**SCIENTIFIC OPINION**

# 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[1]

**EFSA Panel on Contaminants in the Food Chain (CONTAM)**[2, 3]

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## ABSTRACT

T-2 toxin and HT-2 toxin are mycotoxins produced by various *Fusarium* species. The European Commission asked EFSA for a scientific opinion on the risk to human and animal health related to the presence of T-2 and HT-2 toxin in food and feed. A total of 20,519 results for the sum of T-2 and HT-2 toxins in food, feed and unprocessed grains, collected in 2005-2010 from 22 European countries, were used in the evaluation. The highest mean concentrations for the sum of T-2 and HT-2 toxins were observed in grains and grain milling products, notably in oats and oat products. Grains and grain-based foods, in particular bread, fine bakery wares, grain milling products, and breakfast cereals, made the largest contribution to the sum of T-2 and HT-2 toxin exposure for humans. T-2 toxin is rapidly metabolised to a large number of products, HT-2 toxin being a major metabolite. Pigs are amongst the most sensitive animals towards the effects of T-2 toxin, the most sensitive endpoints being immunological or haematological effects. Using these data and a benchmark dose analysis the Panel on Contaminants in the Food Chain established a group tolerable daily intake (TDI) of 100 ng/kg b.w. for the sum of T-2 and HT-2 toxins. Estimates of chronic human dietary exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data are below the TDI for populations of all age groups, and thus not a health concern. For ruminants, rabbits and farmed fish the estimated exposures to the sum of these toxins based on the available occurrence data are considered unlikely to be a health concern, while for pigs, poultry, dogs and horses the risk of adverse health effects is low. For cats the health risk from the exposure to T-2 and HT-2 toxins cannot be assessed.



## KEY WORDS

Mycotoxins, *Fusarium*, HT-2 toxin, T-2 toxin, food, feed, analysis, occurrence, human dietary exposure, animal dietary exposure, risk assessment, toxicity, tolerable daily intake (TDI)

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## SUMMARY

T-2 toxin and HT-2 toxin are mycotoxins and are members of the large group of fungal sesquiterpenes, commonly denoted as trichothecenes. They are produced by various *Fusarium* species. Generally, the *Fusarium* species grow and invade crops under moist cool conditions. T-2 toxin and HT-2 toxin and other trichothecenes are found in cereal grains and products thereof.

Trichothecenes share a common structure with a tetracyclic ring system containing a stable epoxide group between C12 and C13, which seems to account for many of the typical toxic effects of trichothecenes. The structures of T-2 and HT-2 toxins differ only in one functional group, T-2 toxin being acetylated at C-4 whereas HT-2 toxin is not acetylated.

The Scientific Committee on Food (SCF) issued in 2001 an opinion on *Fusarium* toxins Part 5: T-2 and HT-2 toxin. The SCF concluded that the general toxicity, haematotoxicity and immunotoxicity of T-2 toxin are the critical effects and established a combined temporary tolerable daily intake (t-TDI) for the sum of T-2 toxin and HT-2 toxin of 0.06 µg T-2 toxin/kg body weight (b.w.). This was in line with the provisional maximum tolerable daily intake (PMTDI) established for T-2 and HT-2 toxin by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA).

The European Commission (EC) has asked the European Food Safety Authority (EFSA) for a scientific opinion on the risk to human and animal health related to the presence of T-2 and HT-2 toxin in food and feed. In particular the opinion should consider any new results of toxicological studies published since the assessment by the SCF in 2001, in order to assess if the combined t-TDI of 0.06 µg/kg b.w. for T-2 and HT-2 toxin is still appropriate. Additionally, the opinion should include an updated human dietary exposure assessment of T-2 and HT-2 toxins, and a determination of the daily exposure levels of T-2 and HT-2 toxins for different animal species above which signs of toxicity can be observed. It should also include a determination of the daily exposure levels of T-2 and HT-2 toxins above which the level of transfer/carry over of T-2 and HT-2 toxins from the feed to products of animal origin for human consumption results in unacceptable T-2 and HT-2 toxin levels. The EC request also asked for identification of the feed materials which could be considered as sources of T-2 and HT-2 toxins and the characterisation of the distribution of T-2 and HT-2 toxin levels in different feed materials.

