORs (95% CI) were 0.20 (0.09–0.42) for improvement of water quality, 0.15 (0.03–0.70) for change of grain and 0.19 (0.09–0.38) for salt-rich selenium. Apart from methodological limitations (randomization and blind assessment of the trials analysed cannot be guaranteed), these results must be considered with caution, as they only provide indirect evidence of a potential role of T-2: changing grain is proposed as a measure to prevent KBD, as local grain from endemic areas is assumed to be contaminated by *Fusarium* toxins, mainly T-2. However, no actual measurement of T-2 in grains was reported in any of the trials.

Overall, previous results from ecological studies and community trials may suggest that prevalence and development of KBD is associated with the amount of T-2 in food. Moreover, in vitro and in vivo experimental studies have shown chondrocyte toxicity of T-2 (Li et al., 2016). However, the etiology of the disease remains debatable; alternative factors proposed include selenium and iodine deficiency (Yao et al., 2011) and exposure to organic matter (fulvic and humic acid) in contaminated drinking water (Peng et al., 1999). Given the likely multifactorial nature of KBD, the Committee concluded that a causal relationship between exposure to T-2 and KBD could not be established with reasonable confidence. Therefore, the data on KBD had limited relevance for the present assessment.

3. Dose–response analysis

3.1 Identification of key data for risk assessment

3.1.1 Pivotal data from biochemical and toxicological studies

The Committee noted that none of the available studies were ideally suited for dose–response modelling and expressed an overall low confidence in the available toxicological dataset. For example, many of the studies investigated adverse effects at high doses, the actual intake of the test material and the presence of other related mycotoxins in the basal feed was inadequately described, and none of the identified studies that tested low doses (for example ≤25 µg/kg bw per

day) followed standard testing guidelines according to good laboratory practice (GLP). Furthermore, the Committee noted various uncertainties concerning the toxicological relevance of some of the effects observed at low doses (see section 2.2). Nevertheless, dose–response modelling was carried out on selected toxicological end-points in an effort to establish a point of departure.

Table 9 summarizes study results that were considered for the hazard characterization update of T-2/HT-2. To aid comparison, dietary concentrations were converted to doses on a body weight basis in Table 10. However, most of the dietary studies summarized below did not record feed intake. Since feed intake is a sensitive toxicological end-point for T-2, the Committee noted that the estimated doses using default conversion factors may overestimate actual exposures.

The present Committee concurred with the previous evaluation (Annex 1, reference *235*) that effects on feed intake, body weight, and immunological and haematological end-points are sensitive measures of T-2-induced toxicity following acute and repeated oral exposure. Studies published since 2001 also enabled the Committee to identify emesis as a critical end-point for acute exposure.

(a) Acute exposure

Wu W et al. (2016) identified a NOAEL of 5 µg/kg bw T-2 or HT-2 (via gavage) in Standard dark mink based on an increased incidence of emesis at doses ≥50 µg/kg bw T-2 or HT-2. Wu W et al. (2015) also identified a NOAEL of 10 µg/kg bw T-2 or HT-2 in mice, based on statistically significant decreases in feed intake at doses ≥100 µg/kg bw T-2 or HT-2, 0 to 3 hours following single-dose gavage exposure. These effect levels were equally demonstrated following gavage and intraperitoneal exposure using both T-2 and HT-2 and both effects were correlated with plasma concentrations of anorexigenic hormones (Wu W et al., 2016, 2018a; Sheng et al., 2018). Although Sheng et al. (2018) and Wu W et al. (2018a) investigated the dose–response of these hormones to T-2 exposure in mice, the results reported were not adequate to enable dose–response modelling.

Due to the nature (continuous and temporal) of the anorectic effects observed in mice following acute oral exposure to T-2 and HT-2, the Committee noted that BMD modelling using the results of Wu W et al. (2015) would present technical challenges and would not greatly add to the data already presented in section 2.2.6 (a). Consequently, the Committee decided to focus its dose–response analysis on the data from the study by Wu W et al. (2016) on emesis in mink. Given that the anorectic effects induced by T-2 and HT-2 in mice share similar mechanisms with the emetic effects induced by T-2 and HT-2 in mink, the Committee noted that the analysis of the results of the study by Wu W et al. (2016) of emesis in mink is highly relevant to the likely nausea-induced anorectic

Table 9
Summary of key toxicity studies of T-2 and HT-2

Species/study type (route of administration)	Doses	Critical effect(s)	NOAEL	LOAEL
Acute oral toxicity in mink (gavage)				
Wu W et al. (2016)	0, 5, 50, 250 or 500 µg/kg bw T-2 or HT-2	Significantly increased incidence of emesis was observed at doses ≥ 50 µg/kg bw T-2 or HT-2 following single-dose gavage or intraperitoneal exposure	5 µg/kg bw	50 µg/kg bw
Acute oral toxicity in mice (gavage)				
Wu W et al. (2015)	0, 10, 100, 500 or 1000 µg/kg bw T-2 or HT-2	Statistically significant decreases in feed intake were observed at doses ≥100 µg/kg bw T-2 or HT-2 following single-dose gavage or intraperitoneal exposure	10 µg/kg bw	100 µg/kg bw
Short-term oral toxicity in rabbits (gavage)				
Kovács et al. (2013) 65 days	0, 10, 20 or 50 µg/kg bw per day via gavage 0, 0.33 or 0.66 mg/kg diet. Equivalent to 0, 10 or 20 µg/kg bw per day	Decreased feed intake, histopathology in the testes and liver and a slower increase in gonadotropin-releasing hormone (GnRH)-induced testosterone synthesis were observed at doses ≥20 µg/kg bw per day Kovács et al. (2013) showed that the effects noted following gavage exposure were not observed following dietary exposure to T-2 at equivalent doses up to approximately 20 µg/kg bw per day (highest dose tested) for 65 days	10 µg/kg bw per day 20 µg/kg bw per day	20 µg/kg bw per day
Short-term oral toxicity in juvenile pigs (diet)				
Rafai et al. (1995) 21 days	0, 500, 1000, 2000 or 3000 µg/kg diet Equal[a] to 25, 52, 103 or 125 µg/kg bw per day T-2	No NOAEL could be identified, as effects were observed at all doses Significantly reduced feed intake, reduced daily weight gain, reduced leukocyte count, decreased proliferative response of lymphocytes to concanavalin A and reduced horse globulin antibody titre were observed at ≥ 25 µg/kg bw per day See Annex 1, reference *153*: and section 2.2.2(d) for detailed summaries	–	~25 µg/kg bw per day[e]
Meissonnier et al. (2008, 2009) 28 days	0, 540, 1324, 2102 µg/kg diet Equivalent[b] to 0, 27, 68 or 108 µg/kg bw per day T-2	Decreased anti-OVA titres were observed at doses ≥ or equal to approximately 68 µg/kg bw per day. No cellular depletion of the Peyer's patches in the ileum or in the spleen was observed compared to controls. Lymphocyte proliferation in response to concanavalin A and ovalbumin was similar in controls and in all treated animals. Feed intake values were not reported; consequently, dose estimates may not reflect actual dosing	~27 µg/kg bw per day	~68 µg/kg bw per day

Species/study type (route of administration)	Doses	Critical effect(s)	NOAEL	LOAEL
Rafai, Papp & Jakab (2013) 21 days	0, 300 (11.2) or 500 (18.0) µg/kg diet Equal[a] to 0, 11.2 or 18 µg/kg bw per day T-2	No NOAEL could be identified since effects were observed at all doses, i.e., at 11.2 and 18.0 µg/kg bw per day, significantly decreased feed intake (22 and 28% less than control, respectively), terminal body weights (10 and 16% less than control, respectively) and daily body weight gain (24 and 36% less than control, respectively) were observed. The authors stated that no difference from the controls was observed for lymphocyte proliferation (as induced by purified horse globulin, phytohaemagglutinin and concanavalin A) and anti-horse globulin antibody titre	–	11.2 µg/kg bw per day
Verbrugghe et al. (2012) 18 days	0, 15 or 83 µg/kg diet Equivalent[c] to 0.6 or 3.1 µg/kg bw per day T-2	The average daily body weight gain of the high-dose group was significantly lower than in the controls (27% of control) Feed intake was not measured; consequently, dose estimates may not reflect actual dosing	0.6 µg/kg bw per day	3.1 µg/kg bw per day
Subchronic oral toxicity in rats (diet)				
Rahman et al. (2014, 2016, 2021)	0, 500, 750 or 1000 µg/kg diet Equivalent[d] to 0, 50, 75 or 100 µg/kg bw per day T-2	No NOAEL could be identified, as effects were observed at all doses Significantly reduced survival, reduced body weight, reduced feed intake, changes in haematological and clinical chemistry parameters, and histopathology in the kidneys, spleen and thymus were observed at all concentrations. Statistically significant decreases in functional immune responses (anti-SRBC antibody titre, delayed-type hypersensitivity and concanavalin A lymphocyte stimulation) were also reported at all concentrations Feed intake values were not reported; consequently, dose estimates may not reflect actual dosing *Supporting information:* Fadhil, Alkutbi & Nassir (2021) also observed evidence of oxidative stress and/or significant histopathology in the livers and small intestines of rats exposed to dietary concentrations of 470 µg/kg diet T-2 for 90 days. Similarly, histopathological lesions in the spleen, thymus, liver, kidneys, testes, heart and brain of rats exposed to concentrations as low as 250 µg/kg diet for 90 days were reported by Raut et al. (2013)	–	50 µg/kg bw per day

Anti-OVA, anti-ovalbumin; anti-SRBC, anti-sheep red blood cells; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

[a] See Appendix 1 for dose conversion calculations.

[b] Conversion factors of 0.0506 to 0.0516 mg/kg bw per day per mg/kg diet were calculated from Rafai et al., see Appendix 1.

[c] A conversion factor of 0.037 mg/kg bw per day per mg/kg diet was calculated from Rafai et al., see Appendix 1.

[d] A conversion factor of 0.01 mg/kg bw per day per mg/kg diet was used.

[e] The previous Committee (Annex 1, reference *152*) and Rafai et al. report doses of 29, 62, 100 and 130 µg/kg bw per day, which are different from those calculated by the current Committee as described in Appendix 1.

Table 10
Pooled incidence of emesis in mink from oral gavage exposure to T-2 and HT-2 as reported by Wu W et al. (2016)

Dose	Number of animals	Incidence
0	4	0
0.005 mg/kg bw HT-2	4	0
0.005 mg/kg bw T-2	4	0
0.05 mg/kg bw HT-2	4	3
0.05 mg/kg bw T-2	4	3
0.25 mg/kg bw HT-2	4	4
0.25 mg/kg bw T-2	4	4
0.5 mg/kg bw HT-2	4	4
0.5 mg/kg bw T-2	4	4

Fig. 3
Results from key toxicological studies

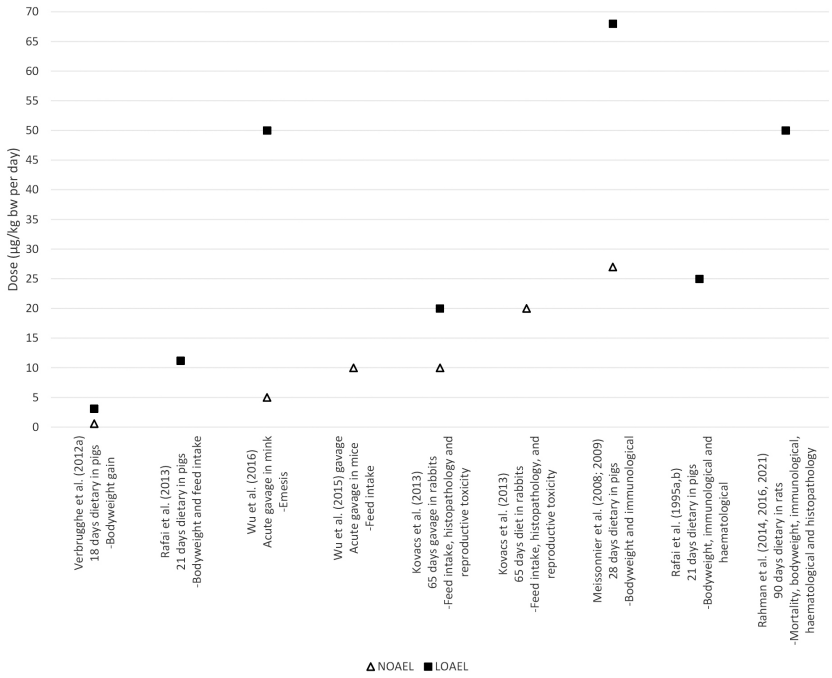


Table 11
Incidence of emesis in mink from oral gavage exposure to DAS as reported by Wu Q et al. (2020)

Dose	Number of animals	Incidence
0	5	0
0.01	5	0
0.025	5	0
0.05	5	0
0.1	5	4
0.25	5	5

effects of T-2 and HT-2 in mice. The Committee also noted that effects following gavage exposure are anticipated to occur at lower doses than effects following dietary exposure. For modelling purposes, the figures on incidence of emesis in mink following exposure to T-2 and HT-2 reported by Wu W et al. (2016) were pooled and are summarized in Table 10.

Considering the potential additive effects of exposure to T-2, HT-2 and DAS (Annex 1, reference *235*), the Committee decided to also conduct dose–response modelling of the emetic effects of DAS in mink following acute oral exposure. This analysis is used to compare the emetic potencies of DAS, T-2 and HT-2. Table 11 summarizes the incidence of emesis in mink reported by Wu Q et al. (2020) following acute gavage exposure to DAS.

(b) Repeated oral exposure

Of the available repeated-dose oral toxicity studies summarized in Table 9, the only ones providing adequate intake estimates of T-2 combined with toxicological observations of the key end-points (i.e. effects on feed intake, body weight and immunological/haematological parameters) were those by Rafai et al. (1995a,b) and Rafai, Papp & Jakab (2013) in juvenile pigs. These studies were all conducted by the same laboratory using the same strain of pigs and similar methods. However, the Committee noted various uncertainties and limitations:

- Statistically significant effects on daily body weight gain were not observed in the study by Rafai et al. (1995a) at doses of 25 and 52 µg/kg bw per day nor in the study by Meissonnier et al. (2008) at doses of approximately 27 and 68 µg/kg bw per day, whereas significant decreases of 24 and 36% in mean body weight gain were observed at doses of 11.2 and 18 µg/kg bw per day by Rafai, Papp & Jakab (2013).
- Significant heterogeneity in the anti-HG titre of the control animals in the study by Rafai et al. (1995b) and Rafai, Papp & Jakab (2013)

was observed (for example 4 versus 6.3 log2) and the responses in all treated animals from the studies by Rafai, Papp & Jakab (2013) and Rafai et al. (1995b) were within the range of control values for anti-HG titre.

- Details of only one of the functional immune parameters (i.e. anti-HG titres) studied by Rafai, Papp & Jakab (2013) were reported.
- Only two treatment groups were included in the study by Rafai, Papp & Jakab (2013).
- Detailed histopathological observations were not reported.

Other longer term dietary studies in rats (Rahman et al., 2014, 2016, 2021) were also considered. However, the Committee identified various limitations associated with these studies, which made their application to the overall hazard characterization difficult:

- Since feed intake was not reported by Rahman et al. (2014, 2016, 2021), accurate dose estimates were not possible.
- The Committee noted that the toxicological relevance of the immunotoxic/haematotoxic effects is questionable for low-dose exposure, considering the severity of other effects noted in the lowest dose group (for example, clinical signs, reduced survival, reduced body weight, reduced feed intake, changes in haematological and clinical chemistry parameters, and histopathology in the kidneys, spleen and thymus).
- The Committee noted that the approximated dose given to the lowest dose group in the studies by Rahman et al. (2014, 2021) is more than fourfold higher than the lowest dose administered in the study by Rafai, Papp & Jakab (2013) in juvenile pigs.

The Committee also expressed concerns over the reliability and reproducibility of the functional immune parameters measured by Rafai et al. (1995b) and Rahman et al. (2021), and the potential for decreased feed intake to affect the immunological and haematological parameters (Annex 1, reference *153*; Friend, Babiuk & Schiefer, 1983; WHO/IPCS, 1996; Poetschke et al., 2000; Savino, 2002; Martin et al., 2008).

Considering all of the uncertainties and limitations associated with the available toxicological literature, the Committee decided to focus dose–response analysis on the juvenile pig study by Rafai, Papp & Jakab (2013) since it investigated more than one treatment level at doses lower than the previously identified LOAEL of 29 µg/kg bw per day (Annex 1, reference *153*), and since accurate

Table 12
Summary of selected effects in juvenile pigs reported by Rafai, Papp & Jakab (2013)

	Dose (µg/kg bw per day)		
Effect	**0**	**11.2**	**18**
Terminal body weight (kg)	23.8 ± 1	21.5 ± 1*	20 ± 0.88*
Daily body weight gain (weeks 1–3; g)	497 ± 63	377 ± 67*	317 ± 96*
Feed intake (weeks 1–3; g/day)	889 ± 99	694 ± 136*	644 ± 98*

n = 10 animals per dose; means presented ± standard deviation; See Appendix 2 for detailed dose calculations; *-p≤0.05.

dose estimates could be derived and background mycotoxin contamination of the basal feed was characterized.

Concerning the haematological effects of T-2 and HT-2, the Committee noted the WHO/IPCS (2012) guidance for immunotoxicity risk assessment for chemicals, which recommends that functional measures of the immune system should be used for hazard characterization rather than observational end-points (for example, leukocyte count). Since Rafai, Papp & Jakab (2013) indicated that significant effects on functional immune parameters were not observed at doses of 11.2 and 18 µg/kg bw per day, the Committee focused on reduced body weight, body weight gain and feed intake as critical effects. Table 12 summarizes the data that were considered in the dose–response assessment.

3.1.2 Pivotal data from human clinical/epidemiological studies

No pivotal data were selected from the human epidemiological studies for dose–response analysis.

3.1.3 Biomarker studies

No pivotal data were selected from the human biomarker studies for dose–response analysis.

3.2 General modelling considerations

For the dose–response analyses, all modelling results were computed using ToxicR version 1.0.0 (https://github.com/NIEHS/ToxicR/releases/tag/v1.0.0). Individual dose–response models were fitted using Bayesian maximum a posteriori (MAP) estimation, which is described by Wheeler et al. (2020, 2022). Dose–response estimates were computed using Bayesian model averaging applying the methodologies described by Wheeler et al. (2020, 2022). For dichotomous model fits (i.e. emesis events), the nine-model suite and suggested priors described by Wheeler et al. (2020) were used. For continuous data, the Committee fitted the

Hill, Exponential-3, Exponential-5 and power models using the normal and normal variance proportional to mean distributions. For the Exponential-3 and Exponential-5 models, the log-normal distribution was used in addition to the two normal distributions. The exact form of the model and priors used is described by Wheeler et al., as mentioned earlier (i.e., Wheeler et al., 2020, 2022). Specifically, for all parameters associated with the shape of the dose–response curve – parameter "d" in Appendix 3 – an informative prior minimized the probability of sharp changes in the dose–response. For all other parameters, vague priors were used. The Committee noted that these priors were developed for gross measurements like body weight and had desirable statistical properties in simulation studies and thus deemed their use acceptable for this analysis. The models, priors and computer code, which can completely reproduce the analyses, is given in Appendix 3.

3.2.1 Acute exposure dose–response modelling and BMD calculations

As recommended in the WHO/IPCS (2020) chapter update on dose–response modelling, model average estimates were computed. Benchmark dose analyses were conducted using the extra risk for quantal data with the benchmark response set to 10%. A benchmark response of 10% is the standard/default value for quantal data (WHO/IPCS, 2020) and was considered appropriate by the Committee for the critical end-point. Similar data for DAS were modelled using the same modelling considerations as for T-2 and HT-2. The results of this analysis are summarized in detail in Appendix 3 and very briefly in Table 13.

For these data, the Committee observed a pattern of no response at zero/low doses and saturated response at high doses. Thus, there is very little information on the shape of the dose–response curve, as the experiment essentially missed the response region. As a result, if no additional information is provided (for example, more experimental doses or Bayesian prior information), one could argue that the BMD may take any value in the missed dose–response region. The Committee selected the $BMDL_{10}$ of 2.6 µg/kg bw per day as the point of departure.

3.2.2 Repeated-dose oral exposure dose–response modelling and BMD calculations

As recommended in the WHO/IPCS (2020) update of the chapter on dose–response modelling, model average estimates were computed. Modelling was carried out using ToxicR, version 22.01 (1.0.0) applying a relative deviation approach. Ideally, a BMR is set numerically so that it reflects the onset of an adverse effect relevant to humans. The Committee considered a reduction in body weight gain in rapidly growing animals as an adverse effect but found it difficult to decide on a minimal level of adversity for such a reduction. In line

Table 13
Dose–response summary statistics for the emetic response in mink following acute gavage exposure to T-2 and HT-2 or DAS

	BMD distribution (µg/kg bw)			
Trichothecene (reference)	**BMD_{10}**	**$BMDL_{10}$**	**$BMDU_{10}$**	**$BMD_{10}/BMDL_{10}$**
T-2/HT-2 (Wu W et al., 2016)	8.6	2.6	24.1	3
DAS (Wu Q et al., 2020)	36.8	14.4	65.2	3

BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower 95% confidence limit on the benchmark dose for a 10% response; $BMDU_{10}$, upper 95% confidence limit on the benchmark dose for a 10% response; DAS, 4,15-diacetoxyscirpenol.

with the guidance in the updated Chapter 5 of EHC 240 (WHO/IPCS, 2020) for such a situation, the Committee chose to consider a range of BMRs, in this case, 5 or 10%, and give specific consideration to the corresponding BMD credible intervals when selecting a point of departure and deciding on the numerical value of uncertainty factors for establishing the health-based guidance value. The results of the modelling are summarized in Tables 14 and 15. Appendix 3 provides a detailed summary of the dose–response analysis.

In the critical study by Rafai, Papp & Jakab (2013), animals were dosed with 11.2 or 18 µg/kg bw per day and, using these data with a BMR of 5%, the BMD and BMDL are considerably below the lowest tested dose of 11.2 µg/kg bw per day. Consequently, the calculation of the BMD is dependent on an extrapolation between 0 and 11.2, and this is true even if model averaging is used. As a result, there is increased statistical uncertainty when calculating the BMDL for low BMRs. For example, when considering daily body weight gain, the BMDL is 1.8 versus 0.6 µg/kg bw per day for BMRs of 10 and 5%, respectively. This calculation represents a threefold decrease in the BMDL, corresponding to a halving of the BMR, which implies increased heterogeneity (at doses below the observed range). The Committee decided that computing the BMD at a 10% BMR was appropriate.

Considering the uncertainty of measuring feed intake by animals housed in groups and the larger model uncertainty associated with the corresponding BMD estimates, the Committee selected the $BMDL_{10}$ of 1.8 µg/kg bw per day based on reduced daily body weight gain for hazard characterization purposes.

Table 14
Dose–response summary statistics for the critical effects in pigs following short-term dietary exposure to T-2 (Rafai, Papp & Jakab, 2013) using a BMR of 5%

	BMD distribution (µg/kg bw per day)			
End-point	**BMD_{05}**	**$BMDL_{05}$**	**$BMDU_{05}$**	**$BMD_{05}/BMDL_{05}$**
Terminal body weight (day 21)	6.4	3.6	9.0	2
Daily body weight gain (weeks 1 to 3)	2.9	0.6	6.2	5
Daily feed intake (weeks 1 to 3)	2.7	0.2	6.3	14

BMD_{05}, benchmark dose for a 5% response; $BMDL_{05}$, lower 95% confidence limit on the benchmark dose for a 5% response; $BMDU_{05}$, upper 95% confidence limit on the benchmark dose for a 5% response.

Table 15
Dose–response summary statistics for the critical effects in pigs following short-term dietary exposure to T-2 (Rafai, Papp & Jakab, 2013) using a BMR of 10%

	BMD distribution (µg/kg bw per day)			
End-point	**BMD_{10}**	**$BMDL_{10}$**	**$BMDU_{10}$**	**$BMD_{10}/BMDL_{10}$**
Terminal body weight (day 21)	11.6	8.8	14.2	1
Daily body weight gain (weeks 1 to 3)	5.4	1.8	9.1	3
Daily feed intake (weeks 1 to 3)	5.6	0.7	9.9	8

BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower 95% confidence limit on the benchmark dose for a 10% response; $BMDU_{10}$, upper 95% confidence limit on the benchmark dose for a 10% response.

4. Comments

4.1 Biochemical aspects

Recent studies have confirmed that T-2 and its conjugates are readily transformed by microbial activity into at least 20 metabolites in the gastrointestinal tract of mammals, with HT-2 being the predominant metabolite (Annex 1, reference *152, 153*; De Angelis et al. 2014; McCormick et al., 2015; Gratz et al., 2017; Yang S et al., 2017; Daud et al. 2020). Following oral dosing with 1 mg/kg bw of HT-2, none could be detected in the plasma of rats. However, a C_{max} for the downstream hydroxylated metabolite, 3′-OH-HT-2 was observed only 10 minutes after oral administration of HT-2 (Yang S et al., 2018). The T-2 metabolites identified using isolated liver cells from several mammalian species in vitro confirmed the potential of phase 1 and phase II metabolism (Annex 1 reference *152*; Wu Q et al., 2011; Yang S et al., 2013, 2018; Slobodchikova et al., 2019). These biotransformations, in combination with the microbial activity in the gastrointestinal tract, ensure that T-2 undergoes substantial presystemic metabolism.