Methods for analysis of T-2 toxin are well established and can be applied for the analysis of cereals, food, feed and biological samples. Accurate quantification of T-2 and HT-2 toxins is mostly carried out by liquid chromatography coupled with (multi-stage) mass spectrometry often within a multianalyte approach. For rapid screening several immunochemical methods have become available but they may suffer from undesired cross reactivity. None of the applied methods have been formally validated in interlaboratory validation studies.

Following a call for data by EFSA in July 2010, a total of 17,683 analytical results for T-2 toxin, 16,536 for HT-2 toxin and 20,519 for the sum of T-2 and HT-2 toxins in food, feed and unprocessed grains, collected between 2005 and 2010 from 22 European countries, were received. Overall, 65 % of the results were below the limit of detection (LOD) or limit of quantification (LOQ). In the quantified results, HT-2 toxin concentration represents about two thirds of the sum of T-2 and HT-2 toxin concentration. The highest mean concentrations for the sum of T-2 and HT-2 toxins were observed in grains and grain milling products, notably in oats and oat products. Levels in unprocessed grains were higher than in grain products for human consumption, suggesting that processing applied to grains results in lower T-2 toxin and HT-2 toxin concentrations. During the milling process T-2 and HT-2 toxins are not destroyed but unevenly redistributed between fractions. Because T-2 and HT-2 toxins are mostly attached to the outer hull of the grain, cleaning, sorting, sieving and de-hulling of grains lead to marked increases in T-2 and HT-2 toxins in cereal by-products, e.g. bran. T-2 and HT-2 toxins are relatively stable compounds during baking and cooking.

The Panel on Contaminants in the food chain (CONTAM Panel) estimated total chronic dietary exposures to the sum of T-2 and HT-2 toxins across 14 European countries, using lower bound (LB) and upper bound (UB) mean concentrations of the sum of T-2 and HT-2 toxins in foods, and consumption data for different age groups. For adults the minimum LB to maximum UB was 3.4 to 18 ng/kg b.w. per day for average consumers, and 7.2 to 39 ng/kg b.w. for high consumers ($95^{th}$ percentile consumption in total population). In elderly and very elderly populations, the chronic dietary exposure to the sum of T-2 and HT-2 toxins was slightly lower compared to other adults. The highest chronic dietary exposure estimates are for toddlers (age ≥ 12 months to < 36 months), at 12 to 43 ng/kg b.w. per day for average consumers, and 23 to 91 ng/kg b.w. for $95^{th}$ percentile consumers.

Grains and grain-based foods, in particular bread, fine bakery wares, grain milling products, and breakfast cereals, made the largest contribution to the sum of T-2 and HT-2 toxin exposure. For infants, the highest contributors were in the food group 'Foods for infants and small children', mainly cereal-based foods. No significant difference in the dietary exposure to the sum of T-2 and HT-2 toxins was found between vegetarians and the general population, although the data were limited.

The available information on the toxicokinetics of T-2 and HT-2 toxins is incomplete. T-2 toxin is rapidly metabolised to a large number of products, HT-2 toxin being a major metabolite. The metabolic pathways include hydrolysis, hydroxylation, de-epoxidation, glucuronidation and acetylation. Distribution and excretion of T-2 toxin and its metabolites are rapid. There are no significant data available on the toxicity of most metabolites. De-epoxidation is believed to be a detoxification process.

T-2 toxin inhibits protein, RNA and DNA synthesis. Recent data also indicate that T-2 toxin induces apoptosis, and in some cell types necrosis, as well as lipid peroxidation affecting cell membrane integrity. T-2 toxin induces haematotoxicity and myelotoxicity associated with impairment of haematopoiesis in bone marrow. The published investigations demonstrate that pigs are among the most sensitive animals towards the effects of T-2 toxin, the most sensitive endpoints being immunological or haematological effects which occur from doses of 29 µg/kg b.w. per day. Since the SCF evaluation in 2001 there is no evidence that the other toxic effects including dermal toxicity, developmental and reproductive toxicity and neurotoxicity occur at doses lower than those causing immunotoxicity and haematotoxicity in pigs. Although cats have been shown to be a very sensitive species their particular sensitivity to T-2 toxin is likely to be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation. Because of this difference in metabolic pathway with that of humans, data for cats are not suitable for human risk assessment.

The assessment by the SCF of the genotoxicity of T-2 toxin indicated a positive effect in several conventional tests for genotoxicity *in vitro* and in rodents *in vivo*, in particular for clastogenic effects, but these effects were observed primarily at concentrations also known to inhibit protein and DNA synthesis and produce cytotoxicity. No new reports on cytogenetic damage caused by T-2 toxin have been identified since then. The SCF reported limited evidence for tumourigenicity of T-2 toxin in experimental animals (induction of hepatocellular- and pulmonary adenomas in male mice). No new data are available on T-2 toxin carcinogenicity in experimental animals or on the carcinogenicity to humans of toxins derived from *Fusarium* sporotrichioides. Also there are no other new epidemiological data in the context of dietary exposure to T-2 and HT-2 toxins and human diseases.

The CONTAM Panel noted that the currently available toxicological studies have some uncertainties. Only one new dose-response study suitable for risk assessment has become available since 2001. The short term study in pigs, used by the SCF as the basis for establishing a t-TDI, was considered by CONTAM Panel to be still the most appropriate study for this purpose, and the immunotoxicological endpoints in this study were found to be the most important biological effects for risk assessment. The lowest-observed-adverse-effect-level (LOAEL) was 29 µg T-2 toxin/kg b.w. per day in this experiment, but no no-observed-adverse-effect-level (NOAEL) was identified. The CONTAM Panel concluded that a reduction in specific antibody response in pigs is the critical effect for human risk assessment.

The data on anti-horse globulin response from this study were used for a benchmark dose (BMD) analysis to identify a reference point for T-2 and HT-2 toxins. The default value for continuous data recommended by EFSA is a benchmark response (BMR) of 5 %. In the absence of statistical or toxicological considerations supporting deviation from the default value the CONTAM Panel chose a BMR of 5 % when applying the BMD approach on the dose-(antibody) response data available. The 95 % lower confidence limit for the benchmark dose response of 5 % ($BMDL_{05}$) calculated for anti-horse globulin titre values was 10 µg T-2 toxin/kg b.w. per day.

In view of the rapid metabolism of T-2 toxin to HT-2 toxin, and the fact that the toxicity of T-2 toxin might at least partly be attributed to HT-2 toxin, a group TDI was established for the sum of T-2 and HT-2 toxins. An uncertainty factor of 100 was applied to the $BMDL_{05}$, to establish a group TDI of 100 ng/kg b.w. for the sum of T-2 and HT-2 toxins. As new relevant evidence has become available since the previous t-TDI was established by the SCF in 2001, and as the present assessment was based on a $BMDL_{05}$, the CONTAM Panel concluded that a full TDI of 100 ng/kg b.w. can now be established.

Estimates of chronic dietary exposure for populations of all age groups to the sum of T-2 and HT-2 toxins based on the available occurrence data are below this group TDI of 100 ng/kg b.w., and therefore there is no health concern.

Animal exposure to the sum of T-2 and HT-2 toxins is primarily from consuming cereal grains and cereal by-products; levels in forages and oilseed meals are generally low. The animals considered were dairy cows, beef cattle, sheep and goats, pigs and piglets, hens, broiler chickens, turkeys, ducks, rabbits, fish, dogs, cats and horses. The highest UB exposure based on the available occurrence data in feed was for milking goats at 3.3 µg/kg b.w. per day and the lowest was for farmed fish at 0.19 µg/kg b.w. per day.

Information on LOAELs and NOAELs for farm and companion animals is limited. In young ruminants, exposure to 300 µg T-2 toxin/kg b.w. per day or more may result in gastrointestinal lesions, altered serum proteins and haematological alterations. In ruminants the effects observed in nutritionally challenged heifers and ewes give rise to the assumption that rumen detoxification of T-2 toxin may not always be complete. For pigs, the published investigations demonstrate that they are among the most susceptible animals towards the effects of T-2 toxin, the most sensitive endpoints being immunological or haematological effects which occur from doses of 29 µg/kg b.w. per day. In poultry, first effects (e.g. mucosal damage in oral cavity) occur at a dose of 40 µg/kg b.w. per day and 48 µg/kg b.w. per day for broiler chickens and fattening turkeys, respectively. In fattening ducks a dose of 40 µg/kg b.w. per day caused a significant reduction in body weight gain. Infertility of eggs and/or reduction of egg production were seen at doses of 120 µg/kg b.w. per day for laying hens.