Four hours after intravenous administration of T-2 to pigs, the largest combined concentration of unchanged T-2 and its metabolites (including glucuronide conjugates) was detected in the gastrointestinal tract (Annex 1 reference *152*). The highest unchanged T-2 concentration was detected in fat tissues of pigs, followed in order by lungs and spleen (Sun et al., 2012, 2014). Probably owing to its lipophilicity, unchanged T-2 has been detected in the milk of nursing mammals (Annex 1 reference *152*; Tanaka et al., 2016).

4.2 Toxicological studies

The Committee (Annex 1, reference *152*) reported that strain and sex differences in susceptibility to the toxicity of T-2 have been observed in mice following acute gavage and inhalation dosing, with female mice exhibiting greater evidence of toxicity at lower doses than male mice. Following acute oral or intraperitoneal exposure, T-2 induces oxidative stress, decreased feed intake, emetic, immunotoxic, haematotoxic, hepatic, renal and neurotoxic effects in a variety of experimental animals. Based on the available evidence, emesis and decreased feed intake in mink and mice, respectively, appear to be sensitive toxicological end-points following acute exposure to both T-2 and HT-2. For example, Wu W et al. (2016) identified a NOAEL of 5 µg/kg bw T-2 or HT-2 (via gavage) in female mink based on an increased incidence of emesis at doses ≥ 50 µg/kg bw T-2 or HT-2 following single-dose gavage or intraperitoneal exposure. Similarly, Wu W et al. (2015) identified a NOAEL of 10 µg/kg bw T-2 or HT-2 in female mice based on statistically significant decreases in feed intake at doses ≥100 µg/kg bw T-2 or HT-2, 0–3 hours following single-dose gavage exposure or 0–6 hours following intraperitoneal exposure. Both emesis in mink and decreased feed intake in mice were associated with elevated plasma concentrations of hormones typically associated with CNS-related mechanisms for satiety that also operate in humans (Wu W et al., 2016; Sheng et al., 2018; Wu W et al., 2018; Köşüş et al., 2015).

In the Committee's previous evaluation of T-2 and HT-2 (Annex 1, reference *152*), the immune system (for example, changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection and a blastogenic response to lectins, and decreased and increased resistance to microbial infection) was identified as the target for T-2 toxicity following short-term exposure. However, it was also noted that feed refusal, reduced weight gain and changes in organ weights are also sensitive toxicological end-points that have been observed in various animal species exposed to T-2 and that the potential effects of reduced feed intake and decreases in body weight gain on the observed immunological end-points could not be evaluated.

The effect of feed refusal and reduced weight gain on immunological end-points was supported by a 6-week dietary study in mice by Friend Babiuk & Schiefer (1983) who used pair-fed control animals. Specifically, Friend, Babiuk & Schiefer (1983) showed that spleen weight, cell counts and lymphoproliferative response were similarly reduced in animals from the pair-fed control group as compared to the animals exposed to T-2 (20 mg/kg diet) for up to 6 weeks. Friend, Babiuk & Schiefer (1983) suggested that the response of the pair-fed control animals was due to protein deficiency, which reduced non-splenic phagocytic cells. This suggestion is supported by WHO/IPCS (1996), which indicates that protein calorie restriction and deficiencies of trace elements such as zinc have been associated with immunosuppression and that nutritional status and stressful conditions influence the pathology of lymphoid organs such as the thymus. Since short-term caloric restriction has been shown to reduce both thymic and splenic weight and, correspondingly, to affect the numbers of thymocytes and lymphocytes (Poetschke et al., 2000; Savino et al., 2002; Martin et al., 2008), the T-2-induced immunotoxicity/haematotoxicity may be partially related to the reduced feed intake caused by T-2 exposure. The Committee (Annex 1, reference *152*) also indicated that the immune response to T-2 exposure varied depending at least in part on the dose and how long after administration the effects were measured. For example, both increased and decreased leukocyte counts and increased and decreased resistance to microbial infection have been reported.

Table 9 summarizes the results of studies that were considered relevant to the hazard characterization update of T-2 and HT-2. For ease of comparison, dietary concentrations were converted to doses in Table 10. However, most of the dietary studies summarized did not record feed intake. Since feed intake is a sensitive toxicological end-point for T-2, the Committee noted that the estimated doses using default conversion factors may overestimate actual exposures. The Committee noted that the toxicological database was limited for the purpose of establishing a health-based guidance value (HBGV). For example, many of the studies investigated adverse effects at high doses. The actual intake of the test material and the presence of other related mycotoxins in the basal feed was inadequately described, and none of the identified studies that reported the effects of low doses (for example, ≤25 µg/kg bw per day) followed standard testing guidelines according to GLP standards. Furthermore, the Committee noted some discordance concerning some of the effects at low doses. Nevertheless, the lowest LOAEL reported is approximately 3 µg/kg bw per day T-2. This comes from a study by Verbrugghe et al. (2012) who noted a significant decrease in daily body weight gain with a NOAEL of approximately 0.6 µg/kg bw per day T-2 in juvenile pigs exposed to diets containing 0, 15 or 83 µg/kg diet T-2 for 18 days. Obremski et al. (2013), Wojtacha et al. (2021), Makowska et al. (2017), Makowska, Obremski & Gonkowski (2018) and Rychlik et al. (2020) observed haematological and

neurochemical changes in the gastrointestinal tract of pigs exposed to low oral doses of T-2 (for example, ~7 to 14.5 µg/kg bw per day) for 42 days.

As expected, gavage exposure induces effects at lower doses than dietary exposure (Kovács et al., 2013). Additionally, effects mediated through the gastrointestinal system (for example, decreased feed intake and/or body weight gain) appear to be observed at or below doses that induce functional effects on the immune system and other systemic effects. For example, Rafai et al. (2013) and Meissonnier et al. (2008) showed that functional effects on the immune system (i.e. decreased antibody responses to horse globulin or ovalbumin) of juvenile pigs were not observed at doses close to or below the previously identified LOAEL of 29 µg/kg bw per day (Annex 1, reference *152*). The Committee also noted that there was inconsistency in the antibody response to horse globulin or ovalbumin in these studies (Rafai et al., 1995; Meissonnier et al., 2008; Rafai, Papp & Jakab, 2013). Although it is difficult to conclusively identify the cause of the inconsistency, the Committee noted that the authors had used a novel protocol. Validated methods to assess T-cell-dependent antibody responses typically use well-characterized antigens such as sheep red blood cells or keyhole limpet haemocyanin, rather than a protein mixture such as horse globulin with its corresponding highly variable antigenicity profile (Peachee et al., 2014). Additionally, there was no information in either the study by Rafai et al. (2013) or the one by Meissonnier et al. (2008) regarding the time interval for a peak antibody (IgM and IgG) response or the use of a positive control (for example, cyclosporin, cyclophosphamide) to validate the performance of the immune function assay.

Significant effects on feed intake were observed at doses as low as 11.2 µg/kg bw per day (Rafai, Papp & Jakab, 2013). Evidence of reduced feed intake and/or growth at doses slightly less than those required for functional changes in the immune system is consistent with what is observed with related trichothecenes such as DAS (Annex 1, reference *235*) and DON (Annex 1, reference *152*). Considering this and the previously mentioned association of reduced feed intake with changes in immunological and haematological parameters (Annex 1, reference *152*; Friend, Babiuk & Schiefer, 1983; WHO/IPCS, 1996; Poetschke et al., 2000; Savino et al., 2002; Martin et al., 2008), the Committee identified reduced body weight, daily body weight gain and daily feed intake observed in juvenile pigs as critical effects for short-term oral T-2 exposure. Subchronic dietary studies of T-2 in rats (Rahman et al., 2014, 2016, 2021) were also considered. However, the Committee identified various limitations associated with these studies, which made their application to the overall hazard characterization difficult:

- The lowest estimated dose was more than fourfold higher than the lowest dose in the Rafai, Papp & Jakab (2013) study in juvenile pigs.

- Severe effects were observed in the lowest dose group, thereby limiting the relevance of the observed effects on haematological and immunological parameters.
- Feed intake was not recorded, thereby limiting accurate dose estimates.
- Other mycotoxins could have been present in the test material.

No additional long-term studies of toxicity and carcinogenicity were identified. Previously, the Committee had summarized the results of a long-term (71-week) dietary study in male and female mice (Annex 1, reference *152*). It noted a statistically significant increase in the incidence of pulmonary and hepatic adenomas in male mice at relatively high dietary concentrations (3 mg/kg diet; equivalent to 450 µg/kg bw per day), with no significant increase in tumour incidence in females. Although positive results have been observed in several in vitro and in vivo genotoxicity tests, the Committee noted that inhibition of DNA and RNA synthesis by T-2 has been reported at concentrations generally exceeding those that cause inhibition of protein synthesis. In line with the Committee's previous conclusions (Annex 1, reference *152*), the current Committee concluded that the mode of action of T-2-induced toxicity is unlikely to include direct interaction with DNA.

Based on the available evidence, reproductive and developmental effects are not expected to occur below doses that have been identified as eliciting reduced feed intake and decreased body weight gain, as well as immunotoxicity or haematotoxicity. For example, the Committee (Annex 1, reference *152*) previously reported that reproductive or gross developmental effects were not observed at doses as low as 220 µg/kg bw per day in a two-generation study in mice (Rousseaux, Schiefer & Hancock, 1986).

4.3 Related trichothecenes

Although not the focus of this evaluation, the comparative effects of T-2 and HT-2 and other mycotoxins were briefly reviewed. In a previous evaluation of DAS, the Committee concluded that T-2 and HT-2 are structurally similar to DAS (Annex 1, reference *235*). There was also evidence that they cause similar effects at the biochemical and cellular levels, have similarities in their toxic effects in vivo and have an additive dose effect when co-exposure occurs. According to the previous Committee (Annex 1, reference *235*), although T-2 appears to be more potent than DAS in vitro and in vivo, the available data were insufficient for establishing relative potencies. In the few studies that considered the combined effects of DAS and T-2, a consistent additive dose effect was observed for end-points such as

in vitro inhibition of protein synthesis and lymphocyte proliferation, oral lethal doses following acute exposure, and the incidence of oral lesions, feed refusal and decreased egg production following short-term dietary exposure in chickens.

Based on more recent information, the current Committee noted that acute oral exposure to DAS has effects on feed intake in mice (Zhang J et al., 2017a,b; 2018a,b) and emetic response in mink (Wu Q et al., 2020), via a similar mode of action to T-2 and HT-2. Other structurally similar trichothecenes (for example, NEO, 3-ADON, 15-ADON, FUS-X and NIV) have also shown similar toxicological effects and biochemical changes to T-2 and HT-2. However, additivity, synergism and antagonism have been observed depending on the mycotoxin mixture, the cell culture or animal species investigated, dose or concentration, duration of exposure and/or the effects measured. The Committee noted that there is limited information concerning co-occurrence of mycotoxins (Annex 1, reference *251*; Battilani et al., 2020; COT, 2021) and that the available literature concerning mycotoxin mixture toxicology is very limited.

As previously recommended (Annex 1, reference *235*), the Committee considered the possibility for additivity with respect to DAS and T-2 and HT-2 exposure. In particular, the present Committee noted that this conclusion is supported by more recent acute toxicity data indicating that DAS exhibits similar emetic effects in mink via a similar mode of action to T-2 and HT-2, but with a lower relative potency (Wu Q et al., 2020). Less comparative information was available with respect to other toxicologically relevant end-points (for example, immunotoxicity and haematotoxicity, hepatotoxicity and renal toxicity) following acute or repeated oral exposure. The current Committee reconfirmed the inclusion of DAS in the group tolerable daily intake (TDI) for T-2 and HT-2, as proposed at the eighty-third JECFA meeting. The current Committee concluded that new data were sufficient to recommend a relative potency factor for DAS. Although comparative longer term data on DAS and T-2 and HT-2 are not available, the Committee concluded that the relative potency factor would be likely to be applicable for exposure scenarios longer than acute, due to the similar critical effects observed following acute and repeated oral exposures.

4.4 Observations in domestic animals/veterinary toxicology

Although a case report of T-2-related poisoning in sheep was identified (Ferreras et al., 2013), the possibility of other mycotoxins contributing to the observed effects could not be adequately ruled out. The previous Committee noted that cats were more susceptible to the toxicity of T-2 than other species, most likely due to their demonstrated deficiency in glucuronide conjugation (Annex 1, reference *152*).

4.5 Observations in humans

Several studies monitoring the presence of type A trichothecenes in urine were identified. All of them used a multi-biomarker approach measuring multiple mycotoxins and their metabolites: a few studies measured levels in the blood as well, but these were below the LOD. Overall, the data available point to a very low level of T-2, HT-2 and DAS in urine. One study compared mycotoxin levels in urine with 24-hour dietary recall data to determine the extent of concordance (De Ruyck et al., 2020). Of the adults for whom urine samples were available, T-2, HT-2 and DAS were detected in 22%, 6.4% and 13%, respectively. As only limited concordance was observed between the exposure estimates and mycotoxin (T-2, HT-2 and DAS) levels in urine, it was suggested that these analytes were insufficient to confidently determine the extent of dietary exposure. No studies of biomarkers of effect were identified.

DAS was investigated in the 1970s and 1980s as a potential chemotherapeutic agent for use in cancer patients (Annex 1, reference *235*). Several phase I and phase II clinical trials were conducted using an intravenous infusion, but they were discontinued due to the lack of efficacy, together with observations of adverse effects. The main adverse effects after acute and repeated exposure included myelosuppression, characterized by decreased levels of lymphocytes and platelets, emesis and hypotension. Mild nausea was reported at estimated doses of 41–65 µg/kg bw; more severe effects (vomiting, hypotension and myelosuppression) were reported at doses of approximately 81 µg/kg bw or above, with a dose-dependent increase in frequency and severity. No clinical or epidemiological studies of the effects of oral administration of DAS in humans have been identified. The Committee concluded that information available from human clinical studies was insufficient or not relevant for hazard characterization of dietary exposure to DAS.

Episodes of acute poisoning associated with ingestion of foods contaminated with Fusarium toxins were reported in 1931–1947 in the former Soviet Union. The pathological pattern included necrotic lesions of the oral cavity, oesophagus and stomach and, in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia, and death. Poisoning events associated with infected grains were also reported in China, Japan, the Korean peninsula (1946–63) and India (1980s–1990s). These events were associated with nausea, vomiting, pharyngeal irritation, abdominal pain, diarrhoea, bloody stools, dizziness and chills. Subsequent analyses of suspected food or grain samples indirectly linked the reported outbreaks to T-2, but the concomitant occurrence with DON, acetyldeoxynivalenol, NIV or other trichothecenes cannot be completely ruled out (Annex 1, reference *152*). No new data on acute poisoning or outbreaks of toxicosis related to T-2 or HT-2 were identified.

Over the past two decades, several epidemiological studies have been published assessing the possible association of Kaschin–Beck disease (KBD) with exposure to trichothecenes. KBD is a form of chronic degenerative osteoarthropathy endemic to several Chinese provinces, the Democratic People's Republic of Korea and south-east Siberia. Results from ecological studies (Lei et al., 2016) and community intervention studies (Yao, Pei & Kang, 2011) suggest that prevalence and development of KBD is associated with the amount of T-2 in food. Moreover in vitro and in vivo experimental studies have shown chondrocyte toxicity of T-2 (Lei et al., 2016). However, the etiology of the disease remains debatable; other risk factors proposed include selenium and iodine deficiency (Yao, Pei & Kang, 2011) and exposure to organic matter (fulvic and humic acid) in contaminated drinking water (Peng et al., 1999). Given the likely multifactorial nature of KBD, the Committee concluded that a causal relationship between T-2 exposure and KBD could not be established with reasonable confidence. Therefore, the data on KBD had limited relevance for the present evaluation.

4.6 Analytical methods

The Committee reviewed the analytical methods for the determination of T-2 and HT-2 developed since the fifty-sixth meeting and noted considerable advances in methodology, particularly the development of multimycotoxin analytical methods based on HPLC-MS.

Although thin-layer chromatography has largely been superseded by more modern methods, reports of its use for T-2 and other trichothecenes can still be found. Screening methods, such as ELISA, lateral flow immunoassays, fluorescence polarization and various biosensors and chemosensors continue to be developed and commercialized based mainly on monoclonal antibodies. These assays can be tailored for detection of T-2 alone or the sum of T-2 and HT-2 combined.

Whereas the Committee noted at its fifty-sixth meeting that gas chromatography (GC) with derivatization and detection by electron capture or MS was the primary technique for quantification, there has been a strong shift away from GC towards the extensive use of HPLC. Depending on the extract clean-up technique, these toxins, either alone or together with other type A and B trichothecenes, can be determined by HPLC with UV or fluorescence detection. For this purpose, several derivatizing agents have been described.

The major advance in routine analysis has been the development of HPLC-MS methods, which enable simultaneous quantification and confirmation. Although capable of targeted single analyte determination, these methods can be used for multimycotoxin determination in which T-2 and HT-2 can be

determined as part of a suite of toxins and/or secondary metabolites. Modern methods achieve LODs in the low or sub-µg/kg range but require consideration of optimum conditions of extraction and extract purification to accommodate the differing chemistries of the target analytes. Two approaches for treating the extract are the "dilute-and-shoot" method in which the extract is injected into the HPLC after solvent dilution or the use of a generic clean-up (QuEChERS) to remove impurities such as lipids. A feature of MS detection, particularly with multimycotoxin determination using limited extract purification, is the occurrence of matrix effects. To overcome these problems, stable isotope-labelled internal standards or matrix-matched standards are used. Quantification can also be performed by the standard addition method. A T-2 and HT-2 certified reference material composed of ground oat flakes is available to aid method development and quality assurance. Modified forms of T-2 and HT-2, including numerous plant metabolites, can be identified by HPLC-MS/MS; however, validation and quantification is limited by the availability of analytical standards (Annex 1, reference *251*).

4.7 Sampling protocols

Currently, sampling methods for the analysis of T-2 and HT-2 in cereal grains use protocols developed for other mycotoxins. Many countries have their own sampling guidelines. For example, China uses GB/T 30642-2014, countries in Europe use EC 401/2006, and Canada and the USA have designated sampling guidelines (United States Department of Agriculture, 1995; Canadian Grain Commission, 2015). Additionally, sampling guidance is available from the Codex Alimentarius Commission (CAC/GL 50-2004). In recent years, the drive towards safer food has highlighted the need to determine levels of T-2 and HT-2 contamination in different food commodities. Therefore, it is important to simplify, harmonize and validate sampling plans for T-2 and HT-2 (Annex 1, reference *251*).

4.8 Effects of processing

In general, T-2 and HT-2 levels can be reduced by various processes commonly used in the food and feed industry. Cleaning and sorting are useful first steps in the reduction of T-2 and HT-2 contamination. T-2 and HT-2 are mostly located in the outer layers of cereal grains, and are recovered in higher concentrations in husk, bran and germ relative to other milling fractions. Therefore, the by-products from sorting and milling should be strictly managed. T-2 and HT-2 concentrations decrease during cooking at about 150 °C. Higher temperatures

increase the extent of degradation of the toxins. Fermentation can reduce levels of contamination by T-2 and HT-2, although pH, moisture, temperature and the fermentation organisms impact concentrations (Annex 1, reference *251*).

4.9 Prevention and control

Information on the prevention and control of T-2 and HT-2 is limited to a small number of studies in a few commodities (primarily oats) and these often agree with the greater volume of information available for the related trichothecene, DON. For preharvest mitigation, decreased concentrations of T-2 and HT-2 are associated with having fewer cereals in rotation and growing resistant cultivars. Ploughing may also be beneficial, depending on the rotational position of the host crops. Unlike with DON, growing maize as a previous crop is not a risk factor and limited studies indicate fungicides do not reduce T-2 and HT-2 contamination. For postharvest mitigation, prevention of further T-2 and HT-2 production by Fusarium species is achieved by storing commodities at low moisture content. Various microbes, enzymes and chemicals have demonstrated ability to metabolize or degrade T-2 and/or HT-2, but these have been mainly tested in liquids and may not be technically feasible for most foodstuffs (Annex 1, reference *251*).

4.10 Levels and patterns of contamination in food commodities

When T-2 and HT-2 were assessed previously at the fifty-sixth meeting of the Committee, the percentages of analyses from 1990–2000 (n = 999) that exceeded 100 µg/kg were 0.4% and 0.9% for T-2 and HT-2, respectively. The value of 100 µg/kg was used by the Committee at that meeting to allow comparison to a previous study due to the wide range of LODs, which decreased over time (World Health Organization, 1990). In the current assessment of data from the GEMS/Food contaminants database, there were 49 912 samples analysed for T-2 and HT-2 from 2001 to 2020. Within this dataset 0.8% and 1.5% of samples exceeded 100 µg/kg T-2 and HT-2, respectively. It cannot be determined if these increases in reported frequency of high concentrations of T-2 and HT-2 are due to increases in the mycotoxin concentrations over time or to a greater focus on sampling in regions and/or commodities with higher levels of T-2 and HT-2.

Based on data from the GEMS/Food contaminants database, comparison of analyses for T-2 and HT-2 across global regions has identified stark differences in the number of tests reported, the distribution of foodstuffs analysed and the analytical results. Most of the analytical records were submitted by the European Region, with limited numbers submitted by a few countries within

the other regions. Some of these countries only submitted results for a single foodstuff (sorghum from four African countries and cassava from the USA). Three countries in the Western Pacific Region submitted analytical results for a wide variety of foodstuffs, but they were mostly negative. Canada also submitted results for a wide variety of foodstuffs, with 1.5% positive samples, a lower bound (LB) mean concentration of 0.6 µg/kg, and a few samples with greater than 100 µg/kg combined T-2 and HT-2. In contrast, T-2 and HT-2 levels reported in Europe were much higher in cereals and any food category that does or may contain cereals. More detailed analysis of the European dataset showed that the highest levels were detected in oat, maize, barley and wheat grain (LB mean concentrations of 241, 24, 17 and 5.2 µg/kg, respectively) with significantly lower concentrations occurring in milled products, excluding bran and by-products.

Although limited in quantity, the literature generally supported the conclusion that T-2 and HT-2 levels are low in all regions of the world outside Europe. For example, a total diet study in sub-Saharan Africa analysed composite food samples (n = 194) representing food intake at eight locations across four countries (Benin, Cameroon, Mali and Nigeria) for numerous mycotoxins (Ingenbleek et al., 2019). No samples had detectable T-2 or HT-2 (LOD = 0.4 and 0.8 µg/kg, respectively).

As with other Fusarium mycotoxins that are produced within the growing crop, their concentrations will fluctuate between growing seasons and regions, depending on climatic conditions. Most studies reporting T-2 and HT-2 concentrations are based on single-year surveys and the effect of seasonal variability cannot be assessed. A 7-year (2002–2008) investigation of Fusarium mycotoxins in harvested oats in the United Kingdom showed the annual combined mean concentration of T-2 and HT-2 ranged from 121 to 727 µg/kg (Edwards et al., 2017).

Recent studies have identified numerous modified mycotoxins that are the result of metabolism in planta; some have also been found to exist in naturally contaminated material. T-2 tetraol and HT-2-3-glucoside can occur at high concentrations compared to the parent mycotoxins. There are also several other metabolites that occur individually at low concentrations compared to the parent molecules but may collectively contribute significantly to the overall type A trichothecene occurrence in cereals and cereal products. In recent studies using host plants inoculated with isotope-labelled mycotoxins, 70–85% of the inoculated T-2 or HT-2 was metabolized (Meng- Nathanail et al. 2015; Reiterer et al., 2016) (Annex 1, reference *251*).

At its ninetieth meeting, the Committee noted that T-2 and HT-2 usually co-occur in food commodities and finished products as they are both produced by the same Fusarium species through the same metabolic pathway (Annex 1, reference *251*). Additionally, the Committee observed that *Fusarium langsethiae*

and *F. sporotrichioides* are capable of producing several other derivatives, such as NEO, T-2 triol and T-2 tetraol, DAS, MAS and SCR. However, it noted that these trichothecenes are not commonly included in mycotoxin surveys, but, where they are, they can be detected as co-contaminants with T-2 and HT-2, particularly where analytical methods with low LODs are used. These few studies are restricted to European cereals, where DAS is detected at low frequency and at low concentrations.

4.11 Food consumption and dietary exposure assessment

4.11.1 Acute dietary exposure

Three studies reported in the scientific literature estimated acute dietary exposure to T-2, HT-2 or the sum of T-2 and HT-2. Two of the studies were duplicate diet studies carried out in the Kingdom of the Netherlands, while the third study, by EFSA, estimated acute dietary exposure for a range of European countries. The EFSA study estimated maximum upper bound (UB) 95th percentile acute dietary exposures to T-2, HT-2 and the sum of T-2 and HT-2 of 137, 165 and 170 ng/kg bw, respectively (EFSA, 2017). These estimates were for infant cohorts, with acute dietary exposure decreasing with increasing age. The duplicate diet studies estimated mean acute dietary exposure to the sum of T-2 and HT-2 for young children (8–12 months) of 40 ng/kg bw (range 10–160 ng/kg bw). For 128 adults, acute dietary exposure to the sum of T-2 and HT-2 was in the range not detected to 18.6 ng/kg bw. The Committee did not present additional national estimates of acute dietary exposure (Annex 1, reference *251*).