For rabbits, doses ranging from 500-2000 µg of T-2 toxin/kg b.w. per day generate decrease of body weight gain and mucosal damage. Only moderate signs including haematological and hormonal effects have been observed for doses ranging from 200-500 µg of T-2 toxin/kg b.w. per day. A NOAEL of 100 µg T-2 toxin/kg b.w. per day was identified. Reduced feed intake, growth and haematocrit values as well as an increased mortality have been reported for fish. The lowest NOAEL of 13 µg T-2 toxin/kg b.w. per day has been identified for catfish. Cats are amongst the most sensitive animal species. This particular sensitivity of cats to T-2 toxin is likely to be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation. Due to the limited data and the severe effects i.e. mortality observed for cats at the low dose levels the available data can not be used to identify a NOAEL or a LOAEL. For dogs no toxicity data are available and a NOAEL or LOAEL cannot be identified. The available data do not provide a NOAEL or a LOAEL for horses.

With regard to animal health risk characterisation, because of the limited knowledge on the effects of T-2 and HT-2 toxins on farm and companion animals, and the absence of a comprehensive database on feed consumption by livestock in the EU, it has not been possible to properly assess the risks of these toxins for animal health. However, the exposure values for the LB and UB concentrations for

the sum of T-2 and HT-2 toxins in diets have been estimated for a number of farm livestock and companion animal categories, based on expected feed intakes and example diets, and these have been compared with identified NOAELs/LOAELs or with the calculated $BMDL_{05}$ for pigs.

The CONTAM Panel used the $BMDL_{05}$ for pigs as a reference point for risk characterisation for both pigs and poultry. The latter was considered acceptable as there was no indication from identified LOAELs that poultry are more sensitive than pigs. In the absence of NOAELs or LOAELs for horses and dogs, the CONTAM Panel also decided to use the same reference point as that derived for pigs to give an indication on the possible risk, since toxicokinetics of T-2 and HT-2 toxins in horses and dogs are not substantially different to that of pigs. However, due to the differences in oral bioavailability and metabolism in ruminants and fish, the $BMDL_{05}$ for pigs was not used for the risk characterisation for these species. The identified NOAELs or LOAELs were used for risk characterisation for ruminants, fish and rabbits. For cats the health risk from the exposure to the sum of T-2 and HT-2 toxins could not be assessed as no NOAEL or LOAEL has been identified, and as there is a lack of sufficient data on the feline-specific biotransformation and toxicodynamics. However, cats seem to be amongst the most sensitive animal species to T-2 toxin and HT-2 toxin intoxication.

Based on estimates of feed intake and the available occurrence data on feedingstuffs, the exposures to the sum of T-2 and HT-2 toxins for ruminants are substantially lower than the LOAELs identified, and are therefore considered unlikely to be a health concern. For pigs and poultry, comparison of the estimates of exposure based on the reported levels of the sum of T-2 and HT-2 toxins in feeds to the $BMDL_{05}$ for pigs indicate that the risk of adverse health effects of feed containing T-2 and HT-2 toxins is low for these species.

The limited data available for rabbits and farmed fish suggest that the estimated exposures to the sum of T-2 and HT-2 toxins in feed at the currently reported concentrations is well below the identified NOAELs, and therefore considered unlikely to be a health concern.

For cats the health risk from the exposure to the sum of T-2 and HT-2 toxins could not be assessed due to the lack of sufficient data. For dogs and horses, the estimates of exposure based on the reported levels of the sum of T-2 and HT-2 toxins in feeds indicate that the risk of adverse health effects as a result of consuming feed containing T-2 and HT-2 toxins is low for these species.