4.11.2 Chronic dietary exposure

Since the previous evaluation, several national or regional estimates of chronic dietary exposure have been published. The Committee considered evaluations from Belgium, China, the Czechia, Ecuador, Europe, France, Ireland, Malawi, Morocco, Netherlands (Kingdom of the), New Zealand, Nigeria, Pakistan, Romania, Serbia, Spain, Sweden, sub-Saharan Africa, Tunisia and the United Republic of Tanzania. These reports include dietary exposure assessments for T-2 (12 studies), HT-2 (14 studies) and the sum of T-2 and HT-2 (12 studies). In several studies, these toxins were not detected or were detected so infrequently that dietary exposure could not be estimated. Estimates of dietary exposure reviewed mainly related to European and north African countries. Table 16 provides a summary of the range of exposure estimates derived from the scientific literature. Exposure estimates have been further separated into those pertaining to children, including infants and toddlers and those pertaining to adults or the general population. Dietary exposure estimates have mostly been presented

Table 16
Summary of the range of estimates of chronic dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2, derived from the literature

	Estimated dietary exposure, range[b] (ng/kg bw per day)			
	Mean		High percentile[c]	
Toxin/population group[a]	LB	UB	LB	UB
T-2				
Children	0.4–26	13–79	5.7[d]–150	27–200
Adults	0.1–6.4	9.1–24	1.6–29	16–66
HT-2				
Children	0.0–27	4.1–91	3.6–64	15–240
Adults	0.0–14	0.4–33	2.4–23	14–59
Sum of T-2 and HT-2				
Children	0.8–53	8.2–169	6.5–210	31–400
Adults	0.3–27	2.7–60	1.9–87	11–120

LB: lower bound, UB: upper bound.
[a] For the purpose of this summary table, "children" were taken to be any population group described as infants, toddlers or children. The maximum age for children varies from study to study, but in all cases "children" will refer to individuals aged 15 years or younger. "Adults" were taken to be any population group described as adults, adolescents, elderly or very elderly. The minimum age for adults varies from study to study, but in all cases "adults" will refer to individuals older than 10 years.
[b] Ranges are presented separately for lower and upper bound estimates of mean and high percentile estimates of dietary exposure.
[c] 95th percentile, unless otherwise indicated.
[d] 90th percentile.

as ranges from an LB to a UB. LB estimates are generally based on mean toxin concentrations calculated with results below the LOD or LOQ being assigned a value of zero. UB estimates are generally based on mean toxin concentrations calculated with results below the LOD or LOQ being assigned a value equal to the LOD or LOQ. Across studies, the foods providing the major contributions to chronic dietary exposure are cereals and cereal-based, particularly wheat and wheat-based, products.

Based on the observed geographical distribution of T-2 and HT-2 contamination of foods (mainly Europe and North America) and available food consumption information, the Committee, at its current meeting, decided it was unnecessary to derive additional national estimates of chronic dietary exposure to T-2 and HT-2.

At the current meeting, the Committee did not present international estimates of dietary exposure to either toxin or the sum of the toxins using the GEMS/Food cluster diets. It was concluded that dietary exposure to T-2 and HT-2 for clusters covering the known geographical distribution of T-2 and HT-2 was suitably covered by existing European estimates of chronic dietary exposure and no international estimates of chronic dietary exposure were derived by the Committee (Annex 1, reference *251*).

4.12 Combined dietary exposure to T-2, HT-2 and DAS

The present Committee re-evaluated the combined dietary exposure to T-2, HT-2 and DAS.

Owing to the high level of left-censorship in the dataset for DAS (up to 100% for some regions), which had heavily impacted the regional estimates of chronic dietary exposure to DAS estimated at the eighty-third meeting, a tiered approach to combined acute or chronic dietary exposure to T-2, HT-2 and DAS was taken at the current meeting by the Committee by initially considering LB estimates of dietary exposure. This would represent the best reliable actual combined dietary exposure scenario to T-2, HT-2 and DAS where, if LB estimates are less than the group acute reference dose (ARfD) or the group TDI, the more conservative UB combined estimates can be examined.

Acute dietary exposure to the sum of T-2 and HT-2 was previously evaluated by the Committee in 2020 (Annex 1 reference *251*) The highest UB (the LB approach was not reported in the EFSA report) 95th percentile exposure estimate of 170 ng/kg bw was reported in infants for European countries. It was also noted that the acute dietary exposure estimates decreased with increasing age. The Committee also noted that acute exposure to DAS was not evaluated at its eighty-third meeting, confirming that at the present meeting, the Committee was unable to carry out an assessment of combined acute dietary exposure to T-2, HT-2 and DAS due to insufficient information.

Chronic dietary exposure to the sum of T-2 and HT-2 was evaluated by the Committee at the present meeting. Based on the data reported in Table 2, based on a review of the literature for the general population, LB estimates of mean exposure to the sum of T-2 and HT-2 are in the range of 0.3–53 ng/kg bw per day, while LB high percentile estimates of dietary exposure are in the range of 1.9–210 ng/kg bw per day. At the eighty-third meeting of the Committee, regional estimates of chronic dietary exposure to DAS were estimated: LB mean estimates were in the range of 0.0–2.8 ng/kg bw per day and LB high percentile (90th) estimates were in the range of 0.0–5.6 ng/kg bw per day.

Combined acute or chronic dietary exposure estimates to T-2, HT-2 and DAS from different studies, regions and population groups should only be made with a high level of caution. The Committee noted that only LB estimates of chronic dietary exposure to DAS were available, and these estimates were much lower than the estimates of combined dietary exposure to T-2 and HT-2. For this reason, a first-tier approach of considering the risks associated with combined acute or chronic dietary exposure to only the sum of T-2 and HT-2 was adopted.

4.13 Dose–response analysis

The Committee determined that effects on feed intake, body weight and immunological and haematological end-points are sensitive measures of T-2-induced toxicity. For the purposes of establishing an ARfD and a group TDI, dose–response analysis was conducted on selected effects observed following acute and repeated-dose oral exposure.

4.13.1 Acute toxicity

For acute toxicity, the Committee reviewed the study in mink by Wu W et al. (2016), which showed that exposure to T-2 and HT-2 significantly increased incidence of emesis in a dose-responsive fashion at doses ≥ 50 µg/kg bw following both single gavage and intraperitoneal exposure. Notably, the emetic effects of T-2 and HT-2 in mink were accompanied by similar biochemical changes (for example, alterations in various anorexigenic hormones) as occurred with the T-2- and HT-2-induced effects on feed intake in mice (Wu W et al., 2015, 2018; Sheng et al., 2018). For modelling purposes, the incidences of emesis in mink for T-2 and HT-2 reported in Wu W et al. (2016) were pooled and are summarized in Table 10.

Considering the potential additive effects of exposure to T-2, HT-2 and DAS (Annex 1 reference *235*), the Committee conducted dose–response modelling of the emetic effects of DAS in mink following acute oral exposure. This analysis was used to compare the emetic potencies of DAS, T-2 and HT-2. Table 11 summarizes the incidence of emesis in mink reported by Wu W et al. (2020) following acute gavage exposure to DAS.

Modelling was carried out for the induction of emesis in mink by acute oral exposure to T-2 and HT-2, using ToxicR, version 22.01 (1.0.0). As recommended in the recent WHO/IPCS (2020) Chapter 5 update on dose–response modelling, model average estimates were computed. Benchmark dose analyses were conducted using the extra risk for quantal data with the benchmark response set to 10%. A benchmark response of 10% is the standard/default value for quantal data (WHO/IPCS, 2020) and was considered appropriate by the Committee for the critical end-point. For relative potency considerations, similar data for DAS (i.e. Wu Q et al., 2020) were modelled using the same modelling considerations as used for T-2 and HT-2. The results of this analysis are summarized in Table 17.

Based on the information in Table 17, the Committee selected the $BMDL_{10}$ of 2.6 µg/kg bw as the point of departure for T-2 and HT-2 and identified a relative potency factor of 0.2 for DAS.

Table 17
Dose–response summary statistics for the emetic response in mink following acute gavage exposure to T-2/HT-2 or DAS

	BMD distribution (μg/kg bw)			
Trichothecene (study)	**BMD_{10}**	**$BMDL_{10}$**	**$BMDU_{10}$**	**$BMD_{10}/BMDL_{10}$**
T-2/HT-2 (Wu W et al., 2016)	8.6	2.6	24.1	3
DAS (Wu Q et al., 2020)	36.8	14.4	65.2	3

BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower 95% confidence limit on the benchmark dose for a 10% response; $BMDU_{10}$, upper 95% confidence limit on the benchmark dose for a 10% response; DAS, 4,15-diacetoxyscirpenol.

4.13.2 Repeated-dose toxicity

The Committee focused the dose–response analysis on the juvenile pig study by Rafai, Papp & Jakab (2013) because it investigated more than one treatment level at doses lower than the previously identified LOAEL of 29 μg/kg bw per day (Annex 1, reference *152*), and because accurate dose estimates could be derived and background mycotoxin contamination of the basal feed was characterized. Table 12 summarizes the data that were considered in the dose–response assessment.

Concerning the immunological effects of T-2 exposure, the Committee noted the WHO/IPCS (2012) guidance on immunotoxicity risk assessment for chemicals, which recommends that functional measures of the immune system be used for hazard characterization rather than observational end-points (for example, leukocyte count). Since Rafai, Papp & Jakab (2013) concluded that the T-cell-dependent antibody response was not observed at doses of either 11.2 or 18 μg/kg bw per day, the Committee focused its dose–response assessment on reduced body weight, daily body weight gain and feed intake. The Committee also expressed concerns over the reliability and reproducibility of the functional immune parameters measured in the key studies summarized previously, and the potential for decreased feed intake to affect the immunological and haematological parameters (Annex 1, reference *152*; Friend, Babiuk & Schiefer, 1983; WHO/IPCS, 1996; Poetschke et al., 2000; Savino, 2002; Martin et al., 2008).

Modelling was carried out with ToxicR, version 22.01 (1.0.0) using a relative deviation approach. Ideally, a BMR is set numerically so that it reflects the onset of a human-relevant adverse effect. The Committee considered a reduction in body weight gain in rapidly growing animals as an adverse effect but found it difficult to decide on a minimal level of adversity for such a reduction. In line with the updated Chapter 5 of EHC 240 guidance for such a situation (WHO/IPCS, 2020), the Committee chose to consider a range of BMRs, in this case, 5% or 10%, and give specific consideration to the corresponding BMD credible intervals (BMDL-BMDU interval) when selecting the point of departure and deciding on

the numerical value of uncertainty factors for establishing the HBGV. The results of the modelling are summarized in Tables 14 and 15.

In the critical study by Rafai, Papp & Jakab (2013), animals were administered doses of 11.2 and 18 µg/kg body weight per day. Using this data with a BMR of 5%, the BMD_{05} and $BMDL_{05}$ are considerably below the lowest tested dose of 11.2 µg/kg body weight per day. Consequently, the calculation of the BMD is dependent on an extrapolation between 0 and 11.2, and this is true even if model averaging is used. As a result, there is increased statistical uncertainty when calculating the BMDL for low BMRs. For example, when considering daily body weight gain, the BMDL is 1.8 versus 0.6 µg/kg bw per day for BMRs of 10 and 5%, respectively. This calculation represents a threefold decrease in the BMDL, corresponding to a halving of the BMR, which implies increased heterogeneity (at doses below the observed range). For all the reasons stated above, the Committee decided that computing the BMD at a 10% BMR was appropriate.

Considering the uncertainties inherent in measuring feed intake of animals that were housed in groups and the larger model uncertainty associated with the corresponding BMD estimates, the Committee selected the $BMDL_{10}$ of 1.8 µg/kg bw per day based on reduced daily body weight gain for hazard characterization purposes.

5. Evaluation

At its ninetieth meeting, the Committee (Annex 1, reference *251*) reviewed the information that had become available since the fifty-sixth meeting on T-2 and HT-2 concerning analytical methods, sampling, effect of processing, prevention and control, occurrence in food commodities and dietary exposure. The toxicological data were addressed at the current meeting and the combined dietary exposure was re-evaluated.

T-2 exposure induces emesis, reduced feed intake, reduced body weight gain, immunotoxicity and haematotoxicity following acute and short-term intake in multiple species. No suitable long-term studies were identified for establishing a tolerable intake for T-2 and HT-2. Nonetheless, based on the critical effects seen in several acute and short-term studies, the Committee concluded that the safety of food contaminated with T-2 or HT-2 could be evaluated.

Furthermore, as previously recommended (Annex 1, reference *235*), the current Committee considered the issue of additivity with respect to DAS exposure. In particular, the current Committee noted that additivity is supported by more recent acute toxicity data indicating that DAS exhibits similar emetic effects in mink via a similar mode of action to T-2 and HT-2, but at a lower

relative potency (Wu Q et al., 2020). Additionally, there is limited evidence that DAS can be detected as co-contaminant with T-2 and HT-2, particularly where analytical methods with low LODs are used.

Although the effects and proposed mechanisms elicited by other trichothecenes (NEO, DON, 3-ADON, 15-ADON, FUS-X and NIV) appear similar, the current Committee concluded that, with the exception of DAS, the evidence for grouping other trichothecenes or establishing relative potency factors, was inadequate and beyond the scope of this addendum.

5.1 Group ARfD

Emesis is a common effect of acute trichothecene exposure in both humans and experimental animals (Annex 1, reference *152, 200, 235*). On this basis, the Committee established a group ARfD for T-2, HT-2 and DAS using the $BMDL_{10}$ of 2.6 µg/kg bw for emesis in mink following acute gavage exposure to T-2 or HT-2 as the point of departure. Based on the available evidence, the Committee decided that an uncertainty factor of 8 (2.5 for interspecies variability in toxicodynamics and 3.16 for intra-human variability in toxicodynamics) was sufficiently protective on the basis that:

1) The mechanisms for emesis in mink are likely to be similar to the mechanisms for emesis in humans (for example, activation of receptors in both the gastrointestinal tract and the CNS).
2) The speed to onset (approximately 30 minutes) and the duration of T-2- and HT-2-induced emesis is proportional to the administered dose suggesting that it is likely to be dependent on Cmax rather than area under the concentration–time curve.
3) The point of departure is based on a gavage study where higher C_{max} concentrations are expected compared with equivalent dietary exposures.

DAS also induces emesis in mink via a similar mode of action, but at a relatively lower potency than T-2 and HT-2 (Wu Q et al., 2020). Furthermore, like T-2 and HT-2, DAS has induced reduced feed intake in mice via a similar mode of action (Zhang et al., 2017a,b; 2018a,b). Accordingly, the Committee established a group ARfD for T-2, HT-2 and DAS of 320 ng/kg bw (rounded down).

Considering the highly comparable nature of the methods used by Wu W et al. (2016) and Wu Qet al. (2020) concerning the emetic effects of T-2, HT-2 and DAS in mink, the Committee recommended a relative potency factor of 0.2 for acute DAS exposure.

5.2 Group TDI

The Committee concluded that the most sensitive reliable and reproducible effects observed following repeated dietary exposure were reported in the 3-week toxicity study in juvenile pigs by Rafai, Papp & Jakab (2013). This study adequately characterized the test material and background exposure to common mycotoxins detected in feed and examined critical toxicological effects at relatively low doses (for example, <25 µg/kg bw per day). The Committee also noted that juvenile pigs have been identified previously as a sensitive species to the emetic and haematotoxic effects of trichothecenes (Coppock et al., 1989; Pestka & Smolinski, 2005). Dose–response analysis of body weights, daily body weight gain and daily feed intake reported by Rafai, Papp & Jakab (2013) was conducted and a $BMDL_{10}$ of 1.8 µg/kg bw per day based on reduced daily body weight gain was selected as the most appropriate point of departure for establishing an HBGV.

Considering that the critical effect (i.e. nausea-induced reductions in feed intake resulting in decreased body weight gain) is likely to be C_{max}-dependent and given the Committee's low confidence in the overall toxicological database, a composite uncertainty factor of 72 was considered appropriate (eightfold as for the group ARfD; threefold for extrapolation from subacute to chronic exposure and threefold for other uncertainties in the database). Accordingly, the Committee established a group TDI of 25 ng/kg bw for T-2, HT-2 and DAS, alone or in combination. The previous group PMTDI of 60 ng/kg bw for T-2 and HT-2, established at the fifty-sixth meeting and amended at the eighty-third meeting to include DAS, was withdrawn.

Although comparative longer term data on DAS and T-2 and HT-2 are not available, the Committee concluded that the relative potency factor of 0.2 is applicable for exposure durations longer than acute, due to the similar critical effects observed following acute and repeated oral exposures. The relative potency factor of 0.2 should be applied in comparing dietary exposure to DAS with the group TDI.

5.3 Risk characterization

5.3.1 Acute dietary exposure

Acute dietary exposure to the sum of T-2 and HT-2 was previously evaluated by the Committee (Annex 1, reference *251*). The highest UB 95th percentile exposure estimate of 170 ng/kg bw was reported for infants in European countries. The Committee also noted that the acute dietary exposure estimates decreased with increasing age. The current Committee noted that at its eighty-third meeting, acute exposure to DAS was not evaluated.

There is insufficient information available to estimate combined acute exposure to T-2, HT-2 and DAS. The dietary exposure estimates made by the Committee for T-2 and HT-2 at its ninetieth meeting are below the ARfD of 320 ng/kg bw. UB estimates of acute dietary exposure to the sum of T-2 and HT-2 (first tier) indicate no health concern but estimates of dietary exposure to DAS in combination with T-2 and HT-2 should be carried out at a future meeting of the Committee when sufficient and suitable data on DAS become available.

5.3.2 Chronic dietary exposure

The estimates of dietary exposure to the sum of T-2 and HT-2 reviewed mainly related to European and north African countries. The estimates of chronic dietary exposure to the sum of T-2 and HT-2 derived from the literature for the general population for the LB mean ranged from 0.3 to 53 ng/kg bw per day and for the LB 95th percentile from 1.9 to 210 ng/kg bw per day. The Committee concluded that dietary exposure estimates for the sum of T-2 and HT-2 at the mean and at the 95th percentile are higher than the group TDI of 25 ng/kg bw, indicating a possible health concern. Estimates of chronic dietary exposure to DAS in combination with T-2 and HT-2 should be carried out at a future meeting of the Committee when sufficient and suitable data on DAS become available.

5.3.3. Recommendations

The Committee recommended the following:

- Development of analytical multimycotoxin methods and standards for the quantification of type A trichothecenes and their various metabolites that occur in planta;
- Research to investigate the spatial distribution of T-2 and HT-2 in agricultural commodities to ensure standard sampling methods for mycotoxins are appropriate;
- That occurrence data for T-2, HT-2 and DAS from a wider range of countries be generated using analytical methods with suitably low LODs, to decrease the uncertainty in dietary exposure estimates and confirm the geographical distribution of these toxins;
- Conducting chronic toxicity studies of T-2, HT-2 and DAS with adequate characterization of T-2, HT-2 and DAS doses as well as the background concentrations of other related mycotoxins in the basal feed; and
- Additional information on the toxicity of relevant mycotoxin mixtures (for example, those that co-occur).

6. References

Agrawal, M, Bhaskar ASB, Rao PVL (2015). Involvement of mitogen-activated protein kinase pathway in T-2 toxin-induced cell cycle alteration and apoptosis in human neuroblastoma cells. Molec Neurobiol. 51:1379–1394. https://doi.org/10.1007/s12035-014-8816-4

Al-Sien AII, Al-Seni MN (2014). Evaluation of gojiextract and charcoal as antioxidant on T-2 toxin administration Onliver male mice. Food Nutr Sci. 05:2124–9. https://doi.org/10.4236/fns.2014.522225

Arce-López B, Lizarraga E, Irigoyen Á, González-Peñas E (2020). Presence of 19 mycotoxins in human plasma in a region of Northern Spain. Toxins (Basel). 12:750.

Arce-López B, Lizarraga E, López de Mesa R, González-Peñas E (2021a). Assessment of exposure to mycotoxins in Spanish children through the analysis of their levels in plasma samples. Toxins (Basel). 13:150.

Arce-López B, Alvarez-Erviti L, De Santis B, Izco M, López-Calvo S, Marzo-Sola ME, et al. (2021b). Biomonitoring of mycotoxins in plasma of patients with Alzheimer's and Parkinson's disease. Toxins (Basel). 13:477

Arunachalam C, Doohan FM (2013). Trichothecene toxicity in eukaryotes: Cellular and molecular mechanisms in plants and animals. Toxicol Lett. 217:149–58. https://doi.org/10.1016/j.toxlet.2012.12.003

Atroshi F, Rizzo A, Sankari S, Biese I, Westermarck T, Veijalainen P (2000). Liver enzyme activities of rats exposed to ochratoxin A and T-2 toxin with antioxidants. Bull Environ Contam Toxicol. 64:586–92. https://doi.org/10.1007/s001280000043

Battilani P, Palumbo R, Giorni P, Dall'Asta C, Dellafiora L, Gkrillas A, et al. (2020). Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach: EFSA Supporting Publications, 17. https://doi.org/10.2903/sp.efsa.2020.en-1757

Behm C, Föllmann W, Degen GH (2012). Cytotoxic potency of mycotoxins in cultures of v79 lung fibroblast cells. J Toxicol Environ Health – Part A: Current Issues, 75:1226–31. https://doi.org/10.1080/15287394.2012.709170

Bouaziz C, Abid-Essefi S, Bouslimi A, El Golli E, Bacha H (2006). Cytotoxicity and related effects of T-2 toxin on cultured Vero cells. Toxicon. 48 :343–52. https://doi.org/10.1016/j.toxicon.2006.06.004

Bouaziz, Chayma, Sharaf el dein, O, El Golli, E, Abid-Essefi, S, Brenner, C, Lemaire, C., Bacha, H (2008). Different apoptotic pathways induced by zearalenone, T-2 toxin and ochratoxin A in human hepatoma cells. Toxicology, 254(1–2), 19–28. https://doi.org/10.1016/j.tox.2008.08.020

Bouaziz C, Martel C, Sharaf el dei, O, Abid-Essefi S, Brenner C, Lemaire C, Bacha H (2009). Fusarial toxin-induced toxicity in cultured cells and in isolated mitochondria involves PTPC-dependent activation of the mitochondrial pathway of apoptosis. Toxicol Sci. 110:363–75. https://doi.org/10.1093/toxsci/kfp117

Calvert TW, Aidoo KE, Candlish AGG, Mohd Fuat AR (2005). Comparison of in vitro cytotoxicity of Fusarium mycotoxins, deoxynivalenol, T-2 toxin and zearalenone on selected human epithelial cell lines. Mycopathologia. 159:413–19. https://doi.org/10.1007/s11046-005-0254-4

Chandratre GA, Telang AG, Badgujar PC, Raut SS, Sharma AK (2014). Toxicopathological alterations induced by high dose dietary T-2 mycotoxin and its residue detection in Wistar rats. Arch Environ Contam Toxicol. 67:124–28. https://doi.org/10.1007/s00244-014-0006-x

Chapman K, Sewell F, Allais L, Delongeas JL, Donald E, Festag, et al. (2013). A global pharmaceutical company initiative: an evidence-based approach to define the upper limit of body weight loss in short term toxicity studies. Regul Toxicol Pharmacol. 67:27–38. doi: 10.1016/j.yrtph.2013.04.003. Epub 2013 Apr 19. PMID: 23602904.

Chattopadhyay P, Upadhyay A, Agnihotri A, Karmakar S, Ghoyary D, Veer V (2013). Comparative hematoxicity of fusirium mycotoxin in experimental sprague-dawley rats. Toxicol Internat. 20:25–29. https://doi.org/10.4103/0971-6580.111552

Chaudhari M, Jayaraj R, Santhosh SR, Rao PVL (2009a). Oxidative damage and gene expression profile of antioxidant enzymes after T-2 toxin exposure in mice. J Biochem Mol Toxicol. 23:212–21. www.interscience.wiley.com

Chaudhari M, Jayaraj R, Bhaskar ASB, Lakshmana Rao PV (2009b). Oxidative stress induction by T-2 toxin causes DNA damage and triggers apoptosis via caspase pathway in human cervical cancer cells. Toxicology. 262:153–61. https://doi.org/10.1016/j.tox.2009.06.002

Chen J, Chu Y, Cao J, Yang Z, Guo X, Wang Z (2006). T-2 toxin induces apoptosis, and selenium partly blocks, T-2 toxin induced apoptosis in chondrocytes through modulation of the Bax/Bcl-2 ratio. Food Chem Toxicol. 44:567–73. https://doi.org/10.1016/j.fct.2005.09.004

Coppock RW, Hoffmann WE, Gelberg HB, Bass D, Buck WB (1989). Hematologic changes induced by intravenous administration of diacetoxyscirpenol in pigs, dogs, and calves. Am J Vet Res. 50:411–15.

COT [Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment]. (2021). Second draft statement on the potential risks of combined exposure to mycotoxins. TOX/2021/04 (https://cot.food.gov.uk/sites/default/files/2021-01/TOX_2021-04%202nd%20Draft%20Statement%20Combined%20Exposure%20Mycotoxins.pdf).

Da Silva EO, Bracarense APFL, Oswald IP (2018). Mycotoxins and oxidative stress: Where are we? World Mycotox J. 11:113–33. https://doi.org/10.3920/WMJ2017.2267

Daud N, Currie V, Duncan G, Busman M, Gratz SW (2020a). Intestinal hydrolysis and microbial biotransformation of diacetoxyscirpenol-α-glucoside, HT-2-β-glucoside and N-(1-deoxy-d-fructos-1-yl) fumonisin B1 by human gut microbiota in vitro. Int J Food Sci Nutr. 71:540–48. https://doi.org/10.1080/09637486.2019.1698015

Daud N, Currie V, Duncan G, Farquharson F, Yoshinari T, Louis P, et al. (2020b). Prevalent human gut bacteria hydrolyse and metabolise important food-derived mycotoxins and masked mycotoxins. Toxins. 12. https://doi.org/10.3390/toxins12100654

De Angelis E, Monaci L, Mackie A, Salt L, Visconti A (2014). Bioaccessibility of T-2 and HT-2 toxins in mycotoxin contaminated bread models submitted to in vitro human digestion. Innovative Food Sci Emerging Technol. 22:248–56. https://doi.org/10.1016/j.ifset.2013.11.011

de Kort, M, Weber, K, Wimmer, B, Wilutzky, K, Neuenhahn, P, Allingham, P, et al. (2020). Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: Acceptable value ranges, definition of severity degrees, and vehicle effects. Toxicol Res Application. 4:239784732093148. https://doi.org/10.1177/2397847320931484

De Ruyck K, Huybrechts I, Yang S, Arcella D, Claeys L, Abbeddou S et al. (2020). Mycotoxin exposure assessments in a multi-center European validation study by 24-hour dietary recall and biological fluid sampling. Environ Int. 137:105539.

Dellafiora L, Galaverna G, Dall'Asta C (2017). In silico analysis sheds light on the structural basis underlying the ribotoxicity of trichothecenes – A tool for supporting the hazard identification process. Toxicol Letters, 270:80–87. https://doi.org/10.1016/j.toxlet.2017.02.015

EFSA [European Food Safety Authority CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)] (2011). Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J. 9:2481. doi:10.2903/j.efsa.2011.2481.

EFSA [European Food Safety Authority CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)] (2017). Scientific opinion on the appropriateness to set a group health based guidance value for T-2 and HT-2 toxin and its modified forms. EFSA J. 15:4655. doi:10.2903/j.efsa.2017.4655

Fadhil AA, Alkutbi SH, Nassir ES (2021). Efficacy of five organic acids combination on T-2- mycotoxicosis in rats. Ind J Forensic Med Toxicol. 15:2081–94. https://doi.org/10.37506/ijfmt.v15i3.15624

Faisal Z, Vörös V, Fliszár-Nyúl E, Lemli B, Kunsági-Máté S, Csepregi R, et al. (2020). Probing the interactions of ochratoxin b, ochratoxin c, patulin, deoxynivalenol, and t-2 toxin with human serum albumin. Toxins. 12. https://doi.org/10.3390/toxins12060392

Fan K, Xu J, Jiang K, Liu X, Meng J, Di Mavungu JD et al. (2019). Determination of multiple mycotoxins in paired plasma and urine samples to assess human exposure in Nanjing, China. Environ Pollut. 248:865–73.

Fang H, Wu Y, Guo J, Rong J, Ma L, Zhao Z, et al. (2012). T-2 toxin induces apoptosis in differentiated murine embryonic stem cells through reactive oxygen species-mediated mitochondrial pathway. Apoptosis. 17 :895–907. https://doi.org/10.1007/s10495-012-0724-3

Ferreras MC, Benavides J, García-Pariente C, Delgado L, Fuertes M, Muñoz M, et al. (2013). Acute and chronic disease associated with naturally occurring T-2 mycotoxicosis in sheep. J Comparative Pathol. 148:236–42. https://doi.org/10.1016/j.jcpa.2012.05.016

Franco LT, Petta T, Rottinghaus GE, Bordin K, Gomes GA, Alvito P, et al. (2019). Assessment of mycotoxin exposure and risk characterization using occurrence data in foods and urinary biomarkers in Brazil. Food Chem Toxicol. 128:21–34.

Frankič T, Salobir J, Rezar V (2008). The effect of vitamin E supplementation on reduction of lymphocyte DNA damage induced by T-2 toxin and deoxynivalenol in weaned pigs. Anim Feed Sci Technol. 141:274–86.

Fribley A, Zhang K, Kaufman RJ (2009). Regulation of apoptosis by the unfolded protein response. Methods Mol Biol (Clifton, NJ). 559:191–204. https://doi.org/10.1007/978-1-60327-017-5_14

Friend SCE, Babiuk LA, Schiefer HB (1983). The effects of dietary T-2 toxin on the immunological function and herpes simplex reactivation in Swiss mice. Toxicol Appl Pharmacol. 69:234–44. https://doi.org/10.1016/0041-008X(83)90304-6

Friend SCE, Hancock DS, Schiefer HB, Babiuk LA (1983). Experimental T-2 toxicosis in sheep. Can J Comparative Med. 47:291–7.

Gaigé S, Djelloul M, Tardivel C, Airault C, Félix B, Jean A, et al. (2014). Modification of energy balance induced by the food contaminant T-2 toxin: A multimodal gut-to-brain connection. Brain, Behav Immun. 37:54–72. https://doi.org/10.1016/j.bbi.2013.12.008

Ge X, Wang J, Liu J, Jiang J, Lin H, Wu J, et al. (2010). The catalytic activity of cytochrome P450 3A22 is critical for the metabolism of T-2 toxin in porcine reservoirs. Catalysis Comm. 12 :71–75. https://doi.org/10.1016/j.catcom.2010.08.003

Gerding J, Cramer B, Humpf HU (2014). Determination of mycotoxin exposure in Germany using an LC-MS/MS multibiomarker approach. Mol Nutr Food Res. 58:2358–68.

Gerding J, Ali N, Schwartzbord J, Cramer B, Brown DL, Degen GH (2015). A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. Mycotoxin Res. 31:127-36.

Goineau S, Castagné V (2016). Comparison of three preclinical models for nausea and vomiting assessment. J Pharm Toxicol Meth. 82:45–53. https://doi.org/10.1016/j.vascn.2016.07.006

Gonkowski S, Gajęcka M, Makowska K (2020). Mycotoxins and the enteric nervous system. Toxins. 12. https://doi.org/10.3390/toxins12070461

Goossens J, Vandenbroucke V, Pasmans F, de Baere S, Devreese M, Osselaere A, et al. (2012a). Influence of mycotoxins and a mycotoxin adsorbing agent on the oral bioavailability of commonly used antibiotics in pigs. Toxins. 4:281–95. https://doi.org/10.3390/toxins4040281

Goossens J, Pasmans F, Verbrugghe E, Vandenbroucke V, De Baere S, Meyer E, et al. (2012b). Porcine intestinal epithelial barrier disruption by the Fusarium mycotoxins deoxynivalenol and T-2 toxin promotes transepithelial passage of doxycycline and paromomycin. BMC Vet Res. 8:245. https://doi.org/10.1186/1746-6148-8-245

Goossens J, Devreese M, Pasmans F, Osselaere A, De Baere S, Verbrugghe E, et al. (2013a). Chronic exposure to the mycotoxin T-2 promotes oral absorption of chlortetracycline in pigs. J Vet Pharmacol Ther. 36:621–4. https://doi.org/10.1111/jvp.12059

Goossens J, De Bock L, Osselaere A, Verbrugghe E, Devreese M, Boussery K, et al. (2013b). The mycotoxin T-2 inhibits hepatic cytochrome P4503A activity in pigs. Food Chem Toxicol. 57:54–6. https://doi.org/10.1016/j.fct.2013.03.009

Gratz SW, Dinesh R, Yoshinari T, Holtrop G, Richardson AJ, Duncan G, et al. (2017). Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota in vitro. Mol Nutr Food Res. 61:1600680. https://doi.org/10.1002/mnfr.201600680

Gratz SW, Currie V, Duncan G, Jackson D (2020). Multimycotoxin Exposure Assessment in UK Children Using Urinary Biomarkers – A Pilot Survey. J Agric Food Chem, 68:351–7.

Grenier B, Oswald IP (2011). Mycotoxin co-contamination of food and feed: Meta-analysis of publications describing toxicological interactions. World Mycotox J. 4:285–313. https://doi.org/10.3920/WMJ2011.1281

Guan F, Li S, Wang ZL, Yang H, Xue S, Wang W, et al. (2013). Histopathology of chondronecrosis development in knee articular cartilage in a rat model of Kashin-Beck disease using T-2 toxin and selenium deficiency conditions. Rheumatol Int. 33:157–66. https://doi.org/10.1007/s00296-011-2335-7

Guerre P, Eeckhoutte C, Burgat V, Galtier P (2000). The effects of T-2 toxin exposure on liver drug metabolizing enzymes in rabbit. Food Add Contam. 17:1019–26. https://doi.org/10.1080/02652030050207819

Guo P, Liu A, Huang D, Wu Q, Fatima Z, Tao Y, et al. (2018). Brain damage and neurological symptoms induced by T-2 toxin in rat brain. Toxicol Lett. 286:96–107. https://doi.org/10.1016/j.toxlet.2018.01.012

Guo P, Qiao F, Huang D, Wu Q, Chen T, Badawy S, et al. (2020). MiR-155-5p plays as a "janus" in the expression of inflammatory cytokines induced by T-2 toxin. Food Chem Toxicol. 140:111258. https://doi.org/10.1016/j.fct.2020.111258

Gürbüzel M, Uysal H, Kızılet H (2015). Assessment of genotoxic potential of two mycotoxins in the wing spot test of *Drosophila melanogaster*. Toxicol Indust Health. 31:261–7. https://doi.org/10.1177/0748233712472528

Hafner D, Bodnár Z, Horvatovich K, Berta G, Kovács M (2012). Preliminary investigations into the effect of feeding mannan oligosaccharide (MOS) on the genotoxic effect of T-2 toxin in rabbits measured by comet assay. Acta Agric Slovenica, 100(suppl. 3):351–5.

Hafner D, Szabó A, D'Costa L, Szabó-Fodor J, Tornyos G, Blochné Bodnár Z, et al. (2016). Individual and combined effects of feed artificially contaminated with with fumonisin B1 and T-2 toxin in weaned rabbits. World Mycotox J. 9:613–22. https://doi.org/10.3920/WMJ2016.2067

Han J, Yu FF, Chang ZP, Yang B, Qu CJ, Zhou TT et al. (2015). Changing grains for the prevention and treatment of Kashin-Beck disease in children: a meta-analysis. Biomed Environ Sci. 28:308–11.

Heyndrickx E, Sioen I, Huybrechts B, Callebaut A, De Henauw S, De Saeger S (2015). Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. Environ Int. 84:82–9.

Holme J, Morrison E, Samuelsen J, Wiger R, Låg M, Schwarze P, et al. (2003). Mechanisms involved in the induction of apoptosis by T-2 and HT-2 toxins in HL-60 human promyelocytic leukemia cells. Cell Biol Toxicol. 19:53–68. https://doi.org/10.1023/a:1022069715399

Horvatovich K, Hafner D, Bodnár Z, Berta G, Hancz C, Dutton M, et al. (2013). Dose-related genotoxic effect of T-2 toxin measured by comet assay using peripheral blood mononuclear cells of healthy pigs. Acta Vet Hungarica. 61:175–86. https://doi.org/10.1556/AVet.2013.010

Huang P, Akagawa K, Yokoyama Y, Nohara K, Kano K, Morimoto K (2007). T-2 toxin initially activates caspase-2 and induces apoptosis in U937 cells. Toxicol Lett. 170:1–10. https://doi.org/10.1016/j.toxlet.2006.05.017

Huybrechts B, Martins JC, Debongnie Ph, Uhlig S, Callebaut A (2015). Fast and sensitive LC–MS/MS method measuring human mycotoxin exposure using biomarkers in urine. Arch Toxicol. 89:1993–2005.

Ishigami N, Shinozuka J, Katayama K, Nakayama H, Doi K (2001). Apoptosis in mouse fetuses from dams exposed to T-2 toxin at different days of gestation. Exper Toxicol Pathol. 52:493–501. https://doi.org/10.1016/s0940-2993(01)80004-x

Iurlaro R, Muñoz-Pinedo C (2016). Cell death induced by endoplasmic reticulum stress. FEBS J. 283:2640–52. https://doi.org/10.1111/febs.13598

Iwahashi Y, Kitagawa E, Iwahashi H (2008). Analysis of mechanisms of T-2 toxin toxicity using yeast DNA microarrays. Int J Mol Sci. 9:2585–600. https://doi.org/10.3390/ijms9122585

Jaćević V, Vukajlovic A, Lazarević M, Bocarov-Stancic A, Resanović R, Djordjevic S (2009). Influence of various adsorbents on basic physiological parameters in rats acutely intoxicated by T-2 toxin: A comparative study. J Vet Pharmacol Ther. 32(suppl. 1), 99–100. https://doi.org/10.1111/j.1365-2885.2009.01090.x

Jaćević V, Dumanović J, Lazarević M, Nepovimova E, Resanović R, Milovanović Z, et al. (2020). Antidotal potency of the novel, structurally different adsorbents in rats acutely intoxicated with the T-2 toxin. Toxins. 12:643. https://doi.org/10.3390/toxins12100643

Jain AK, Singh D, Dubey K, Maurya R, Mittal S, Pandey AK (2018). Models and methods for in vitro toxicity. in in vitro toxicology. In: Dhawan A, Kwon S, editors. In vitro toxicology. Cambridge (MA): Academic Press. https://doi.org/10.1016/B978-0-12-804667-8.00003-1

Jiang J, Zhu J, Liu Q, Zhang T, Wen J, Xia J, et al. (2021). Role of DNA methylation-related chromatin remodeling in aryl hydrocarbon receptor-dependent regulation of T-2 toxin highly inducible Cytochrome P450 1A4 gene. FASEB J. 35:e21469. doi: 10.1096/fj.202002570RR. PMID: 33788981.

Kang R, Perveen A, Li C (2020). Effects of maternal T-2 toxin exposure on the hepatic glycolipid metabolism in young mice. Ecotoxicol Environ Safety. 196:110530. https://doi.org/10.1016/j.ecoenv.2020.110530

Kang P, Yao Y, Yang J, Shen B, Zhou Z, Pei F (2013). An animal model of Kashin-Beck disease induced by a low-nutrition diet and exposure to T-2 toxin. Osteoarthritis Cartilage. 21:1108–15. https://doi.org/10.1016/j.joca.2013.05.005

Karacaoğlu E, Selmanoğlu G (2017). T-2 toxin induces cytotoxicity and disrupts tight junction barrier in SerW3 cells. Environ Toxicol Pharmacol. 56:259–67. https://doi.org/10.1016/j.etap.2017.10.005

Kasimir M, Behrens M, Schulz M, Kuchenbuch H, Focke C, Humpf HU (2020). Intestinal metabolism of α- and β-glucosylated modified mycotoxins T-2 and HT-2 toxin in the pig cecum model. J Agr Food Chem. 68:5455–61. https://doi.org/10.1021/acs.jafc.0c00576

Kong L, Zhu L, Yi X, Huang Y, Zhao H, Chen Y, et al. (2021). Betulinic acid alleviates spleen oxidative damage induced by acute intraperitoneal exposure to t-2 toxin by activating nrf2 and inhibiting mapk signaling pathways. Antioxidants. 10:1–17. https://doi.org/10.3390/antiox10020158

Königs M, Mulac D, Schwerdt G, Gekle M, Humpf HU (2009). Metabolism and cytotoxic effects of T-2 toxin and its metabolites on human cells in primary culture. Toxicology. 258:106–15. https://doi.org/10.1016/j.tox.2009.01.012

Köşüş A, Köşüş N, Usluoğullari B, Hizli D, Namuslu M, Ayyildiz A (2015). Gut satiety hormones and hyperemesis gravidarum. Arch Gynecol Obstet. 292:1225–30. https://doi.org/10.1007/s00404-015-3751-9

Kovács M, Tornyos G, Matics Z, Kametler L, Rajli V, Bodnár Z, et al. (2011). Subsequent effect of subacute T-2 toxicosis on spermatozoa, seminal plasma and testosterone production in rabbits. Animal. 5:1563–9. https://doi.org/10.1017/S1751731111000644

Kovács M, Tornyos G, Matics Z, Mézes M, Balogh K, Rajli V, et al. (2013). Effect of chronic T-2 toxin exposure in rabbit bucks, determination of the No Observed Adverse Effect Level (NOAEL). Animal Reprod Sci. 137:245–52. https://doi.org/10.1016/j.anireprosci.2013.01.006

Kraus VB (2015). Rare osteoarthritis: ochronosis and Kashin-Beck disease. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology, 6th edition. Philadelphia: Mosby Elsevier; 1536–40.

Kravchenko, LV, Avren'eva LI, Guseva GV, Posdnyakov AL, Tutel'yan VA (2001). Effect of nutritional indoles on activity of xenobiotic metabolism enzymes and T-2 toxicity in rats. Bull Exp Biol Med. 131:544–7. https://doi.org/10.1023/A:1012346401315

Kruber P, Trump S, Behrens J, Lehmann I (2011). T-2 toxin is a cytochrome P450 1A1 inducer and leads to MAPK/p38- but not aryl hydrocarbon receptor-dependent interleukin-8 secretion in the human intestinal epithelial cell line Caco-2. Toxicology. 284:34–41. https://doi.org/10.1016/j.tox.2011.03.012

Le Dréan G, Auffret M, Batina P, Arnold F, Sibiril Y, Arzur D, et al. (2005). Myelotoxicity of trichothecenes and apoptosis: An in vitro study on human cord blood CD34+ hematopoietic progenitor. Toxicol in Vitro. 19:1015–24. https://doi.org/10.1016/j.tiv.2005.03.017

Lei R, Jiang N, Zhang Q, Hu S, Dennis BS, He S et al. (2016). Prevalence of selenium, T-2 toxin, and deoxynivalenol in Kashin-Beck disease areas in Qinghai Province, Northwest China. Biol Trace Elem Res. 171:34–40.

Li D, Han J, Guo X, Qu C, Yu F, Wu, X (2016). The effects of T-2 toxin on the prevalence and development of Kashin-Beck disease in China: A meta-analysis and systematic review. Toxicol Res. 5:731–51. https://doi.org/10.1039/c5tx00377f

Li M, Cuff CF, Pestka JJ (2006). T-2 toxin impairment of enteric reovirus clearance in the mouse associated with suppressed immunoglobulin and IFN-γ responses. Toxicol Appl Pharmacol. 214:318–25. https://doi.org/10.1016/j.taap.2006.01.007

Li M, Harkema JR, Islam Z, Cuff CF, Pestka JJ (2006). T-2 toxin impairs murine immune response to respiratory reovirus and exacerbates viral bronchiolitis. Toxicol Appl Pharmacol. 217:76–85. https://doi.org/10.1016/j.taap.2006.08.007

Lin NN, Chen J, Xu B, Wei X, Guo L, Xie JW (2015). The roles of carboxylesterase and CYP isozymes on the in vitro metabolism of T-2 toxin. Military Med Res. 2:13. https://doi.org/10.1186/s40779-015-0041-6

Lin X, Shao W, Yu F, Xing K, Liu H, Zhang F, et al. (2019). Individual and combined toxicity of T-2 toxin and deoxynivalenol on human C-28/I2 and rat primary chondrocytes. J Appl Toxicol. 39:343–53. https://doi.org/10.1002/jat.3725

Liu Q, Wen J, Zhu J, Zhang T, Deng Y, Jiang J (2019). Aromatic hydrocarbon receptor regulates chicken cytochrome P450 1A5 transcription: A novel insight into T-2 toxin-induced gene expression and cytotoxicity in LMH cells. Biochem Pharmacol. 168:319–29. https://doi.org/10.1016/j.bcp.2019.07.023

Liu X, Wen J, Chen R, Zhang T, Jiang J, Deng Y (2016). T-2 toxin induces the expression of porcine CYP3A22 via the upregulation of the transcription factor, NF-Y. Biochim Biophys Acta – General Subjects. 1860: 2191–201. https://doi.org/10.1016/j.bbagen.2016.05.009

Liu Y, Yang Y, Dong R, Zhang Z, Jia F, Yu H, et al. (2020). Protective effect of selenomethionine on intestinal injury induced by T- 2 toxin. Res Vet Sci: 132:439–47. https://doi.org/10.1016/j.rvsc.2020.07.018

Liu Y, Wang H, Zhang M, Wang J, Zhang Z, Wang Y, et al. (2021a). Protective effect of selenomethionine on T-2 toxin-induced liver injury in New Zealand rabbits. BMC Vet Res. 17:153. https://doi.org/10.1186/s12917-021-02866-1

Liu Y, Dong R, Yang Y, Xie H, Huang Y, Chen X, et al. (2021b). Protective effect of organic selenium on oxidative damage and inflammatory reaction of rabbit kidney induced by T-2 toxin. Biol Trace Element Res. 199:1833–42. https://doi.org/10.1007/s12011-020-02279-5

Lu M, Cao J, Liu F, Li S, Chen J, Fu Q et al. (2012). The effects of mycotoxins and selenium deficiency on tissue-engineered cartilage. Cells Tissues Organs. 196:241–50.

Lu Q, Hu S, Guo P, Zhu X, Ren Z, Wu Q, et al. (2021). PPAR-γ with its anti-fibrotic action could serve as an effective therapeutic target in T-2 toxin-induced cardiac fibrosis of rats. Food Chem Toxicol. 152:112183. https://doi.org/10.1016/j.fct.2021.112183

Luo C, Huang C, Zhu L, Kong L, Yuan Z, Wen L, et al. (2020). Betulinic acid ameliorates the t-2 toxin-triggered intestinal impairment in mice by inhibiting inflammation and mucosal barrier dysfunction through the NF-κB signaling pathway. Toxins. 12:794. https://doi.org/10.3390/toxins12120794

Makowska K, Obremski K., Gonkowski S (2018). The impact of T-2 toxin on vasoactive intestinal polypeptide-like immunoreactive (VIP-LI) nerve structures in the wall of the porcine stomach and duodenum. Toxins. 10. https://doi.org/10.3390/toxins10040138

Makowska K, Obremski K, Zielonka L, Gonkowski S (2017). The influence of low doses of zearalenone and t-2 toxin on calcitonin gene related peptide-like immunoreactive (CGRP-LI) neurons in the ENS of the porcine descending colon. Toxins. 9. https://doi.org/10.3390/toxins9030098

Male D, Wu W, Mitchell NJ, Bursian S, Pestka JJ, Wu F (2016a). Modeling the emetic potencies of food-borne trichothecenes by benchmark dose methodology. Food Chem Toxicol. 94:178–85. https://doi.org/10.1016/j.fct.2016.06.009

Male D, Mitchell NJ, Wu W, Bursian S, Pestka J, Wu F (2016b). Modelling the anorectic potencies of food-borne trichothecenes by benchmark dose and incremental area under the curve methodology. World Mycotox J. 9:279–88.

Martin LB 2nd, Navara KJ, Bailey MT, Hutch CR, Powell ND, Sheridan JF, et al. (2008). Food restriction compromises immune memory in deer mice (*Peromyscus maniculatus*) by reducing spleen-derived antibody-producing B cell numbers. Physiol Biochem Zool. 81:366–72. doi: 10.1086/587090. PMID: 18419561; PMCID: PMC2727600.

McCormick SP, Kato T, Maragos CM, Busman M, Lattanzio VMT, Galaverna G, et al. (2015). Anomericity of T-2 toxin-glucoside: Masked mycotoxin in cereal crops. J Agric Food Chem. 63:731–8. https://doi.org/10.1021/jf504737f

McKean C, Tang L, Billam M, Tang M, Theodorakis CW, Kendall RJ, et al. (2006). Comparative acute and combinative toxicity of aflatoxin B1 and T-2 toxin in animals and immortalized human cell lines. J Appl Toxicol. 26:139–47. https://doi.org/10.1002/jat.1117

Meissonnier GM, Laffitte J, Raymond I, Benoit E, Cossalter AM, Pinton P, et al. (2008). Subclinical doses of T-2 toxin impair acquired immune response and liver cytochrome P450 in pigs. Toxicology. 247:46–54. https://doi.org/10.1016/j.tox.2008.02.003

Meissonnier GM, Raymond I, Laffitte J, Cossalter AM, Pinton P, Benoit E, et al. (2009). Dietary glucomannan improves the vaccinal response in pigs exposed to aflatoxin B or T-2 toxin. World Mycotox J. 2:161–72.

Minervini F, Fornelli F, Lucivero G, Romano C, Visconti A (2005). T-2 toxin immunotoxicity on human B and T lymphoid cell lines. Toxicology. 210 :81–91. https://doi.org/10.1016/j.tox.2005.01.007

Monaci L, Garbetta A, Angelis E. De Visconti A, Minervini F (2015). Assessment of toxic potential of mycotoxin contaminated bread during in vitro human digestion on human B lymphoid cell line. Toxicol Lett. 232:106–12. https://doi.org/10.1016/j.toxlet.2014.09.021

Mu P, Xu M, Zhang L, Wu K, Wu J, Jiang J, et al. (2013). Proteomic changes in chicken primary hepatocytes exposed to T-2 toxin are associated with oxidative stress and mitochondrial enhancement. Proteomics. 13:3175–88. https://doi.org/10.1002/pmic.201300015

Murshedul A, Nagase M, Yoshizawa T, Sakato N (2000). Thymocyte apoptosis by T-2 toxin in vivo in mice is independent of Fas/Fas ligand system. Biosci Biotechnol Biochem. 64:210. https://doi.org/10.1271/bbb.64.210

Nagase M, Alam MM, Tsushima A, Yoshizawa T, Sakato N (2001). Apoptosis induction by T-2 toxin: activation of caspase-9, caspase-3, and DFF-40/CAD through cytosolic release of cytochrome c in HL-60 cells. Biosci Biotechnol Biochem. 65:1741–7. https://doi.org/10.1271/bbb.65.1741

Nagata T, Suzuki H, Ishigami N, Shinozuka J, Uetsuka K, Nakayama H, et al. (2001). Development of apoptosis and changes in lymphocyte subsets in thymus, mesenteric lymph nodes and PEYER'S patches of mice orally inoculated with T-2 toxin. Exp Toxicol Pathol. 53:309–15. https://doi.org/10.1078/0940-2993-00196

Nakajima K, Tanaka T, Masubuchi Y, Ito Y, Kikuchi S, Woo GH, et al. (2019). Developmental exposure of mice to T-2 toxin increases astrocytes and hippocampal neural stem cells expressing metallothionein. Neurotox Res. 35:668–83. https://doi.org/10.1007/s12640-018-9981-4

Nayakwadi S, Ramu R, Sharma AK, Gupta VK, Rajukumar K, Kumar V, et al. (2020). Toxicopathological studies on the effects of T-2 mycotoxin and their interaction in juvenile goats. PLoS ONE, 15(3), e0229463. https://doi.org/10.1371/journal.pone.0229463

Niknejad F, Escrivá L, Adel Rad KB, Khoshnia M, Barba FJ, Berrada H (2021). Biomonitoring of multiple mycotoxins in urine by GC-MS/MS: A pilot study on patients with esophageal cancer in Golestan Province, Northeastern Iran. Toxins (Basel). 13:243.