The available data describing possible effects of combined exposure to T-2 and HT-2 toxins with other mycotoxins are too limited to draw any conclusions.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

T-2 and HT-2 toxins are type A trichothecene mycotoxins, which are closely-related epoxy sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 toxins in grains such as wheat, maize, oats, barley, rice, beans, and soya beans as well in their derived products. *Fusarium langsethiae* seems to be the main *Fusarium* producing T-2 and HT-2 toxins, but it may not be the only one responsible because other species, such as *Fusarium poae* or *Fusarium sporotrichioides* were also identified to possibly produce T-2 and HT-2 toxins.

### The opinion from the Scientific Committee for Food and the JECFA

The Scientific Committee on Food (SCF) issued on 30 May 2001 an opinion on *Fusarium* toxins Part 5: T-2 and HT-2 toxin.[4] The SCF concluded that the general toxicity, haematotoxicity and immunotoxicity of T-2 toxin are the critical effects. The haematotoxicity and immunotoxicity of T-2 toxin in pigs in a short-term study were used as the basis for the safety assessment.

To account for the deficiencies in the studies e.g. study duration, pair feeding of control animals, comparative studies on metabolism and toxicokinetics and the use of a lowest-observed-adverse-effect-level (LOAEL), presumably close to the no-observed-adverse-effect-level (NOAEL), an extra uncertainty factor of 5 was included by the SCF, giving an overall uncertainty factor of 500. This led to the establishment of a temporary tolerable daily intake (t-TDI) of 0.06 µg T-2 toxin/kg b.w. This t-TDI value would also protect against the other chronic, subchronic and reproductive effects observed in the studies.

The acute toxicity of T-2 toxin and HT-2 toxin are within the same range and T-2 toxin is rapidly metabolised to HT-2 toxin. The toxicity of T-2 toxin might well at least partly be attributed to HT-2 toxin. Hence, the SCF concluded therefore that it was appropriate to establish a combined t-TDI for the sum of T-2 toxin and HT-2 toxin.

This combined t-TDI was confirmed by the SCF in their opinion on *Fusarium* toxins - Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol adopted on 26 February 2002.[5]

This t-TDI is in line with the TDI derived for T-2 and HT-2 toxins by the JECFA.[6]

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risk to human and animal health related to the presence of T-2 and HT-2 toxins in food and feed.

In particular the opinion should

a) consider any new results of toxicological studies published since the latest assessment by the Scientific Committee on Food of 30 May 2001 on T-2 toxin and HT-2 toxin in food in order to assess if the combined temporary tolerable intake of 0.06 µg/kg b.w. for T-2 and HT-2 toxins is still appropriate;

[4] Opinion of the Scientific Committee on Food on *Fusarium* toxins – Part 5:T-2 Toxin and HT-2 Toxin, adopted on 30 May 2001. (SCF/CS/CNTM/MYC/25 Rev 6 Final) http://ec.europa.eu/food/fs/sc/scf/out88_en.pdf

[5] Opinion of the Scientific Committee on Food on *Fusarium* toxins – Part 6: Group evaluation of T-2 toxin, HT-2 Toxin, nivalenol and deoxynivalenol adopted on 26 February 2002. (SCF/CS/CNTM/MYC/26 Final) http://ec.europa.eu/food/fs/sc/scf/out88_en.pdf

[6] JECFA (2001) Summary and Conclusions of the Fifty-sixth meeting Geneva, 6- 15 February 2001. Mycotoxins. http://www.fao.org/waicent/faoinfo/economic/esn/jecfa/jecfa56.pdf

b) contain an updated dietary exposure assessment of T-2 toxin and HT-2 toxin taking into account recent analytical results on the occurrence of T-2 and HT-2 toxins in food and the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc);

c) determine the daily exposure levels of T-2 toxin and HT-2 toxin for the different animal species (difference in sensitivity between animal species) above which

- signs of toxicity can be observed (animal health/impact on animal health) or
- the level of transfer/carry over of T-2 toxin and HT-2 toxin from the feed to the products of animal origin for human consumption results in unacceptable levels of T-2 toxin and HT-2 toxin
- identify feed materials which could be considered as sources of contamination by T-2 toxin and HT-2 toxin and the characterisation, insofar as possible, of the distribution of levels of contamination for the different (groups of) feed materials