Obremski K, Podlasz P, Zmigrodzka M, Winnicka A, Woźny M, Brzuzan P, et al. (2013). The effect of T-2 toxin on percentages of CD4+, CD8+, CD4+CD8+and CD21+ lymphocytes, and mRNA expression levels of selected cytokines in porcine ileal Peyer's patches. Polish J Vet Sci. 16:341–349. https://doi.org/10.2478/pjvs-2013-0046

Peachee VL, Smith MJ, Beck MJ, Stump DG, White KL, Jr (2014). Characterization of the T-dependent antibody response (TDAR) to keyhole limpet hemocyanin (KLH) in the Göttingen minipig. J Immunotoxicol. 11:376–82. https://doi.org/10.3109/1547691X.2013.853716

Peng A, Wang WH, Wang CX, Wang ZJ, Rui HF, Wang WZ, et al. (1999). The role of humic substances in drinking water in Kashin-Beck disease in China. Environ Health Perspect. 107:293–6. https://doi.org/10.1289/ehp.99107293

Perveen A, Shen J, Kaka NA, Li C (2020). Maternal exposure to T-2 toxin affects puberty genes and delays estrus cycle in mice offspring. Animals. 10:471. https://doi.org/10.3390/ani10030471

Pestka JJ, Smolinski AT (2005). Deoxynivalenol: Toxicology and potential effects on humans. J Toxicol Environ Health – Part B: Critical Reviews. 8:39–69. https://doi.org/10.1080/10937400590889458

Pierozan CR, Agostini PS, Gasa J, Novais AK, Dias CP, Santos RSK, et al. (2016). Factors affecting the daily feed intake and feed conversion ratio of pigs in grow-finishing units: the case of a company. Porcine Health Manag. 2:7. doi: 10.1186/s40813-016-0023-4. PMID: 28405433; PMCID: PMC5382519.

Pinton P, Tsybulskyy D, Lucioli J, Laffitte J, Callu P, Lyazhri F, et al. (2012). Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: Differential effects on morphology, barrier function, tight junction proteins, and mitogen-activated protein kinases. Toxicol Sci. 130:180–90. https://doi.org/10.1093/toxsci/kfs239

Poetschke HL, Klug D, Perkins SN, Wang TT, Richie ER, Hursting SD (2000). Effects of calorie restriction on thymocyte growth, death and maturation. Carcinogenesis. 21:1959–64. doi: 10.1093/carcin/21.11.1959. PMID: 11062154.

Pomothy JM, Barna RF, Pászti EA, Babiczky Á, Szóládi Á, Jerzsele Á, et al. (2020). Beneficial effects of rosmarinic acid on IPEC-J2 cells exposed to the combination of deoxynivalenol and T-2 Toxin. Mediators Inflamm. 2020:8880651. https://doi.org/10.1155/2020/8880651

Rafai P, Papp Z, Jakab L (2013). Biotransformation of trichothecenes alleviates the negative effects of T-2 toxin in pigs. Acta Vet Hungarica. 61:333–43. https://doi.org/10.1556/AVet.2013.025

Rafai P, Bata A, Vanyi A, Papp Z, Brydl E, Jakab L, et al. (1995a). Effect of various levels of T-2 toxin on the clinical status, performance and metabolism of growing pigs. Vet Record. 136:485–9. https://doi.org/10.1136/vr.136.19.485

Rafai P, Tuboly S, Bata A, Tilly P, Ványi A, Papp Z, et al. (1995b). Effect of various levels of T-2 toxin in the immune system of growing pigs. Vet Record. 136:511–14. https://doi.org/10.1136/vr.136.20.511

Rahman S, Sharma AK, Singh ND, Telang AG, Azmi S, Prawez S (2014). Clinico-haematological changes in T-2 toxicosis in Wistar rats. Indian J Vet Pathol. 38:22. https://doi.org/10.5958/0973-970x.2014.01129.8

Rahman S, Sharma AK, Singh ND, Prawez S (2016). T-2 toxin induced nephrotoxicity in Wistar rats. Indian J Vet Pathol. 40:320. https://doi.org/10.5958/0973-970x.2016.00074.2

Rahman S, Sharma AK, Singh ND, Prawez S (2021). Immunopathological effects of experimental T-2 mycotoxicosis in Wistar rats. Human Exp Toxicol. 40:772–90. https://doi.org/10.1177/0960327120968852

Rakkestad KE, Skaar I, Ansteinsson VE, Solhaug A, Holme JA, Pestka JJ, et al. (2010). DNA damage and DNA damage responses in THP-1 Monocytes after exposure to spores of either *Stachybotrys chartarum* or *Aspergillus versicolor* or to T-2 toxin. Toxicol Sci. 115:140–55. https://doi.org/10.1093/toxsci/kfq045

Rani MU, Reddy AG, Reddy GD, Raj MA (2009). Oxidative stress due to ochratoxin and T-2 toxin either alone or in combination and evaluation of protective role of *Curcuma longa*, Zingiber officinale, toxichek and activated charcoal. Toxicol Internat. 16:63–8.

Raut S, Sharma A, Chandratre G, Telang A (2013). Experimentally induced sub-chronic toxicity of T-2 toxin in male Wistar rats. Indian J Vet Pathol. 37:41–8.

Rocha O, Ansari K, Doohan FM (2005). Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Addit Contam. 22:369–78. https://doi.org/10.1080/02652030500058403

Rodríguez-Carrasco Y, Moltó JC, Mañes J, Berrada H (2014). Exposure assessment approach through mycotoxin/creatinine ratio evaluation in urine by GC-MS/MS. Food Chem Toxicol. 72:69–75.

Romero A, Ares I, Ramos E, Castellano V, Martínez M, Martínez-Larrañaga MR, et al. (2016). Mycotoxins modify the barrier function of Caco-2 cells through differential gene expression of specific claudin isoforms: Protective effect of illite mineral clay. Toxicology. 353–4, 21–33. https://doi.org/10.1016/j.tox.2016.05.003

Rousseaux CG, Schiefer HB, Hancock DS (1986). Reproductive and teratological effects of continuous low-level dietary T-2 toxin in female CD-1 mice for two generations. J Appl Toxicol. 6:179–84. https://doi.org/10.1002/jat.2550060308

Ruiz MJ, Franzova P, Juan-García A, Font G (2011a). Toxicological interactions between the mycotoxins beauvericin, deoxynivalenol and T-2 toxin in CHO-K1 cells in vitro. Toxicon. 58:315–26. https://doi.org/10.1016/j.toxicon.2011.07.015

Ruiz MJ, Macáková P, Juan-García A, Font G (2011b). Cytotoxic effects of mycotoxin combinations in mammalian kidney cells. Food Chem Toxicol. 49:2718–24. https://doi.org/10.1016/j.fct.2011.07.021

Rychlik A, Gonkowski S, Kaczmar E, Obremski K, Calka J, Makowska K (2020). The T-2 toxin produced by fusarium spp. Impacts porcine duodenal nitric oxide synthase (nnos)-positive nervous structures—the preliminary study. Internat J Molec Sci. 21:1–12. https://doi.org/10.3390/ijms21145118

Sakai A, Suzuki C, Masui Y, Kuramashi A, Takatori K, Tanaka N (2007). The activities of mycotoxins derived from Fusarium and related substances in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). Mutat Res – Genetic Toxicol Environ Mutagenesis. 630:103–11. https://doi.org/10.1016/j.mrgentox.2007.03.005

Salimian, J, Arefpour, M. A, Riazipour, M., Poursasan, N (2014). Immunomodulatory effects of selenium and vitamin e on alterations in T lymphocyte subsets induced by T-2 toxin. Immunopharmacology and Immunotoxicology, 36(4), 275–281. https://doi.org/10.3109/08923973.2014.931420

Savino, W (2002). The thymus gland is a target in malnutrition. Eur J Clin Nutr. 56 Suppl 3:S46–9. doi: 10.1038/sj.ejcn.1601485. PMID: 12142962.

Scanes CG (2020). Avian physiology: are birds simply feathered mammals? Frontiers Physiol. 11:1–11. https://doi.org/10.3389/fphys.2020.542466

Schelstraete, W, Devreese, M., Croubels, S (2019). Impact of subacute exposure to T-2 toxin and zearalenone on the pharmacokinetics of midazolam as CYP3A probe drug in a porcine animal model: A pilot study. Frontiers Pharmacol. 10:399. https://doi.org/10.3389/fphar.2019.00399

Schiefer HB, Rousseaux CG, Hancock DS, Blakley BR (1987). Effects of low-level long-term oral exposure to T-2 toxin in CD-1 mice. Food Chem Toxicol. 25:593–601. https://doi.org/10.1016/0278-6915(87)90020-2

Sehata S, Teranishi M, Atsumi F, Uetsuka K, Nakayama H, Doi K (2003). T-2 toxin-induced morphological changes in pregnant rats. J Toxicol Pathol. 16:59–65. https://doi.org/10.1293/tox.16.59

Sehata S, Kiyosawa N, Sakuma K, Ito K, Yamoto T, Teranishi M, et al. (2004a). Gene expression profiles in pregnant rats treated with T-2 toxin. Exper Toxicol Pathol. 55:357–66. https://doi.org/10.1078/0940-2993-00342

Sehata S, Kiyosawa N, Makino T, Atsumi F, Ito K, Yamoto T, et al. (2004b). Morphological and microarray analysis of T-2 toxin-induced rat fetal brain lesion. Food Chem Toxicol. 42:1727–36. https://doi.org/10.1016/j.fct.2004.06.006

Sehata S, Kiyosawa N, Atsumi F, Ito K, Yamoto T, Teranishi M, et al. (2005). Microarray analysis of T-2 toxin-induced liver, placenta and fetal liver lesions in pregnant rats. Exper Toxicol Pathol. 57:15–28. https://doi.org/10.1016/j.etp.2005.02.005

Semenov EI, Mishina NN, Tanaseva SA, Kadikov IR, Tremasova AM, Papunidi KK, et al. (2018). Systemic anaphylaxis due to combined mycotoxicosis in wister rats. Indian Vet J. 95:16–19.

Shakhov AG, Sashnina LY, Mas'yanov YN, Vostroilova GA (2016). Cytokine profile of albino rats' changes under the impact of Salmonella against the background of subacute T-2 toxicosis. Russian Agric Sci. 42:271–274. https://doi.org/10.3103/s1068367416030198

Shang Y (2013). Chicken cytochrome P450 1A5 is the key enzyme for metabolizing T-2 toxin to 3'OH-T-2. Internat J Molec Sci. 14:10809–18. https://doi.org/10.3390/ijms140610809

Shen J, Perveen A, Kaka N, Li Z, Dai P, Li C (2020). Maternal exposure to T-2 toxin induces changes in antioxidant system and testosterone synthesis in the testes of mice offspring. Animals. 10 :74. https://doi.org/10.3390/ani10010074

Sheng K, Zhang H, Yue J, Gu W, Gu C, Zhang H, et al. (2018). Anorectic response to the trichothecene T-2 toxin correspond to plasma elevations of the satiety hormone glucose-dependent insulinotropic polypeptide and peptide YY3-36. Toxicology. 402–3, 28–36. https://doi.org/10.1016/j.tox.2018.04.007

Shi H, Li S, Bai Y, Prates LL, Lei Y, Yu P (2018). Mycotoxin contamination of food and feed in China: Occurrence, detection techniques, toxicological effects and advances in mitigation technologies. Food Control. 91:202–15. https://doi.org/10.1016/j.foodcont.2018.03.036

Shokri F, Heidari M, Gharagozloo S, Ghazi-Khansari M (2000). In vitro inhibitory effects of antioxidants on cytotoxicity of T-2 toxin. Toxicology. 146:171–76. https://doi.org/10.1016/S0300-483X(00)00172-4

Slobodchikova I, Sivakumar R, Rahman MS, Vuckovic D (2019). Characterization of phase i and glucuronide phase ii metabolites of 17 mycotoxins using liquid chromatography—high-resolution mass spectrometry. Toxins. 11:433. https://doi.org/10.3390/toxins11080433

Šmerák P, Bárta I, Polívková Z, Bártová J, Sedmíková M (2001). Mutagenic effects of selected trichothecene mycotoxins and their combinations with aflatoxin B1. Czech J Food Sci. 19:90–6. https://doi.org/10.17221/6583-cjfs

Smith MC, Hymery N, Troadec S, Pawtowski A, Coton E, Madec S (2017). Hepatotoxicity of fusariotoxins, alone and in combination, towards the HepaRG human hepatocyte cell line. Food Chem Toxicol. 109:439–51. https://doi.org/10.1016/j.fct.2017.09.022

Smith MC, Madec S, Troadec S, Coton E, Hymery N (2018). Effects of fusariotoxin co-exposure on THP-1 human immune cells. Cell Biol Toxicol. 34:191–205. https://doi.org/10.1007/s10565-017-9408-7

Somoskői B, Kovács M, Cseh S (2018). Effects of T-2 and Fumonisin B1 combined treatment on in vitro mouse embryo development and blastocyst quality. Toxicol Indust Health. 34:353–60. https://doi.org/10.1177/0748233718764039

Sun L, Cui S, Deng Q, Liu H, Cao Y, Wang S et al. (2019). Selenium content and/or T-2 toxin contamination of cereals, soil, and children's hair in some areas of Heilongjiang and Gansu Provinces, China. Biol Trace Elem Res. 191:294–9.

Sun LY, Li Q, Meng FG, Fu Y, Zhao ZJ, Wang LH (2012). T-2 toxin contamination in grains and selenium concentration in drinking water and grains in Kaschin-Beck disease endemic areas of Qinghai Province. Biol Trace Elem Res. 150:371–5.

Su N, Liu CL, Chen XP, Fan XX, Ma YC (2020). T-2 toxin cytotoxicity mediated by directly perturbing mitochondria in human gastric epithelium GES-1 cells. J Appl Toxicol. 40:1141–52. https://doi.org/10.1002/jat.3973

Sun LY, Meng FG, Li Q, Zhao ZJ, He CZ, Wang SP et al. (2014). Effects of the consumption of rice from non-KBD areas and selenium supplementation on the prevention and treatment of paediatric Kaschin-Beck disease: an epidemiological intervention trial in the Qinghai Province. Osteoarthritis Cartilage. 22:2033–40.

Sun YX, Zhao HY, Liu YJ, Dai ZQ, Fang BH (2012). Toxicokinetics of T-2 toxin, HT-2 toxin and t-2 triol after intravenously administrated T-2 toxin in swine. J Anim Vet Adv. 11:1977–81. https://doi.org/10.3923/javaa.2012.1977.1981

Sun Y, Zhang G, Zhao H, Zheng J, Hu F, Fang B (2014). Liquid chromatography-tandem mass spectrometry method for toxicokinetics, tissue distribution, and excretion studies of T-2 toxin and its major metabolites in pigs. J Chromatogr B: Anal Technol Biomed Life Sci. 958:75–82. https://doi.org/10.1016/j.jchromb.2014.03.010

Szabó A, Szabó-Fodor J, Fébel H, Romvári R, Kovács M (2014). Individual and combined haematotoxic effects of fumonisin B1 and T-2 mycotoxins in rabbits. Food Chem Toxicol. 72:257–64. https://doi.org/10.1016/j.fct.2014.07.025

Szabó A, Szabó-Fodor J, Fébel H, Mézes M, Bajzik G, Kovács M (2016). Oral administration of fumonisin B1 and T-2 individually and in combination affects hepatic total and mitochondrial membrane lipid profile of rabbits. Physiol Internat. 103:321–33. https://doi.org/10.1556/2060.103.2016.3.5

Szabó-Fodor J, Szeitzné-Szabó M, Bóta B, Schieszl T, Angeli C, Gambacorta L, et al. (2021). Assessment of human mycotoxin exposure in Hungary by urinary biomarker determination and the uncertainties of the exposure calculation: A case study. Foods, 11:15.

Tajima O, Schoen ED, Feron VJ, Groten JP (2002). Statistically designed experiments in a tiered approach to screen mixtures of Fusarium mycotoxins for possible interactions. Food Chem Toxicol. 40:685–95. https://doi.org/10.1016/S0278-6915(01)00124-7

Tanaka T, Abe H, Kimura M, Onda N, Mizukami S, Yoshida T, et al. (2016). Developmental exposure to T-2 toxin reversibly affects postnatal hippocampal neurogenesis and reduces neural stem cells and progenitor cells in mice. Arch Toxicol. 90:2009–24. https://doi.org/10.1007/s00204-015-1588-4

Taroncher M, Rodríguez-Carrasco Y, Ruiz MJ (2021). Interactions between T-2 toxin and its metabolites in HepG2 cells and in silico approach. Food Chem Toxicol. 148:111942. https://doi.org/10.1016/j.fct.2020.111942

Toutounchi NS, Hogenkamp A, Varasteh S, van't Land B, Garssen J, Kraneveld AD, et al. (2019). Fusarium mycotoxins disrupt the barrier and induce IL-6 release in a human placental epithelium cell line. Toxins. 11. https://doi.org/10.3390/toxins11110665

Tran VN, Viktorova J, Augustynkova K, Jelenova N, Dobiasova S, Rehorova K, et al. (2020). In silico and in vitro studies of mycotoxins and their cocktails; Their toxicity and its mitigation by silibinin pretreatment. Toxins. 12:148. https://doi.org/10.3390/toxins12030148

Verbrugghe E, Vandenbroucke V, Dhaenens M, Shearer N, Goossens J, De Saeger S, et al. (2012a). T-2 toxin induced *Salmonella Typhimurium* intoxication results in decreased Salmonella numbers in the cecum contents of pigs, despite marked effects on Salmonella-host cell interactions. Vet Res. 43:22. https://doi.org/10.1186/1297-9716-43-22

Verbrugghe E, Croubels S, Vandenbroucke V, Goossens J, De Backer P, Eeckhout M, et al. (2012b). A modified glucomannan mycotoxin-adsorbing agent counteracts the reduced weight gain and diminishes cecal colonization of *Salmonella Typhimurium* in T-2 toxin exposed pigs. Res Vet Sci. 93:1139–41. https://doi.org/10.1016/j.rvsc.2012.07.007

Vilà B, Jaradat ZW, Marquardt RR, Frohlich AA (2002). Effect of T-2 toxin on in vivo lipid peroxidation and vitamin E status in mice. Food Chem Toxicol. 40:479–86. https://doi.org/10.1016/S0278-6915(01)00122-3

Wan Q, Wu G, He Q, Tang H, Wang Y (2015a). The toxicity of acute exposure to T-2 toxin evaluated by the metabonomics technique. Mol BioSystems. 11:882–91. https://doi.org/10.1039/c4mb00622d

Wan D, Wang X, Wu Q, Lin P, Pan Y, Sattar A, et al. (2015b). Integrated transcriptional and proteomic analysis of growth hormone suppression mediated by trichothecene T-2 toxin in rat GH3 cells. Toxicol Sci. 147:326–38. https://doi.org/10.1093/toxsci/kfv131

Wang J, Jiang J, Zhang H, Wang J, Cai H, Li C, et al. (2011). Integrated transcriptional and proteomic analysis with in vitro biochemical assay reveal the important role of CYP3A46 in T-2 toxin hydroxylation in porcine primary hepatocytes. Mol Cell Proteomics. 10. https://doi.org/10.1074/mcp.M111.008748

Wang, Ji, Yang, C, Yuan, Z, Yi, J., Wu, J (2018). T-2 toxin exposure induces apoptosis in tm3 cells by inhibiting mammalian target of rapamycin/serine/threonine protein kinase(Mtorc2/akt) to promote ca2+ production. Int J Molec Sci. 19. https://doi.org/10.3390/ijms19113360

Warensjö LE, Montano Montes A, Schmidt J, Cramer B, Humpf HU, Moraeus L, Olsen M (2020). Mycotoxins in blood and urine of Swedish adolescents-possible associations to food intake and other background characteristics. Mycotoxin Res. 36:193–206.

Warth B, Petchkongkaew A, Sulyok M, Krska R (2014). Utilising an LC-MS/MS-based multi-biomarker approach to assess mycotoxin exposure in the Bangkok metropolitan area and surrounding provinces. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 31:2040–6.

Weidner M, Welsch T, Hübner F, Schwerdt G, Gekle M, Humpf HU (2012). Identification and apoptotic potential of T-2 toxin metabolites in human cells. J Agric Food Chem. 60:5676–84. https://doi.org/10.1021/jf300634k

Welsch T, Humpf HU (2012). HT-2 toxin 4-glucuronide as new T-2 toxin metabolite: enzymatic synthesis, analysis, and species specific formation of T-2 and HT-2 toxin glucuronides by rat, mouse, pig, and human liver microsomes. J Agric Food Chem. 60:10170–8. doi: 10.1021/jf302571y. Epub 2012 Sep 28. PMID: 22967261.

Wheeler MW, Blessinger T, Shao K, Allen BC, Olszyk L, Davis JA, et al. (2020). Quantitative risk assessment: developing a Bayesian approach to dichotomous dose–response uncertainty. Risk Anal. 40:1706–22.

Wheeler MW, Cortinas J, Aerts M, Gift JS, Davis JA (2022). Continuous model averaging for benchmark dose analysis: averaging over distributional forms. Environmetrics. 3 e2728.

WHO/IPCS [World Health Organization & International Programme on Chemical Safety] (1996). Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Geneva: World Health Organization. https://inchem.org/documents/ehc/ehc/ehc180.htm

WHO/IPCS [World Health Organization & International Programme on Chemical Safety] (2012). Guidance for immunotoxicity risk assessment for chemicals. Geneva: World Health Organization. https://apps.who.int/iris/handle/10665/330098

WHO/IPCS [World Health Organization & International Programme on Chemical Safety] (2020). Principles and methods for the risk assessment of chemicals in food, Chapter 5 (decond edition). Environmental Health Criteria 240. Geneva: World Health Organization. https://cdn.who.int/media/docs/default-source/food-safety/publications/chapter5-dose-response.pdf?sfvrsn=32edc2c6_5

Wigley, P (2017). Immunology of birds. In: eLS. 1–8 DOI: 10.1002/9780470015902.a0026259

Wojtacha P, Trybowski W, Podlasz P, Żmigrodzka M, Tyburski J, Polak-Śliwińska M, et al. (2021). Effects of a low dose of T-2 toxin on the percentage of T and B lymphocytes and cytokine secretion in the porcine ileal wall. Toxins. 13. https://doi.org/10.3390/toxins13040277

Wu J, Tu D, Yuan LY, Yuan H, Wen LX (2013). T-2 toxin exposure induces apoptosis in rat ovarian granulosa cells through oxidative stress. Environ Toxicol Pharmacol. 36:493–500. https://doi.org/10.1016/j.etap.2013.03.017

Wu J, Huang W, Xiao H, Xie Y, Yuan Z, Yi J, et al. (2018). Procyanidins B2 reverses the T-2 toxin-induced mitochondrial apoptosis in TM3 Leydig cells. J Functional Foods. 45:118–28. https://doi.org/10.1016/j.jff.2018.03.038

Wu Q, Dohnal V, Huang Li, Kuca K, Yuan Z (2010). Metabolic pathways of trichothecenes. Drug Metab Rev. 42:250–67. doi: 10.1080/03602530903125807. PMID: 19678805.

Wu Q, Huang L, Liu Z, Yao M, Wang Y, Dai M, et al. (2011). A comparison of hepatic in vitro metabolism of T-2 toxin in rats, pigs, chickens, and carp. Xenobiotica. 41:863–73. https://doi.org/10.3109/00498254.2011.593206

Wu Q, Engemann A, Cramer B, Welsch T, Yuan Z, Humpf HU (2012). Intestinal metabolism of T-2 toxin in the pig cecum model. Mycotox Res. 28:191–8. https://doi.org/10.1007/s12550-012-0134-y

Wu Q, Kuca K, Nepovimova E, Wu W (2020). Type A trichothecene diacetoxyscirpenol-induced emesis corresponds to secretion of peptide yy and serotonin in mink. Toxins (Basel). 12:419. https://doi.org/10.3390/toxins12060419

Wu W, Zhou HR, Pan X, Pestka JJ (2015). Comparison of anorectic potencies of the trichothecenes T-2 toxin, HT-2 toxin and satratoxin G to the ipecac alkaloid emetine. Toxicol Rep. 2:238–51. https://doi.org/10.1016/j.toxrep.2014.12.010

Wu W, Zhou HR, Bursian SJ, Link JE, Pestka JJ (2016). Emetic responses to T-2 toxin, HT-2 toxin and emetine correspond to plasma elevations of peptide YY3–36 and 5-hydroxytryptamine. Arch Toxicol. 90:997–1007. https://doi.org/10.1007/s00204-015-1508-7

Wu W, Sheng K, Xu X, Zhang H, Zhou G (2018). Potential roles for glucagon-like peptide-17–36 amide and cholecystokinin in anorectic response to the trichothecene mycotoxin T-2 toxin. Ecotoxicol Environ Safety. 153:181–87. https://doi.org/10.1016/j.ecoenv.2018.02.003

Yang F, Li L, Chen K, Li C, Wang Y, Wang G (2019). Melatonin alleviates β-zearalenol and HT-2 toxin-induced apoptosis and oxidative stress in bovine ovarian granulosa cells. Environ Toxicol Pharmacol. 68:52–60. https://doi.org/10.1016/j.etap.2019.03.005

Yang R, Wang YM, Zhang LS, Zhang L, Zhao ZM, Zhao J, et al. (2015). Delay of the onset of puberty in female rats by prepubertal exposure to T-2 toxin. Toxins. 7:4668–83. https://doi.org/10.3390/toxins7114668

Yang J, Guo W, Wang J, Yang X, Zhang Z, Zhao Z (2020). T-2 toxin-induced oxidative stress leads to imbalance of mitochondrial fission and fusion to activate cellular apoptosis in the human liver 7702 cell line. Toxins. 12. https://doi.org/10.3390/toxins12010043

Yang R, Wang YM, Zhang L, Zhao ZM, Zhao J, Peng SQ (2016). Prepubertal exposure to T-2 toxin advances pubertal onset and development in female rats via promoting the onset of hypothalamic-pituitary-gonadal axis function. Human Exper Toxicol. 35:1276–85. https://doi.org/10.1177/0960327116629529

Yang S, Li Y, Cao X, Hu D, Wang Z, Wang Y, et al. (2013). Metabolic pathways of T-2 toxin in in vivo and in vitro systems of Wistar rats. J Agric Food Chem. 61:9734–43. https://doi.org/10.1021/jf4012054

Yang S, Van Poucke C, Wang Z, Zhang S, De Saeger S, De Boevre M (2017). Metabolic profile of the masked mycotoxin T-2 toxin-3-glucoside in rats (in vitro and in vivo) and humans (in vitro). World Mycotox J. 10:349–62. https://doi.org/10.3920/WMJ2017.2224

Yang S, Zhang H, De Boevre M, Zhang J, Li Y, Zhang S, et al. (2018). Toxicokinetics of HT-2 toxin in rats and its metabolic profile in livestock and human liver microsomes. J Agric Food Chem. 66:8160–8. https://doi.org/10.1021/acs.jafc.8b02893

Yang X, Zhang X, Zhang J, Ji Q, Huang W, Zhang X, Li Y (2019a). Spermatogenesis disorder caused by T-2 toxin is associated with germ cell apoptosis mediated by oxidative stress. Environ Pollution. 251:372–9. https://doi.org/10.1016/j.envpol.2019.05.023

Yang X, Zhang X, Yao Q, Song M, Han Y, Shao B, Li Y (2019b). T-2 toxin impairs male fertility by disrupting hypothalamic-pituitary-testis axis and declining testicular function in mice. Chemosphere. 234:909–16. https://doi.org/10.1016/j.chemosphere.2019.06.145

Yao YF, Kang P-de, Li XB, Yang J, Shen B, Zhou ZK, et al. (2010). Study on the effect of T-2 toxin combined with low nutrition diet on rat epiphyseal plate growth and development. Internat Orthopaed. 34:1351–6. https://doi.org/10.1007/s00264-010-0966-z

Yao Y, Pei F, Kang P (2011). Selenium, iodine, and the relation with Kashin-Beck disease. Nutrition. 27:1095–100. https://doi.org/10.1016/j.nut.2011.03.002

Ye W, Lin R, Chen X, Chen J, Chen R, Xie X, et al. (2019). T-2 toxin upregulates the expression of human cytochrome P450 1A1 (CYP1A1) by enhancing NRF1 and Sp1 interaction. Toxicol Lett. 315:77–86. https://doi.org/10.1016/j.toxlet.2019.08.021

Yi Y, Zhao F, Wang N, Liu H, Yu L, Wang A, Jin Y (2018). Endoplasmic reticulum stress is involved in the T-2 toxin-induced apoptosis in goat endometrium epithelial cells. J Appl Toxicol. 38:1492–501. https://doi.org/10.1002/jat.3655

You Y, Cheng AC, Wang MS, Jia RY, Sun KF, Yang Q. et al. (2017). The suppression of apoptosis by α-herpesvirus. Cell Death Dis. 8:1–12. https://doi.org/10.1038/cddis.2017.139

Young JC, Zhou T, Yu H, Zhu H, Gong J (2007). Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem Toxicol. 45:136–43. https://doi.org/10.1016/j.fct.2006.07.028

Yu FF, Lin XL, Yang L, Liu H, Wang X, Fang H, et al. (2017). Comparison of T-2 toxin and HT-2 toxin distributed in the skeletal system with that in other tissues of rats by acute toxicity test. Biomed Environ Sci. 30:851–4. https://doi.org/10.3967/bes2017.115

Yu FF, Qi X, Shang YN, Ping ZG, Guo X (2019). Prevention and control strategies for children Kashin-Beck disease in China. A systematic review and meta-analysis. Medicine (Baltimore). 98:e16823.

Yuan Y, Zhou X, Yang J, Li M, Qiu X (2013). T-2 toxin is hydroxylated by chicken CYP3A37. Food Chem Toxicol. 62:622–7. https://doi.org/10.1016/j.fct.2013.09.031

Yuan Z, Matias, FB, Yi JE, Wu, J (2016). T-2 toxin-induced cytotoxicity and damage on TM3 Leydig cells. Comp Biochem Physiol C Toxicol Pharmacol. 181–2, 47–54. https://doi.org/10.1016/j.cbpc.2015.12.005

Zhang F, Wang L, Yang Z-H, Liu Z-T, Yue W, Wang Yue P (2006). Value of mink vomit model in study of anti-emetic drugs. World J Gastroenterol. 12:1300–2. www.wjgnet.com http://www.wjgnet.com/1007-9327/12/1300.asp

Zhang J, Liu S, Zhang, H, Li Y, Wu W, Zhang H (2017a). Gut satiety hormones cholecystokinin and glucagon-like Peptide-17-36 amide mediate anorexia induction by trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol. Toxicol Appl Pharmacol. 335:49–55. https://doi.org/10.1016/j.taap.2017.09.020

Zhang J, Jia H, Wang Q, Zhang Y, Wu W, Zhang, H (2017b). Role of peptide YY3-36 and glucose-dependent insulinotropic polypeptide in anorexia induction by trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and neosolaniol. Toxicol Sci. 159:203–10. https://doi.org/10.1093/toxsci/kfx128

Zhang J, Zhang HH, Liu S, Wu W, Zhang HH (2018a). Comparison of anorectic potencies of type a trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and neosolaniol. Toxins. 10:179. https://doi.org/10.3390/toxins10050179

Zhang J, Sheng K, Wu W, Zhang H (2018b). Anorectic responses to T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol correspond to plasma elevations of neurotransmitters 5-hydroxytryptamine and substance P. Ecotoxicol Environ Safety. 161:451–58. https://doi.org/10.1016/j.ecoenv.2018.06.005

Zhang X, Wang Y, Velkov T, Tang S, Dai C (2018). T-2 toxin-induced toxicity in neuroblastoma-2a cells involves the generation of reactive oxygen, mitochondrial dysfunction and inhibition of Nrf2/HO-1 pathway. Food Chem. Toxicol. 114:88–97. https://doi.org/10.1016/j.fct.2018.02.010

Zhang X, Wang Y, Yang X, Liu M, Huang W, Zhang J, et al. (2021). The nephrotoxicity of T-2 toxin in mice caused by oxidative stress-mediated apoptosis is related to Nrf2 pathway. Food Chem Toxicol. 149:112027. https://doi.org/10.1016/j.fct.2021.112027

Zhang X, Wang Q, Zhang J, Song M, Shao B, Han Y. et al. (2022). The Protective effect of selenium on T-2-induced nephrotoxicity is related to the inhibition of ROS-mediated apoptosis in mice kidney. Biol Trace Element Res. 200:206–16. https://doi.org/10.1007/s12011-021-02614-4

Zhang YF, Su PK, Wang LJ, Zheng HQ, Bai XF, Li P, et al. (2018). T-2 toxin induces apoptosis via the Bax-dependent caspase-3 activation in mouse primary Leydig cells. Toxicol Mech Meth. 28:23–8. https://doi.org/10.1080/15376516.2017.1354413

Zhang YF, Yang JY, Meng XP, Nie N, Tang MC, Yang XL (2020). L-Arginine protects mouse Leydig cells against T-2 toxin-induced apoptosis in vitro. Toxicol Indust Health. 36:1031–8. https://doi.org/10.1177/0748233720964312

Zhang Z, Xu Y, Wang J, Xie H, Sun X, Zhu X, et al. (2022). Protective effect of selenomethionine on T-2 toxin–induced rabbit immunotoxicity. Biol Trace Element Res. 200:172–82. https://doi.org/10.1007/s12011-021-02625-1

Zhong W, Hutchinson TE, Chebolu S., Darmani NA (2014). Serotonin 5-HT3 receptor-mediated vomiting occurs via the activation of Ca2+/CaMKII-dependent ERK1/2 signaling in the least shrew (Cryptotis parva). PLoS ONE. 9. https://doi.org/10.1371/journal.pone.0104718

Zhu CC, Zhang Y, Duan X, Han J, Sun SC (2016). Toxic effects of HT-2 toxin on mouse oocytes and its possible mechanisms. Arch Toxicol. 90:1495–505. https://doi.org/10.1007/s00204-015-1560-3

Zhu L, Yi X, Ma C, Luo C, Kong L, Lin X, et al. (2020). Betulinic acid attenuates oxidative stress in the thymus induced by acute exposure to T-2 toxin via regulation of the MAPK/Nrf2 signaling pathway. Toxins. 12:540. https://doi.org/10.3390/toxins12090540

Zhuang Z, Yang D, Huang Y, Wang S (2013). Study on the apoptosis mechanism induced by t-2 toxin. PLoS ONE. 8:8–14. https://doi.org/10.1371/journal.pone.0083105

Appendix 1. Search strategy

Search completed by the Health Canada Library (Eva-Marie Neumann) on 5 February 2021 and 24 December 2021 for Abdul Afghan.

A1.1. Number of results

Database search 5 February 2021

Table A1.1

Database search	Total results from databases exported to RefWorks	Duplicates removed in RefWorks	Final results in RefWorks folder
	2083	888	1195

Database search update, 24 December 2021					
MEDLINE	Embase	Global Health	All results before removing duplicates	All results after removing duplicates	Duplicates removed
53	108	67	228	146	82

A1.2. Original request

Research question/main concepts and keywords you would like included in the search

The authors were asked to compile *all available toxicological data* related to T-2 and HT-2 toxin in experimental animals (e.g. rats, mice, monkeys, rabbits, guinea-pigs, pigs, etc.…) and *humans* since the Joint FAO/WHO Expert Committee on Food Additives (JECFA)'s last assessment (circa 2001). The reviews by the Committee (Annex 1 reference *153*) and the European Food Safety Authority (EFSA, 2017) are good examples of the aims of the assessment in 2022.

Keywords

- T-2-toxin OR HT-2 toxin

Topics:

1. Toxicokinetics/pharmacokinetics:
   a. **Oral OR Buccal** AND (absorption; distribution; metabolism; biotransformation; enzymes; excretion; elimination; half-life; plasma; AUC)

2. Toxicity/pharmacodynamics/adverse effects (in humans or animals)
   a. Mechanisms/Mode of action
   b. Acute OR subchronic OR chronic [captured by toxic*]
   c. Genotoxicity (mutation OR mutagen OR mutagenicity OR chromosomal aberration OR clastogenicity OR clastogen OR micronucleus)
   d. Carcinogenicity (carcinogen OR cancer OR tumor)
   e. Reproductive toxicity OR developmental toxicity OR teratogenicity
   f. Nephrotoxicity OR neurotoxicity OR hepatotoxicity OR immunotoxicity OR haemotoxicity OR hematotoxicity OR cytotoxicity OR thyroid OR endocrine OR emetic OR anorectic OR immunotoxic OR haematotoxicity OR myelotoxicity
   g. Gene expression (e.g., genomics, transcriptomics, proteomics, and metabolomics)
3. Biomarkers of exposure (human)
   a. Detection OR identification OR forensic
   b. Biological marker OR case study OR epidemiology OR poisoning OR incidental poisoning

Not limited to humans but also other laboratory (e.g. rats, mice, rabbits, guinea-pigs) and farm animals (e.g. pigs, cattle, chicken, etc…)

Publication year limits: 2000–current

Sample of key/relevant articles (2–3 at least) already identified

1. Wu W, Zhou H, Bursian SJ, Link JE, Pestka JJ 2016. Emetic responses to T-2 toxin, HT-2 toxin and emetine correspond to plasma elevations of peptide YY3-36 and 5-hydroxytryptamine. Arch Toxicol. 90:997–1007. (https://link.springer.com/article/10.1007/s00204-015-1508-7).

2. Rahman S, Sharma AK, Singh ND, Telang AG, Azmi S, Prawez S (2014). Clinico-haematological changes in T-2 toxicosis in Wistar rats. Indian J Vet Pathol. 38:22–8. (https://www.indianjournals.com/ijor.aspx?target=ijor:ijvp&volume=38&issue=1&article=006)

3. Rafai P, Tuboly S, Bata A, Tilly P, Ványi A, Papp Z, Jakab L, Túry E (1995). Effect of various levels of T-2 toxin in the immune system of growing pigs. Vet Record. 136:511–14 (https://pubmed.ncbi.nlm.nih.gov/7660548/).

4. Raut SS, Sharma AK, Chandratre G, Telang AG (2013). Experimentally induced sub-chronic toxicity of T-2 toxin in male Wistar rats. Indian J Vet Pathol. 38:41–8. (https://www.indianjournals.com/ijor.aspx?target=ijor:ijvp&volume=37&issue=1&article=010).

A1.3. Search strategy

Embase

Database(s): **Embase** 1974 to 23 December 2021

Table A1.2

Embase search strategy

#	Searches	Results
1	T2-toxin/ or HT 2 toxin/	2171
2	("fusariotoxin t 2" or fusaritoxin* or (ht-2 adj2 toxin*) or (ht2 adj2 toxin*) or (t-2 adj2 toxin*) or (t2 adj2 toxin*) or (t-2 adj2 mycotoxin*) or (t2 adj2 mycotoxin*) or "21259-20-1" or "26934-87-2").tw,kw,rn.	2650
3	1 or 2 [T2 HT2 toxin]	2651
4	adverse event/ or exp body weight/ or exp death/ or exp hypersensitivity/ or side effect/	2229509
5	(advers* or allerg* or anaphyla* or anorectic* or anorex* or body weight* or dead or death? or deleterious or emesis or emetic* or fatal* or harm? or harmful* or hazard* or hypersensit* or lethal* or life-threat* or mortal* or necros* or poison* or (risk* adj2 assessment*) or safety or (side adj2 effect*) or vomit*).tw,kw.	5805669
6	exp allergenicity/ or exp carcinogenicity/ or exp hypersensitivity/ or exp neoplasm/ or exp toxicity/ or exp toxicity testing/	5988920
7	(toxic* or mycotoxic* or cardiotoxic* or cytotoxic* or dermatotoxic* or dermotoxic* or embryotoxic* or fetotoxic* or genotoxic* or h?ematoxic* or h?ematotoxic* or h?emotoxic* or hepatotoxic* or hepatoxic* or immunocytotoxic* or immunosuppress* or immun* suppress* or immunotoxic* or maternotoxic* or myelotoxic* or nephrotoxic* or neurotoxic* or ototoxic* or pharmacotoxic* or reprotoxic* or toxigen*).tw,kw.	1669683
8	(teratogen* or adenocarcin* or cancer* or carcin* or hepatocarcinogen* or malign* or neoplasm* or nephrocarcinogen* or tumor* or tumour*).tw,kw.	4839047
9	endocrine disrupter/ or exp endocrine function/ or exp endocrine system/	1196166
10	(adrenal* or endocrine* or gonad* or hypophys* or hypothalamus or neuroendocrine or ovary or ovaries or pancrea* or paragangli* or parathyroid* or pineal body or testicle* or teste or testes or testis or thymic or thymocit* or thymus or thyroid*).tw,kw.	1478707
11	(androgen* or estradiol or estrogen* or gestagen* or hormone* or oestrogen* or progesteron* or testosteron*).tw,kw.	911369
12	exp cell damage/ or exp genetic damage/ or exp genetic disorder/ or exp mutagenesis/ or exp mutagen testing/ or oxidative stress/ or reactive oxygen metabolite/	2613648
13	(ames test* or comet assay* or microtox* test* or micronucle* or reactive oxygen species or reactive oxygen metabolite? or oxidative stress*).tw,kw.	435672
14	(clastogen* or metabolomic* or mutagen* or mutation* or proteomic* or transcriptomic* or ((chromosom* or dna or gene* or genomic*) adj3 (aberrat* or adduct? or apoptos* or break* or cytostas* or damag* or fragment* or loss* or necro* or non-disjunction* or nondisjunction* or repair*)) or single strand break* or double strange break* or point mutation* or point deletion*).tw,kw.	1532158
15	exp pharmacokinetics/ or exp pharmacokinetic parameters/ or exp pharmacodynamics/ or exp pharmacodynamic parameters/	4575332
16	(pharmacokinetic* or pharmacodynamic* or ADME or absorb* or absorp* or accumulat* or bioavailab* or biotransformation* or clearance* or distribution* or eliminat* or excret* or half-life or halflife or half-lives or halflives or metabolis* or metaboliz* or ((mode* or mechanism*) adj3 action*) or MoA or plasma).tw,kw.	4839677
17	congenital malformation/ or developmental toxicity/ or exp fertility/ or exp pregnancy disorder/ or exp prenatal development/ or exp reproduction/	1649670
18	(abnormal* or deformit* or defect? or developmental or embryo* or fertil* or gestat* or infertil* or malformation* or pregnan* or prenatal* or reproduction* or reproductive or retardation*).tw,kw.	3358402
19	((f?etal* or f?etus* or implantation or litter* or neonat* or offspring*) adj4 (complicat* or losing or loss* or lost or prematur* or resorp*)).tw,kw.	46957
20	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	20464668

21	3 and 20 [T2 HT2 toxin + adverse events toxicity PK PD]	1834
22	exp animal experiment/ or exp animal model/ or exp human/	25550096
23	(human or humans or adolescen* or adult* or baby or babies or boy? or child* or consumer? or elderly or f?etal* or f?etus* or girl? or individual? or infant? or inpatient* or man or men or neonate? or neo nate? or newborn* or new born* or participant* or p?ediatric* or people* or preteen* or preadolescen* or toddler* or teen* or school age* or schoolage* or senior? or participant* or patient? or people* or person? or pregnan* or volunteer* or woman or women or worker* or youth? or (animal* adj3 experiment*) or (animal adj3 farm*) or (animal* adj3 laboratory) or ape or apes or beagle* or bird or birds or boar? or bovine* or broiler? or calf or calve* or canine* or cat or cats or cattle or chick? or chicken* or chimpanzee* or cockerel* or cow? or dams or dog or dogs or donkey* or duck or ducks or duckling* or equine or ewe? or feline* or frog or frogs or gerbil* or goat* or "guinea-pig*" or guineapig* or hamster* or hare or hares or heifer* or hen or hens or horse? or lamb? or livestock* or macaque* or mammal? or mare? or marmoset* or mice or mink? or monkey* or mouse or murine or ovine or pig or piglet* or pigs or porcine or pork* or poultry or primate* or rabbit* or rat or rats or rodent? or ruminant* or sheep or sow or sows or swine* or turkey or turkeys).tw,kw.	21904779
24	exp cell line/ or exp in vitro study/	6675419
25	(cell line* or "ex vivo" or "in vivo" or "in vitro").tw,kw.	2758051
26	22 or 23 or 24 or 25 [human, animal, cell line]	30276946
27	21 and 26	1519
28	limit 27 to yr=2000-current	920
29	("20210203" or "20210204" or "20210205" or "20210206" or "20210207" or "20210208" or "20210209" or "2021021*" or "2021022*" or 202103* or 202104* or 202105* or 202106* or 202107* or 202108* or 202109* or 202110* or 202111* or 202112* or 2022*).dc,dd.	2133913
30	("202105" or "202106" or "202107" or "202108" or "202109" or 20211* or 20212* or 20213* or 20214* or 20215*).em.	2918648
31	28 and (29 or 30) [UPDATE FEB 3 2021-DEC 24 2021]	108

MEDLINE

Database(s): **Ovid MEDLINE(R) ALL** 1946 to 23 December 2021

Table A1.3
MEDLINE search strategy

#	Searches	Results
1	T2-toxin/	1281
2	("fusariotoxin t 2" or fusaritoxin* or (ht-2 adj2 toxin*) or (ht2 adj2 toxin*) or (t-2 adj2 toxin*) or (t2 adj2 toxin*) or (t-2 adj2 mycotoxin*) or (t2 adj2 mycotoxin*) or "21259-20-1" or "26934-87-2").tw,kw,kf.	2056
3	1 or 2 [T2 HT2 toxin]	2149
4	exp "Drug-Related Side Effects and Adverse Reactions"/ or exp body weight/ or exp death/ or exp hypersensitivity/	1083193
5	(advers* or allerg* or anaphyla* or anorectic* or anorex* or body weight* or dead or death? or deleterious or emesis or emetic* or fatal* or harm? or harmful* or hazard* or hypersensit* or lethal* or life-threat* or mortal* or necros* or poison* or (risk* adj2 assessment*) or safety or (side adj2 effect*) or vomit*).tw,kw,kf.	4243852
6	exp allergens/ or exp carcinogens/ or exp neoplasms/ or exp toxicity tests/	3816764
7	(toxic* or mycotoxic* or cardiotoxic* or cytotoxic* or dermatotoxic* or dermotoxic* or embryotoxic* or fetotoxic* or genotoxic* or h?ematoxic* or h?ematotoxic* or h?emotoxic* or hepatotoxic* or hepatoxic* or immunocytotoxic* or immunosuppress* or immun* suppress* or immunotoxic* or maternotoxic* or myelotoxic* or nephrotoxic* or neurotoxic* or ototoxic* or pharmacotoxic* or reprotoxic* or toxigen*).tw,kw,kf.	1267745
8	(teratogen* or adenocarcin* or cancer* or carcin* or hepatocarcinogen* or malign* or neoplasm* or nephrocarcinogen* or tumor* or tumour*).tw,kw,kf.	3714597

9	exp endocrine disruptors/ or exp endocrine system/	465787
10	(adrenal* or endocrine* or gonad* or hypophys* or hypothalamus or neuroendocrine or ovary or ovaries or pancrea* or paragangli* or parathyroid* or pineal body or testicle* or teste or testes or testis or thymic or thymocit* or thymus or thyroid*).tw,kw,kf.	1244944
11	(androgen* or estradiol or estrogen* or gestagen* or hormone* or oestrogen* or progesteron* or testosteron*). tw,kw,kf.	777938
12	exp chromosome aberrations/ or exp dna damage/ or Genetic Diseases, Inborn/ or exp mutagenesis/ or mutagens/ or exp mutation/ or Oxidative Stress/ or Reactive Oxygen Species/	1158834
13	(ames test* or comet assay* or microtox* test* or micronucle* or reactive oxygen species or reactive oxygen metabolite? or oxidative stress*).tw,kw,kf.	340167
14	(clastogen* or metabolomic* or mutagen* or mutation* or proteomic* or transcriptomic* or ((chromosom* or dna or gene* or genomic*) adj3 (aberrat* or adduct? or apoptos* or break* or cytostas* or damag* or fragment* or loss* or necro* or non-disjunction* or nondisjunction* or repair*)) or single strand break* or double strange break* or point mutation* or point deletion*).tw,kw,kf.	1194892
15	exp pharmacokinetics/ or exp molecular mechanisms of pharmacological action/	4059232
16	(pharmacokinetic* or pharmacodynamic* or ADME or absorb* or absorp* or accumulat* or bioavailab* or biotransformation* or clearance* or distribution* or eliminat* or excret* or half-life or halflife or half-lives or halflives or metabolis* or metaboliz* or ((mode* or mechanism*) adj3 action*) or MoA or plasma).tw,kw,kf.	4141403
17	Abnormalities, Drug-Induced/ or exp fertility/ or exp pregnancy complications/ or exp "Embryonic and Fetal Development"/ or exp reproduction/ or teratogens/	1357032
18	(abnormal* or deformit* or defect? or developmental or embryo* or fertil* or gestat* or infertil* or malformation* or pregnan* or prenatal* or reproduction* or reproductive or retardation*).tw,kw,kf.	2771436
19	((f?etal* or f?etus* or implantation or litter* or neonat* or offspring*) adj4 (complicat* or losing or loss* or lost or prematur* or resorp*)).tw,kw,kf.	32886
20	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	16982862
21	3 and 20 [T2 HT2 toxin + adverse events toxicity PK PD]	1491
22	animal experimentation/ or Models, animal/ or humans/	20058676
23	(human or humans or adolescen* or adult* or baby or babies or boy? or child* or consumer? or elderly or f?etal* or f?etus* or girl? or individual? or infant? or inpatient* or man or men or neonate? or neo nate? or newborn* or new born* or participant* or p?ediatric* or people* or preteen* or preadolescen* or toddler* or teen* or school age* or schoolage* or senior? or participant* or patient? or people* or person? or pregnan* or volunteer* or woman or women or worker* or youth? or (animal* adj3 experiment*) or (animal adj3 farm*) or (animal* adj3 laboratory) or ape or apes or beagle* or bird or birds or boar? or bovine* or broiler? or calf or calve* or canine* or cat or cats or cattle or chick? or chicken* or chimpanzee* or cockerel* or cow? or dams or dog or dogs or donkey* or duck or ducks or duckling* or equine or ewe? or feline* or frog or frogs or gerbil* or goat* or "guinea-pig*" or guineapig* or hamster* or hare or hares or heifer* or hen or hens or horse? or lamb? or livestock* or macaque* or mammal? or mare? or marmoset* or mice or mink? or monkey* or mouse or murine or ovine or pig or piglet* or pigs or porcine or pork* or poultry or primate* or rabbit* or rat or rats or rodent? or ruminant* or sheep or sow or sows or swine* or turkey or turkeys). tw,kw,kf.	17569095
24	exp cell lines/ or exp in vitro techniques/	1653088
25	(cell line* or "ex vivo" or "in vivo" or "in vitro").tw,kw,kf.	2236437
26	22 or 23 or 24 or 25 [human, animal, cell line]	26305577
27	21 and 26	1227
28	limit 27 to yr=2000-current	657
29	("20210203" or "20210204" or "20210205" or "20210206" or "20210207" or "20210208" or "20210209" or "2021021*" or "2021022*" or 202103* or 202104* or 202105* or 202106* or 202107* or 202108* or 202109* or 202110* or 202111* or 202112* or 2022*).ez,dt.	1400919
30	28 and 29 [UPDATE FEB 3 2021-DEC 24 2021]	53