## ASSESSMENT

### 1. Introduction

T-2 toxin and HT-2 toxin are mycotoxins and are members of a large group of fungal sesquiterpenes, commonly denoted as trichothecenes. They are produced by various Fusarium species, including *F. sporotrichoides*, *F. poae*, *F equiseti*, *F. acumninatum*, as well as species from the genera *Myrothecium*, *Cephalosporum*, *Verticimonosporum*, *Trichoderma, Trichothecium* and *Stachybotrys*. Generally, the *Fusarium* species grow and invade crops, and may produce T-2 and HT-2 toxins under moist cool conditions already prior to harvest. T-2 toxin and HT-2 toxin and other trichothecenes are predominantly found in cereal grains (particularly in oats) and products thereof.

T-2 toxin and HT-2 toxin are toxic to all animal species as well as to humans. Historical cases of human intoxications associated with the consumption of overwintered, mouldy grains are described as Alimentary Toxic Aleukia (ATA), characterised by sepsis and haemorrhages and a general pancytopenia.

The toxic effects exerted by T-2 toxin and HT-2 toxin include the inhibition of protein synthesis, affecting also the synthesis of immunoglobulins and in turn the humoral immunity. Alteration of cell membrane functions and lipid peroxidation account for many of the acute effects of T-2 and HT-2 toxins, including the necrotic lesions observed at the contact sites. Apoptosis of proliferating cells including bone marrow cells (inhibition of haematopoesis) and cells of the immune system (lymphoid depletion) account for the systemic toxicity following dietary exposure.

Comparable symptoms have been described in farm animal species, often accompanied by local necroses in the upper gastro-intestinal tract. The significant differences in the sensitivity to T-2 and HT-2 toxin of monogastric species and ruminants is attributable to the effective presystemic elimination (de-epoxidation) of the toxins by the rumen microbial flora.

Only T-2 toxin and HT-2 toxin are considered in this opinion although combined dietary exposures for humans and animals with other tricothecenes and mycotoxins may occur.

### 1.1. Previous assessments

In 2001, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and Scientific Committee on Food (SCF) assessed T-2 and HT-2 toxins. In its 56$^{th}$ meeting the JECFA assessed various mycotoxins including T-2 toxin and HT-2 toxin (FAO/WHO, 2001). The Committee concluded that the safety of food contaminated with T-2 toxin could be evaluated from the lowest-observed-effect-level (LOEL) of 0.029 mg/kg body weight (b.w.) per day for changes in white and red blood cell counts identified in the 3-week dietary study in pigs (Rafai et al., 1995b). This LOEL was the lowest LOEL for adverse effects in the studies on T-2 toxin. It was assumed that this level was close to the no-observed-effect-level (NOEL), as the effects on blood cell counts were subtle and reversible. Furthermore, other studies in pigs showed no adverse effects at this dose. The Committee used this LOEL and a safety factor of 500 to derive a provisional maximum tolerable daily intake (PMTDI) for T-2 toxin of 60 ng/kg b.w. per day. The safety factor of 500 was used because there was no clear NOEL in the 3-week study in pigs and there were deficiencies in the database, including insufficient study of long-term administration of T-2 toxin and sex, species, and individual variations in sensitivity. The Committee further concluded that the toxic effects of T-2 toxin and its metabolite HT-2 toxin could not be differentiated, and that the toxicity of T-2 toxin *in vivo* might be due at least partly to effects of HT-2 toxin. Hence, HT-2 toxin

was included in the PMTDI, resulting in a group PMTDI of 60 ng/kg b.w. per day for T-2 and HT-2 toxins, alone or in combination.

The Scientific Committee on Food (SCF) issued an opinion on *Fusarium* toxins Part 5: T-2 and HT-2 toxin (SCF, 2001). This evaluation was primarily based on the report of the Nordic Council of Ministers (1998). The SCF concluded that the general toxicity, haematotoxicity and immunotoxicity of T-2 toxin are the critical effects. The haematotoxicity and immunotoxicity of T-2 toxin in pigs in the short-term study of Rafai et al. (1995b) were used as the basis for the assessment.