Global health

Database(s): **Global Health** 1973 to 2021 (week 50)

Table A1.4
Global health search strategy

#	Searches	Results
1	T-2 toxin/ or HT-2 toxin/	1479
2	("fusariotoxin t 2" or fusaritoxin* or (ht-2 adj2 toxin*) or (ht2 adj2 toxin*) or (t-2 adj2 toxin*) or (t2 adj2 toxin*) or (t-2 adj2 mycotoxin*) or (t2 adj2 mycotoxin*) or "21259-20-1" or "26934-87-2").ti,ab.	1864
3	1 or 2 [T2 HT2 toxin]	1971
4	adverse effects/ or body weight/ or exp death/ or exp hypersensitivity/	176528
5	(advers* or allerg* or anaphyla* or anorectic* or anorex* or body weight* or dead or death? or deleterious or emesis or emetic* or fatal* or harm? or harmful* or hazard* or hypersensit* or lethal* or life-threat* or mortal* or necros* or poison* or (risk* adj2 assessment*) or safety or (side adj2 effect*) or vomit*).ti,ab.	856052
6	allergens/ or carcinogens/ or exp neoplasms/ or exp toxicity/ or exp toxicology/	393043
7	(toxic* or mycotoxic* or cardiotoxic* or cytotoxic* or dermatotoxic* or dermotoxic* or embryotoxic* or fetotoxic* or genotoxic* or h?ematoxic* or h?ematotoxic* or h?emotoxic* or hepatotoxic* or hepatoxic* or immunocytotoxic* or immunosuppress* or immun* suppress* or immunotoxic* or maternotoxic* or myelotoxic* or nephrotoxic* or neurotoxic* or ototoxic* or pharmacotoxic* or reprotoxic* or toxigen*).ti,ab.	231858
8	(teratogen* or adenocarcin* or cancer* or carcin* or hepatocarcinogen* or malign* or neoplasm* or nephrocarcinogen* or tumor* or tumour*).ti,ab.	317327
9	exp endocrine diseases/ or endocrine system/ or exp sex hormones/	158829
10	(adrenal* or endocrine* or gonad* or hypophys* or hypothalamus or neuroendocrine or ovary or ovaries or pancrea* or paragangli* or parathyroid* or pineal body or testicle* or teste or testes or testis or thymic or thymocit* or thymus or thyroid*).ti,ab.	97995
11	(androgen* or estradiol or estrogen* or gestagen* or hormone* or oestrogen* or progesteron* or testosteron*).ti,ab.	72684
12	exp chromosome aberrations/ or chromosome breakage/ or dna repair/ or genetic disorders/ or exp mutagenesis/ or mutagenicity/ or exp mutagens/ or mutations/ or oxidative stress/ or reactive oxygen species/	97359
13	(ames test* or comet assay* or microtox* test* or micronucle* or reactive oxygen species or reactive oxygen metabolite? or oxidative stress*).ti,ab.	72951
14	(clastogen* or metabolomic* or mutagen* or mutation* or proteomic* or transcriptomic* or ((chromosom* or dna or gene* or genomic*) adj3 (aberrat* or adduct? or apoptos* or break* or cytostas* or damag* or fragment* or loss* or necro* or non-disjunction* or nondisjunction* or repair*)) or single strand break* or double strange break* or point mutation* or point deletion*).ti,ab.	112952
15	exp pharmacodynamics/ or exp metabolism/	148709
16	(pharmacokinetic* or pharmacodynamic* or ADME or absorb* or absorp* or accumulat* or bioavailab* or biotransformation* or clearance* or distribution* or eliminat* or excret* or half-life or halflife or half-lives or halflives or metabolis* or metaboliz* or ((mode* or mechanism*) adj3 action*) or MoA or plasma).ti,ab.	651782
17	exp abnormalities/ or embryonic development/ or fetal development/ or exp fertility/ or exp pregnancy complications/ or prenatal development/ or exp reproduction/ or exp reproductive disorders/	195801
18	(abnormal* or deformit* or defect? or developmental or embryo* or fertil* or gestat* or infertil* or malformation* or pregnan* or prenatal* or reproduction* or reproductive or retardation*).ti,ab.	305019
19	((f?etal* or f?etus* or implantation or litter* or neonat* or offspring*) adj4 (complicat* or losing or loss* or lost or prematur* or resorp*)).ti,ab.	4745
20	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	2033477
21	3 and 20 [T2 HT2 toxin + adverse events toxicity PK PD]	1515
22	animal experiments/ or animal models/ or man/	2446650

23	(human or humans or adolescen* or adult* or baby or babies or boy? or child* or consumer? or elderly or f?etal* or f?etus* or girl? or individual? or infant? or inpatient* or man or men or neonate? or neo nate? or newborn* or new born* or participant* or p?ediatric* or people* or preteen* or preadolescen* or toddler* or teen* or school age* or schoolage* or senior? or participant* or patient? or people* or person? or pregnan* or volunteer* or woman or women or worker* or youth? or (animal* adj3 experiment*) or (animal adj3 farm*) or (animal* adj3 laboratory) or ape or apes or beagle* or bird or birds or boar? or bovine* or broiler? or calf or calve* or canine* or cat or cats or cattle or chick? or chicken* or chimpanzee* or cockerel* or cow? or dams or dog or dogs or donkey* or duck or ducks or duckling* or equine or ewe? or feline* or frog or frogs or gerbil* or goat* or "guinea-pig*" or guineapig* or hamster* or hare or hares or heifer* or hen or hens or horse? or lamb? or livestock* or macaque* or mammal? or mare? or marmoset* or mice or mink? or monkey* or mouse or murine or ovine or pig or piglet* or pigs or porcine or pork* or poultry or primate* or rabbit* or rat or rats or rodent? or ruminant* or sheep or sow or sows or swine* or turkey or turkeys).ti,ab.	2706024
24	cell lines/ or in vitro/	174431
25	(cell line* or "ex vivo" or "in vivo" or "in vitro").ti,ab.	280921
26	22 or 23 or 24 or 25 [human, animal, cell line]	3175243
27	21 and 26	941
28	limit 27 to yr=2000-current	686
29	("20210131" or "202102*" or 202103* or 202104* or 202105* or 202106* or 202107* or 202108* or 202109* or 202110* or 202111* or 202112*).up.	276324
30	28 and 29 [UPDATE FEB 3 2021-DEC 24 2021]	67

A1.4. Guide – Ovid syntax – search fields operators

Table A1.5
Ovid syntax – search fields operators[a]

/	At the end of a term or phrase, searches for term or phrase as a subject heading (index term). Databases use different subject headings systems (or thesaurus) to index content, for example: MEDLINE: MeSH (Medical Subject Headings) Embase: Emtree PsycINFO: American Psychological Association index
*	'*' is an "unlimited" truncation symbol. It substitutes for any number of characters at the end of a term or the root of a term, to retrieve plurals and variant spellings Before a subject heading, indicates that the subject heading is a main topic of the article
?	'?' is a "wildcard" symbol. It substitutes for one or no characters
.ab	Abstract field code
ab. /freq=2	Searches for terms that are present at least twice in the abstract
.kf	Keyword Heading Word available in MEDLINE only – retrieves a particular word in the Keyword Heading field
.kw	Keyword Heading field code – assigned by authors
.mh	MeSH (Medical Subject Heading) – MEDLINE medical index term
.mp	Multipurpose field code – searches in title, abstract, drug trade name (tn) and additional fields
.pt	Publication type
.ti	Title field code
.tw	Text Word field code. In Embase includes title, abstract, and drug trade name (tn)
/ae	Embase/MEDLINE subheading: Adverse Events [drug subheading]
/ci	MEDLINE subheading: Chemically Induced [disease subheading]
/si	Embase subheading: Chemically Induced [disease subheading]

/to	Embase/MEDLINE subheading: Toxicity [drug subheading]
adj#	Operator that searches for terms adjacent within # number of words, in any order
exp	"Explodes" a subject heading by searching for the main subject heading and for all Narrower Terms

[a] Unless otherwise stated, search terms are free text terms.

Reference

EFSA [European Food Safety Authority CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)]. (2017). Scientific opinion on the appropriateness to set a group health based guidance value for T-2 and HT-2 toxin and its modified forms. EFSA J. 15:4655. doi:10.2903/j.efsa.2017.4655

Appendix 2. Dose calculations

Table A2.1

Diet (mg/kg diet)	Initial mean body weight (kg)	Mean daily weight gain (g/day)	Body weight (kg)	Mean daily feed intake (g/day)	Dose (µg/kg bw per day)
Week 1					
0	9.3	404.6	12.13	630.7	0
0.5	9.1	291.4	11.14	565.4	25
1	9.4	354.1	11.88	644.8	54
2	8.6	223.9	10.17	471.3	93
3	8.7	146.1	9.72	401.7	124
Week 2					
0	12.13	451.4	15.29	845.1	0
0.5	11.14	387.1	13.85	704.6	25
1	11.88	487.3	15.29	923.7	60
2	10.17	348.6	12.61	727.6	115
3	9.72	280	11.68	512	131
Week 3					
0	15.29	534.3	19.03	976	0
0.5	13.85	505.7	17.39	872.9	25
1	15.29	490.5	18.72	751.5	40
2	12.61	382.9	15.29	765.6	100
3	11.68	240	13.36	533.8	120

Estimated doses and conversion factors		
Diet (mg/kg diet)	**Average doses weeks 1–3**	**Conversion factors**
0.5	25 µg/kg bw per day	50.6 µg/kg bw per day PER mg/kg diet
1	52 µg/kg bw per day	51.6 µg/kg bw per day PER mg/kg diet
2	103 µg/kg bw per day	51.4 µg/kg bw per day PER mg/kg diet
3	125 µg/kg bw per day	41.7 µg/kg bw per day PER mg/kg diet

Sources: Rafai P, Bata A, Ványi A, Papp Z, Brydl E, Jakab L, Tuboly S, Túry E. (1995a). Effect of various levels of T-2 toxin on the clinical status, performance and metabolism of growing pigs. Vet Record. 136:485–9. https://doi.org/10.1136/vr.136.19.485
Rafai P, Tuboly S, Bata A, Tilly P, Ványi A, Papp Z, Jakab L, Túry E. (1995b). Effect of various levels of T-2 toxin in the immune system of growing pigs. Vet Record. 136:511–4. https://doi.org/10.1136/vr.136.20.511

Table A2.2

Diet (mg/kg diet)	Initial mean body weight (kg)	Day 7 mean body weight (kg)	Mean daily feed intake (kg)	Dose (µg/kg bw per day)
Week 1				
0	13.4	16.9	0.774	0.0
0.3	13.6	15.9	0.571	10.8
0.5	13.3	16	0.608	19.0
Week 2				
0	13.4	20.4	0.919	0.0
0.3	13.6	18.3	0.649	10.6
0.5	13.3	17.6	0.574	16.3
Week 3				
0	13.4	23.8	0.973	0.0
0.3	13.6	21.5	0.863	12.0
0.5	13.3	20	0.75	18.8

Estimated doses and conversion factors		
Diet (mg/kg diet)	**Average doses weeks 1–3**	**Conversion factors**
0.3	11.2 µg/kg bw per day	37.2 µg/kg bw per day PER mg/kg diet
0.5	18.0 µg/kg bw per day	36.0 µg/kg bw per day PER mg/kg diet

Sources: Rafai P, Papp Z, Jakab L (2013). Biotransformation of trichothecenes alleviates the negative effects of T-2 toxin in pigs. Acta Vet Hungarica. 61:333–43. https://doi.org/10.1556/AVet.2013.025

Appendix 3. Detailed output of benchmark dose analysis

A3.1. Acute exposure

This code analyses the emetic response data from the studies by Wu et al. (2016) and Wu et al. (2020). A model average benchmark dose (BMD) analysis is conducted for dichotomous data and maximum a posteriori (MAP) estimates are given. The analysis uses all models and prior information in the manuscript of Wheeler et al. (2020). These priors were developed for general dichotomous dose–response data and showed good performance in simulation studies.

Dichotomous response models

Dichotomous response models assume that the response has a probability $\pi(x)$ of occurring at some dose x where $\pi(x)$ is a parametric function of dose that needs to be estimated. The following nine models, with corresponding parameter prior, are used in the model average:

Probit

$$\pi(x) = \Phi(a + b \times x)$$

With $a \sim N(0,1)$ and $b \sim N(0,1)$.

Log-Probit

$$\pi(x) = a+(1 - a)\Phi(c + d \times x)$$

With logit[a] ~ N(0,2), c ~ N(0,1), and log[d] ~ N(log[2], 0.5)

Logistic

$$\pi(x) = \frac{1}{1+\exp(-[a + b \times x])'}$$

With a ~ N(0,1) and log[b] ~ N(0.1, 1)

Log-logistic

$$\pi(x) = a + \frac{1 - a}{1+\exp(-[c + d \times x])}$$

With logit[a] ~ N(0,2), c ~ N(0,1), and log[d] ~ N(log[2], 0.5)

Hill

$$\pi(x) = a + \frac{b \times (1 - a)}{1+\exp(-[c + d \times x])}$$

With logit[a] ~ N(–1,2), logit[b] ~ N(4,2), c ~ N(0,0.25), and log[d] ~ N(log[2], 0.5)

Weibull

$$\pi(x) = a + (1 - a)(1-\exp[- b \times x^d])$$

With logit[a] ~ N(0,2), b ~ N(0,1.5), and log[d] ~ N(log[2], 0.5)

Gamma

$$\pi(x) = a + (1 - a) \int_0^{b \times x} t^{d-1} \exp(-t) \mathrm{d}t$$

With logit[a] ~ N(0,2), log[b] ~ N(0,1) and log[d] ~ N(log[2], 0.424)

Quantal-linear

$$\pi(x) = a + (1 - a)(1 - \exp[-b \times x])$$

With logit[a] ~ N(0,2) and log[b] ~ N(0.15,1)

Multistage

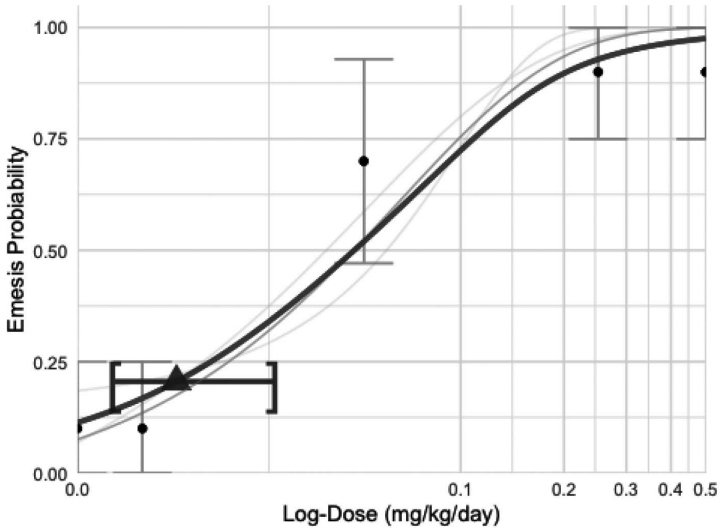
$$\pi(x) = a + (1 - a)(1 - \exp[-b_1 \times x - b_2 \times x^2])$$

With logit[a] ~ N(0,2), log[b_1] ~ N(0,0.5) and log[b_2] ~ N(0,1.0).

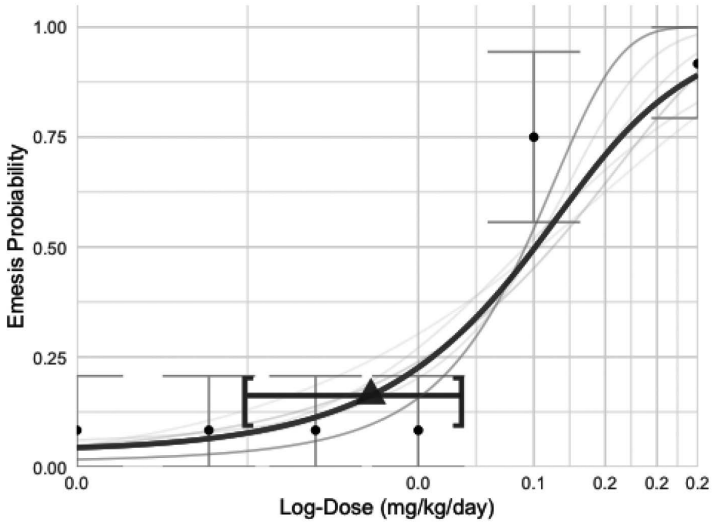
```
library(ToxicR)
library(readxl)
WU_2016 <- read_excel("WU-2016.xlsx")
WU_2020 <- read_excel("WU-2020.xlsx")
library(ggploT-2)
library(scales)

my_trans = trans_new("log_p1", function(x)(log(x+0.01)), function(x)
(exp(x)-0.01), domain = c(0, Inf))

fancy_scientific <- function(l) {
 # turn in to character string in scientific notation
 l <- as.numeric(l)
 l <- sprintf("%1.1f",l)
 l
}
fit <- ma_dichotomous_fit(WU_2016[,1],WU_2016[,3],WU_2016[,2])
plot(fit)+ggtitle("")+scale_x_continuous( trans=my_trans,labels=fancy_
scientific) +xlab("Log-Dose (mg/kg/day)") + ylab("Emesis Probiability")
```




```
library(ggploT-2)
fiT-2 <- ma_dichotomous_fit(WU_2020[,1],WU_2020[,3],WU_2020[,2])
plot(fiT-2)+ggtitle("")+scale_x_continuous(trans=my_trans,labels=fancy_
scientific)+xlab("Log-Dose (mg/kg/day)") +
 ylab("Emesis Probiability")
```



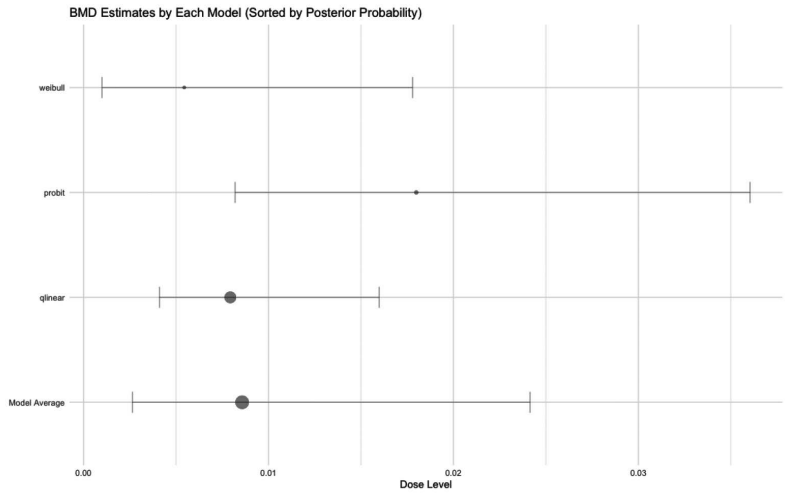
The following is the final BMD analysis used in the study.

```
library(knitr)
BMDS <- rbind(fit$bmd,fiT-2$bmd)
rownames(BMDS) <- c("Wu (2016)","Wu (2020)")
kable(BMDS,digits=4,caption="BMD in (mg/kg/day)")
```

BMD in (mg/kg/day)

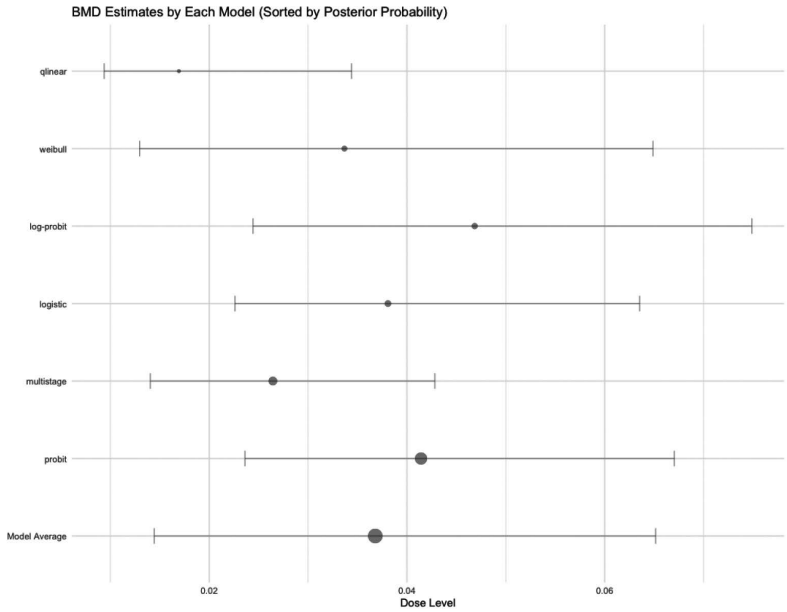
	BMD	BMDL	BMDU
Wu (2016)	0.0086	0.0026	0.0241
Wu (2020)	0.0368	0.0144	0.0652

```
cleveland_plot(fit)
```



Model	BMD	Model weights
Quantal-Linear	0.008 (0.004,0.016)	0.610
Probit	0.018 (0.008,0.036)	0.127
Weibull	0.005 (0.001,0.018)	0.125
Gamma	0.009 (0.002,0.024)	0.044
Hill	0.008 (0.002,0.020)	0.038
Logistic	0.020 (0.009,0.040)	0.027
Log-Probit	0.010 (0.003,0.025)	0.018
Multistage	0.019 (0.010,0.057)	0.008
Log-Logistic	0.006 (0.001,0.022)	0.003

```
cleveland_plot(fiT-2)
```



Model	BMD	Model weights
Probit	0.041 (0.024,0.067)	0.490
Multistage	0.026 (0.014, 0.043)	0.152
Logistic	0.038 (0.023, 0.064)	0.080
Log-Probit	0.047 (0.024, 0.075)	0.072
Weibull	0.034 (0.013, 0.065)	0.065
Quantal-Linear	0.017 (0.009, 0.034)	0.054
Hill	0.039 (0.018, 0.065)	0.032
Gamma	0.029 (0.012, 0.056)	0.030
Log-Logistic	0.038 (0.015, 0.071)	0.024

A3.2. Repeated-dose exposure

The following analysis is based upon data from Rafai, Papp & Jakab (2013). In what follows, ToxicR, version 22.04 (1.0.1), is used for the analysis. Model average estimates are computed using the Laplace estimates (Wheeler 2020, 2022). Unless otherwise specified, all benchmark dose (BMD) analyses are done using the relative deviation. The Committee looked at two benchmark response (BMR) values of 5 and 10%. This Appendix provides the code for the 10% value.

A3.2.1 Continuous dose–response models and priors

For dose–response modelling, we observe $(\overline{y}_1, \overline{y}_2, \ldots \overline{y}_n)'$ means and $\sigma = (\widehat{\sigma}_1, \widehat{\sigma}_2, \ldots \widehat{\sigma}_n)'$ estimated standard deviations corresponding to the experiment. For each mean, 10 animals were observed. Here, each mean y_i, $1 \leq i \leq n$, corresponds to a dose x_i from Rafai, Papp & Jakab (2013). Given these data, we assume all observations are independently drawn from a common error distribution such that the central tendency changes as a smooth function of dose. This function, $f(x)$, is the dose–response. It determines changes in the response as the dose increases, and its exact role depends on the data distribution. For example, if the y_i are normally distributed, $f(x_i)$ is the mean given x_i and if one assumes each y_i follows a log-normal distribution, $f(x_i)$ is the median given x_i.

In this analysis the Hill, Exponential-3, Exponential-5 and Power dose–response models are used.

$$f_{\text{hill}}(x \mid \theta = \{\alpha, b, c, d\}) = \alpha + \frac{bx^d}{c^d + x^d}$$

$$f_{exp-3}(x \mid \theta = \{\alpha, b, d\}) = \alpha[\exp(-\{bx\}^d)],$$

$$f_{exp-5}(x \mid \theta = \{\alpha, b, c, d\}) = \alpha[c - (1 - c)\exp(-\{bx\}^d)],$$

$$f_{power}(x \mid \theta = \{\alpha, b, d\}) = \alpha + bx^d.$$

Additionally, we assume three error distributions, corresponding to the normal, normal non-constant variance (NCV), and the log-normal distribution. That is, the observed data $(y_1, \ldots, y_{10})$ are such that $\overline{y}_k = \frac{\sum_{i=1}^{10} y_{ik}}{10}$. We assume that these individual observations can arise from the following distributions: $y \sim N(f(x), \sigma^2)$, $y \sim N(f(x), \sigma^2) \times f(x)^\alpha$, or $y \sim LN(f(x), \sigma^2)$, where $N(\cdot)$ is the normal distribution and $LN(\cdot)$ is the log-normal distribution, respectively.

For the benchmark dose analysis, Bayesian model averaging was used. The four above models were fitted in this model average, assuming the normal and normal NCV distributions. Additionally, the Exponential-3 and Exponential-5 models were fitted using the log-normal distributions. Ten models were fitted to the data, and the BMD was computed using model averaging.

As specified in the main text of the monograph, priors were based upon the study of Wheeler et al. (2022), which defines general priors relative to the scale of response that can be used for gross toxicology observations (for example body weight). For example, in the study by Rafai, Papp & Jakab (2013), we analyse grams (in the hundreds) and kilograms (in the tens). The priors are set relative to

the magnitude of the response. These distributions are scaled to the background response. For example, parameter α is given a prior distribution centred on the mean response at control, with the variance being the mean squared. Below, we give the unscaled distributions relative to the control response equal to one. The method of rescaling is given in Wheeler et al. (2022), but as an example, if $\alpha \sim N(1,1)$ and the control response is 23.4, then the prior used in the analysis is $N(23.4,547.56)$. In the lists below, the only parameters that are not rescaled are the model's shape parameters d. These are informative priors meant to prevent biologically unrealistic dose–response curves.

Hill model parameters

$$\alpha \sim \mathrm{N}(1,1)_{1(-100,100)}$$
$$b \sim \mathrm{N}(0,1)_{1(-100,100)}$$
$$c \sim \mathrm{LN}(0,2)_{1(-100,100)}$$
$$d \sim \mathrm{LN}(\log[1.6],0.421)_{1(0,100)}$$

Exponential-3 model parameters

$$\alpha \sim \mathrm{LN}(0,1)_{1(0,100)}$$
$$b \sim \mathrm{LN}(0,2)_{1(0,100)}$$
$$d \sim \mathrm{LN}(\log[1.6], 0.421)_{1(0,100)}$$

Exponential-5 model

$$\alpha \sim \mathrm{LN}(0,1)_{1(0,100)}$$
$$b \sim \mathrm{N}(0,2)_{1(-100,100)}$$
$$c \sim \mathrm{LN}(0,2)_{1(0,100)}$$
$$d \sim \mathrm{LN}(\log[1.6], 0.421)_{1(0,100)}$$

Power model

$$\alpha \sim \mathrm{N}(1,1)_{1(0,100)}$$
$$b \sim \mathrm{N}(0,2)_{1(-100,100)}$$
$$d \sim \mathrm{LN}(\log[1.6], 0.421)_{1(0,100)}$$

A3.2.2 Analysis code

The R package ToxicR (1.0.1) was used to model these data. The following gives a step-by-step code analysis of each dataset in the study by Rafai, Papp & Jakab (2013).

```
library(ToxicR)
```

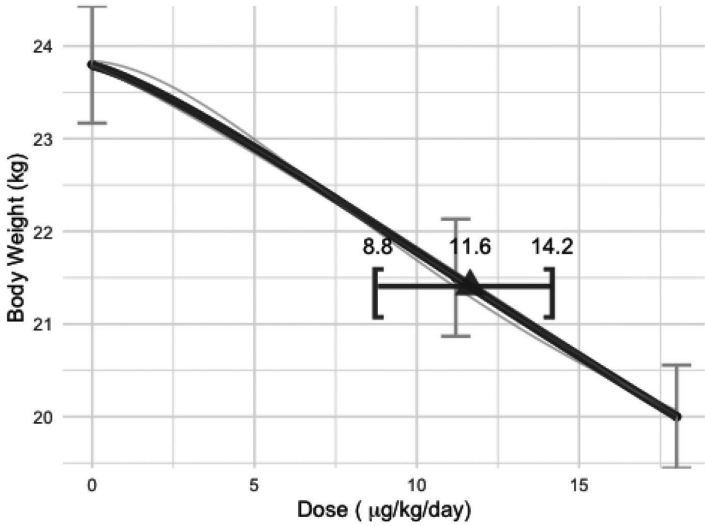
```
library(readxl)
library(ggploT-2)
library(ggpubr)

Rafai_BW <- read_excel("Body weight-2013.xlsx")
Rafai_BWG <- read_excel("BodyWeightGain-2013.xlsx")
Rafai_FI <- read_excel("Feed Intake.xlsx")
```

Body weight

The following is the code used to analyse the body weight data from this study.

```
dose <- Rafai_BW$dose
Y <- as.matrix(Rafai_BW[,c(3,2,4)])
fit_BW <- ma_continuous_fit(dose,Y,BMD_TYPE="rel",BMR=0.1)
p1 <- plot(fit_BW)+ggtitle("")+ylab("Body Weight (kg)") +
 xlab(expression(paste("Dose ( ", mu, "g/kg/day)"))) +
 annotate("text",x = fit_BW$bmd, y=21.85,
 label=sprintf("%1.1f",fit_BW$bmd))
p1
```

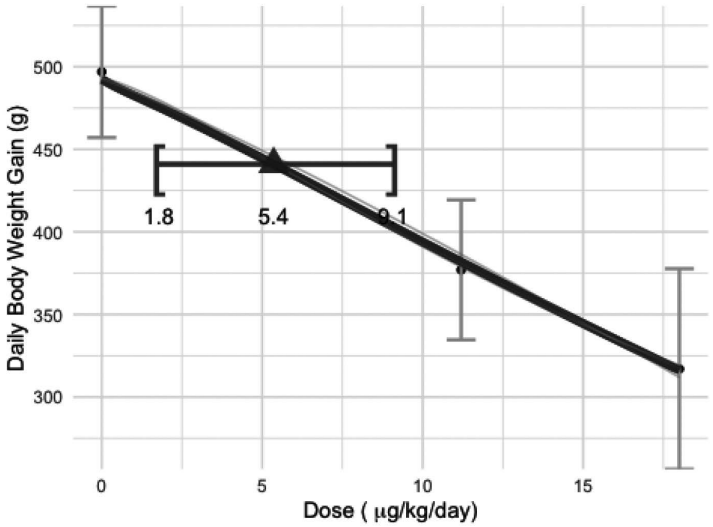


Body weight gain

The following is the code used to analyse body weight gain data from this study.

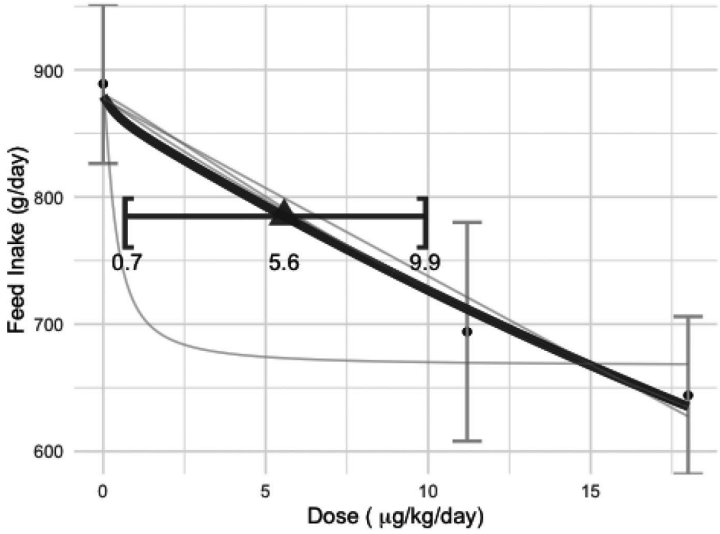
```
dose <- Rafai_BWG$Dose
Y <- as.matrix(Rafai_BWG[,c(3,2,4)])
```

```
fit_BWG <- ma_continuous_fit(dose,Y,BMD_TYPE="rel",BMR=0.1)
p1 <- plot(fit_BWG)+ggtitle("")+ylab("Daily Body Weight Gain (g)") +
 xlab(expression(paste("Dose ( ", mu, "g/kg/day)")))+
 annotate("text",x = fit_BWG$bmd, y=410,
 label=sprintf("%1.1f",fit_BWG$bmd))
p1
```



Feed intake

```
dose <- Rafai_FI$Dose
Y <- as.matrix(Rafai_FI[,c(3,2,4)])
fit_FI <- ma_continuous_fit(dose,Y,BMD_TYPE="rel",BMR=0.1)
p1 <- plot(fit_FI)+ggtitle("")+ylab("Feed Inake (g/day)") +
 xlab(expression(paste("Dose ( ", mu, "g/kg/day)"))) +
 annotate("text",x = fit_FI$bmd, y=750,
 label=sprintf("%1.1f",fit_FI$bmd))
p1
```



A3.3 BMD analysis

The following table gives model average BMD analyses for the three critical end-points. Here the BMD is defined using the relative effect with a 10% relative change in the mean specified as the BMR.

```
BMD_FI <- rbind(fit_FI$bmd)
BMD_BW <- rbind(fit_BW$bmd)
BMD_BWG <- rbind(fit_BWG$bmd)
final_table <- rbind(BMD_BW,BMD_BWG,BMD_FI)
rownames(final_table) <- c("Body Weight","Body Weight Gain","Feed
Intake")
library(knitr)
kable(final_table,digits=1,caption="Analysis of Rafai (2013) data".)
```

The BMD analysis for the BMR=10% for the three end-points in the Rafai (2013) study.

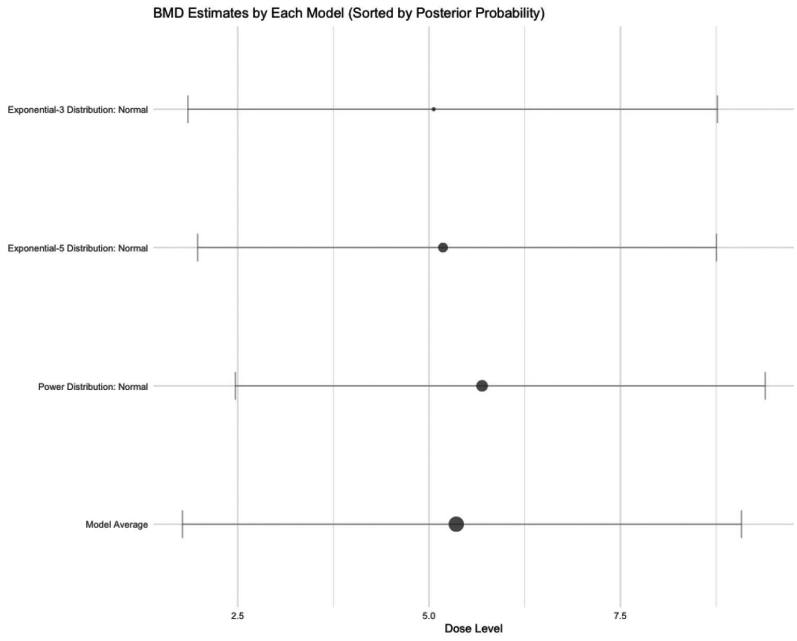
	BMD	BMDL	BMDU
Body weight	11.6	8.8	14.2
Body weight gain	5.4	1.8	9.1
Feed intake	5.6	0.7	9.9

Critical end-point

The Committee determined that body weight gain was the critical response in the study. The following table gives the constituent models used in the model average. The log-normal distributions were removed from this table as they had zero weight.

Dose-response	BMD (BMDL,BMDU)	Model weights
Power distribution: normal	5.7 (2.5, 9.4)	0.464
Exponential-5 distribution: normal	5.2 (2.0, 8.8)	0.326
Exponential-3 distribution: normal	5.1 (1.8, 8.8)	0.175
Hill distribution: normal	0.2 (0.0, 7.4)	0.025
Exponential-5 distribution: normal-NCV	5.5 (2.0, 9.8)	0.007
Hill distribution: normal-NCV	0.3 (0.0, 6.7)	0.001
Exponential-3 distribution: normal-NCV	5.4 (1.9, 10.0)	0.001
Power distribution: normal-NCV	6.3 (2.6, 10.7)	0.001

BMD, benchmark dose; BMDL, benchmark dose (lower confidence limit); BMDU, benchmark dose (upper confidence limit).



References

Rafai P, Papp Z, Jakab L (2013). Biotransformation of trichothecenes alleviates the negative effects of T-2 toxin in pigs. Acta Vet Hungarica. 61:333–43. https://doi.org/10.1556/AVet.2013.025

Wheeler MW, Blessinger T, Shao K, Allen BC, Olszyk L, Davis JA, et al. (2020). Quantitative risk assessment: developing a Bayesian approach to dichotomous dose–response uncertainty. Risk Anal. 40:1706–22.

Wheeler MW, Cortinas J, Aerts M, Gift JS, Davis JA (2022). Continuous model averaging for benchmark dose analysis: averaging over distributional forms. Environmetrics. 3 e2728.

Wu W, Zhou HR, Bursian SJ, Link JE, Pestka JJ (2016). Emetic responses to T-2 toxin, HT-2 toxin and emetine correspond to plasma elevations of peptide YY3–36 and 5-hydroxytryptamine. Arch Toxicol. 90:997–1007. https://doi.org/10.1007/s00204-015-1508-7

Wu Q, Kuca K, Nepovimova E, Wu W (2020). Type A trichothecene diacetoxyscirpenol-induced emesis corresponds to secretion of peptide yy and serotonin in mink. Toxins (Basel). 12:419. https://doi.org/10.3390/toxins12060419

ANNEX 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). Subsequently revised and published as: Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants. Rome: Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/ WHO Expert Committee on Food Additives). Subsequently revised and published as: Specifications for identity and purity of food additives, Vol. II. Food colours. Rome: Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/ WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. Evaluation of food additives: some enzymes, modified starches, and certain other substances. Toxicological evaluations and specifications and a review of the technological efficacy of some 87 Annex 1 antioxidants

(Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.

28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.

43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.

46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.

47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.

48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.

49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.

50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.

51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.

52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.

53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.

54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.

55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.

56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.

57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.

58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.

59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.

61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.

65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.

66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.

68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.

69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.

70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.

71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.

72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20, 1987.

73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.

74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21, 1987.

75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.

76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70, 1987 (out of print; https://apps.who.int/iris/handle/10665/37578).

77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.

78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22, 1988.

79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.

80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23, 1988.

82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.

83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.

84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24, 1989.

85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.

86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.

87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.

88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.

89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.

90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.

91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.

92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.

93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.

94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.

95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.

96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome: Food and Agriculture Organization of the United Nations, 1992 (2 volumes).

97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.

98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.

99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.

100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.

101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.

103. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 1, 1992.

104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.

105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.

106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.

107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.

108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.

109. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 2, 1993.

110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.

111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.

112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.

113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.

114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.

115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.

116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.

117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.

118. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 3, 1995.

119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.

120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.

121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.

122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.

123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 4, 1996.

125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.

126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.

127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.

128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.

129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.

130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.

131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.

132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.

133. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 5, 1997.

134. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.

135. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, 1998.

136. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/11, 1999.

137. Evaluation of certain food additives (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.

138. Safety evaluation of certain food additives. WHO Food Additives Series, No. 42, 1999.

139. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 6, 1998.

140. Evaluation of certain veterinary drug residues in food (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.

141. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 43, 2000.

142. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.

143. Evaluation of certain food additives and contaminants (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.

144. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 44, 2000.

145. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 7, 1999.

146. Evaluation of certain veterinary drug residues in food (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.

147. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 45, 2000.

148. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/13, 2000.

149. Evaluation of certain food additives and contaminants (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 901, 2001.

150. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.

151. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 8, 2000.

152. Evaluation of certain mycotoxins in food (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906, 2002.

153. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper, No. 74, 2001.

154. Evaluation of certain food additives and contaminants (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.

155. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.

156. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 9, 2001.

157. Evaluation of certain veterinary drug residues in food (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.

158. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 49, 2002.

159. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/14, 2002.

160. Evaluation of certain food additives and contaminants (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.

161. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 50, 2003.

162. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 10, 2002.

163. Evaluation of certain veterinary drug residues in food (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.

164. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 51, 2003.

165. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/15, 2003.

166. Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.

167. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 52, 2004.

168. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 11, 2003.

169. Evaluation of certain veterinary drug residues in food (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.

170. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/16, 2004.

171. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 53, 2005.

172. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 12, 2004.

173. Evaluation of certain food additives (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.

174. Safety evaluation of certain food additives. WHO Food Additives Series, No. 54, 2005.

175. Compendium of food additive specifications. FAO Food and Nutrition Paper, No. 52, addendum 13 (with errata), 2005.

176. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.

177. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 55; FAO Food and Nutrition Paper, No. 82, 2006.

178. Evaluation of certain food additives (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.

179. Safety evaluation of certain food additives. WHO Food Additives Series, No. 56, 2006.

180. Combined compendium of food additive specifications. FAO JECFA Monographs, No. 1, Volumes 1–4, 2005, 2006.

181. Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.

182. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 2, 2006.

183. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 57, 2006.

184. Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.

185. Compendium of food additive specifications. FAO JECFA Monographs, No. 3, 2006.

186. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 58, 2007.

187. Evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 59, 2008.

189. Compendium of food additive specifications. FAO JECFA Monographs, No. 4, 2007.

190. Evaluation of certain food additives (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.

191. Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, 2009.

192. Compendium of food additive specifications. FAO JECFA Monographs, No. 5, 2009.

193. Evaluation of certain veterinary drug residues in food (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.

194. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 61, 2009.

195. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 6, 2009.

196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.

197. Safety evaluation of certain food additives. WHO Food Additives Series, No. 62, 2010.

198. Compendium of food additive specifications. FAO JECFA Monographs, No. 7, 2009.

199. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.

200. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63; FAO JECFA Monographs, No. 8, 2011.

201. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 9, 2010.

202. Evaluation of certain food additives and contaminants (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.

203. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64, 2011.

204. Compendium of food additive specifications. FAO JECFA Monographs, No. 10, 2010.

205. Evaluation of certain food additives and contaminants (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.

206. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65, 2011.

207. Compendium of food additive specifications. FAO JECFA Monographs, No. 11, 2011.

208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.

209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.

210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 12, 2012.

211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.

212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs, No. 13, 2012.

214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.

215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.

216. Compendium of food additive specifications. FAO JECFA Monographs, No. 14, 2013.

217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.

218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.

219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 15, 2014.

220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.

221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.

222. Compendium of food additive specifications. FAO JECFA Monographs, No. 16, 2014.

223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.

224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.

225. Compendium of food additive specifications. FAO JECFA Monographs, No. 17, 2015.

226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.

227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.

228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 18, 2016.

229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.

230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.

231. Compendium of food additive specifications. FAO JECFA Monographs, No. 19, 2016.

232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.

233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.

234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.

235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, 2018.

236. Compendium of food additive specifications. FAO JECFA Monographs, No. 20, 2017.

237. Safety evaluation of certain food additives. WHO Food Additives Series, No. 75, 2019.

238. Evaluation of certain veterinary drug residues in food (Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1008, 2018.

239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs, No. 21, 2018.

240. Compendium of food additive specifications. FAO JECFA Monographs, No. 22, 2018.

241. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019.

242. Evaluation of certain food additives (Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1014, 2019.

243. Evaluation of certain food additives (Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1020, 2019.

244. Compendium of food additive specifications. FAO JECFA Monographs, No. 23, 2019.

245. Evaluation of veterinary drug residues in food (Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1023, 2020.

246. Safety evaluation of certain food additives. WHO Food Additives Series, No. 77, 2020.

247. Safety evaluation of certain food additives. WHO Food Additives Series, No. 78, 2020.

248. Evaluation of certain food additives (Ninety-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1037, 2020.

249. Evaluation of certain food additives (Eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1027, 2021.

250. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 79, 2021.

251. Evaluation of certain contaminants in food (Ninetieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1032, 2022.

252. Evaluation of certain food additives and contaminants (Ninety-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1036, 2022.

253. Safety evaluation of certain food additives. WHO Food Additives Series, No. 80, 2022.

254. Evaluation of certain contaminants in food (Ninety-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1040, 2023.

255. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 82, 2023.

256. Safety evaluation of certain food additives. WHO Food Additives Series, No. 83, 2023.

257. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 85, 2023.

ANNEX 2

Abbreviations and acronyms used in the monograph

3-ADON	3-acetyldeoxynivalenol
5-HT	5-hydroxytryptamine
15-AcDON	15-acetyldeoxynivalenol
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphate
ALT	alanine transaminase
AP	alkaline phosphatase
AST	aspartate aminotransferase
BMD	benchmark dose
BMR	benchmark response
BUN	blood urea nitrogen
CAT	catalase
CCCF	Codex Committee on Contaminants in Foods
CCK	cholecystokinin
CD	circular dichroism
C_{max}	maximum concentration
CYP	cytochrome P450
DAS	diacetoxyscirpenol
DLC	differential leukocyte count
DON	deoxynivalenol
DTH	delayed-type hypersensitivity
EFCOVAL	European food consumption validation
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
ENS	enteric nervous system
ER	endoplasmic reticulum
FBS	fetal bovine serum
GALT	gut-associated lymphoid tissue
GD	gestational day
GGT	gamma glutamyltransferase
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-17–36 amide
GnRH	gonadotropin-releasing hormone
GSH	glutathione
GSH-Px	glutathione peroxidase

GST	glutathione-S-transferase
HGF	human gingival fibroblast
HO	haem oxygenase
HPLC-MS	high-performance liquid chromatography–mass spectrometry
HPTLC	high-performance thin-layer chromatography
HSA	human serum albumin
HSP70	heat shock protein 70
HT	haemagglutination test
IFN- γ	interferon gamma
IL-2	interleukin-2
IL-4	interleukin-4
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JNK	c-Jun N-terminal kinase 1
KBD	Kashin-Beck disease
LC-MS	liquid chromatography–mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest observed adverse effect level
MAP	maximum a posteriori
MAPK	mitogen-activated protein kinase
MC	mast cells
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDA	malondialdehyde
MeSH	Medical Subject Heading
MLN	mesenteric lymph nodes
NCV	non-constant variance
NEO	neosolaniol
NIV	nivalenol
NHLF	normal human lung fibroblasts
NOAEL	no-observed adverse effect level
NOEL	no-observed-effect level
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PCV	packed cell volume
PFU	plaque-forming units
PMTDI	provisional maximum tolerable daily intake
PND	postnatal day
PYY_{3-36}	peptide YY_{3-36}
qPCR	real-time polymerase chain reaction

ROS	reactive oxygen species
RPTEC	renal proximal tubule epithelial cells
SCF	Scientific Committee on Food
SDI	sperm deformity index
SOD	superoxide dismutase
T-AOC	total antioxidant capacity
TAS	total antioxidants
TCC	thrombocyte count
TEC	total erythrocyte count
TEER	transepithelial electrical resistance
TLC	total leukocyte count
TMI	tolerable monthly intake
TNF	tumour necrosis factor
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
TWI	tolerable weekly intake
TZI	teratozoospermia index
UPLC-MS	ultra high-performance liquid chromatography–mass spectrometry
UPLC-Q/TOF	ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry
ZEA	zearalenone

ANNEX 3

Participants in the ninety-third meeting of the Joint FAO/WHO Expert Committee on Food Additives

Virtual meeting, 24 March–1 April 2022

Members

Dr A. Agudo, Unit of Nutrition and Cancer, Catalan Institute of Oncology, Barcelona, Spain

Dr S. Barlow, Brighton, East Sussex, United Kingdom

Dr D.J. Benford, Cheddington (Bucks), England *(Chairperson)*

Dr N. Fletcher, Food Standards Australia New Zealand, Canberra, ACT, Australia

Dr U. Mueller, Perth, Australia *(Rapporteur)*

Mr M. Feeley, Ottawa, Canada

Dr J. Schlatter, Zurich, Switzerland

WHO temporary advisers

Mr A. Afghan, Health Products and Foods Branch, Health Canada/Government of Canada, Canada

Mr P.J. Cressey, Institute of Environmental Science and Research Limited (ESR), Christchurch, New Zealand

Dr L. Edler, Dudenhofen, Germany

Dr Y. Kiparissis, Health Products and Foods Branch, Health Canada/Government of Canada, Canada

Dr E. Kirrane, US Environmental Protection Agency's Center for Public Health and Environmental Assessment, Research Triangle Park, NC, United States of America

Dr J.-C. LeBlanc, Laboratory for Food Safety, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort Cedex, France

Dr M. Wheeler, NIH/NIEHS Biostatistics and Computational Biology Branch, Research Triangle Park, NC, United States of America

FAO experts

Professor S. Edwards, Harper Adams University, Shropshire, United Kingdom

Professor P.W. Li, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, China

Dr G.S. Shephard, Cape Town, South Africa

Secretariat

Dr V. Fattori, Food Systems and Food Safety Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Secretariat)*

Ms N.Y. Ho, Department of Nutrition and Food Safety, World Health Organization, Geneva, Switzerland *(WHO Joint Secretariat)*

Dr M. Lipp, Food Systems and Food Safety Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Secretariat)*

Mr K. Petersen, Department of Nutrition and Food Safety, World Health Organization, Geneva, Switzerland *(WHO Joint Secretary)*

This volume contains a monograph prepared at the ninety-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met virtually from 24 March–1 April 2022.

The monograph addendum in this volume summarizes the data on a contaminant group (trichothecenes T-2 and HT-2 toxins) discussed at the ninety-third meeting. A monograph on the other features of this contaminant group, which were discussed at a previous meeting in 2001, are published in WHO Food Additives Series 47.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

9789240092525