To account for the deficiencies in the studies e.g. short study duration, lack of pair feeding of control animals, lack of comparative studies on metabolism and toxicokinetics and the use of a lowest-observed-adverse-effect-level (LOAEL), presumably close to the no-observed-adverse-effect-level (NOAEL), an extra uncertainty factor of 5 was included by the SCF, giving an overall uncertainty factor of 500. This led to the establishment of a temporary TDI (t-TDI) of 0.06 µg T-2 toxin/kg b.w. (60 ng T-2 toxin/kg b.w.). This t-TDI value would also protect against the other chronic, subchronic and reproductive effects observed in the studies.

The acute toxicity and the *in vitro* cytotoxicity of T-2 toxin compared to HT-2 toxin are within the same range and T-2 toxin is rapidly metabolised to HT-2 toxin. The toxicity of T-2 toxin might at least partly be attributed to HT-2 toxin. Hence, the SCF concluded that it was appropriate to establish a combined t-TDI for the sum of T-2 toxin and HT-2 toxin.

This combined t-TDI was confirmed by the SCF in their opinion on *Fusarium* toxins - Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol in 2002 (SCF, 2002).

Earlier, the International Agency on Research on Cancer (IARC) evaluated T-2 toxin and concluded that 'There is limited evidence in experimental animals for the carcinogenicity of T-2 toxin'. Therefore T-2 toxin was not classified as to its carcinogenicity in humans (IARC, 1993). The overall IARC evaluation was that toxins derived from *Fusarium sporotrichioides* are not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 1993).

### 1.2. Chemistry of T-2 and HT-2 toxins

T-2 toxin[7] and HT-2 toxin[8] (see Figure 1) belong to a large group of approximately 180 trichothecenes discovered so far which are produced by *Fusarium* species. Trichothecenes have a common tetracyclic, sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system and are divided into four groups (A-D) according to their different chemical functionalities. The stable epoxide group between C12 and C13 seems to account for many of the typical toxic effects of trichothecenes. Epidemiological surveys have demonstrated that the predominant type A and B trichothecenes are widely distributed in cereals and feeds as natural pollutants, whereas C (characterised by a second epoxide at C7,8 or C9,10) and D trichothecenes (containing an ester-linked macrocycle at C4,16) occur rarely in food and feed. Type A trichothecenes include T-2 toxin, HT-2 toxin and 4,15-diacetoxyscirpenol, and type B toxins include deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and nivalenol.

[7] Other synonyms for T-2 toxin: Fusariotoxin T2; insariotoxin; Mycotoxin T2; T-2 mycotoxin; toxin T2; $T_2$-toxin; $T_2$-trichothecene. Other chemical names for T-2 toxin used in previous assessments by JECFA and SCF include 4β, 15-diacetoxy-3α -hydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytricothec-9-ene; 12,13-Epoxytrichothec-9-ene-3,4,8,15-tetraol 4,15-diacetate 8-(3-methylbutanoate); (3α,4β,8α)-12,13-epoxytrichothec-9-ene-3,4,8,15-tetrol 4,15-diacetate 8-(3-methylbutyrate)

[8] Other synonyms for HT-2 toxin: Fusariotoxin HT-2; Mycotoxin HT-2; Toxin HT 2. Other chemical names for HT-2 toxin used in previous assessments by JECFA and SCF include 15-acetoxy-3α,4β- dihydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytricothec-9-ene; 12,13-epoxytrichothec-9-a,4-b,8-a,15-tetraol 15 acetate 8-isovalerate; (3α,4β,8α)-12,13-epoxytrichothec-9-ene-3,4,8,15-tetrol 15-acetate 8-(3-methylbutyrate)

Type A trichothecenes possess an ester function at the C8 position whereas for type B trichothecenes, a carbonylic functionality at C8 is characteristic. Both T-2 and HT-2 toxins are non-volatile compounds, which are stable at neutral and acidic pH. The fewer free hydroxyl groups and the lacking keto group at C8 of type A trichothecenes make them less polar compared with the related type B trichothecenes. Therefore, different methods of analysis are usually employed for the determination of type A and type B trichothecenes (see Section 3).

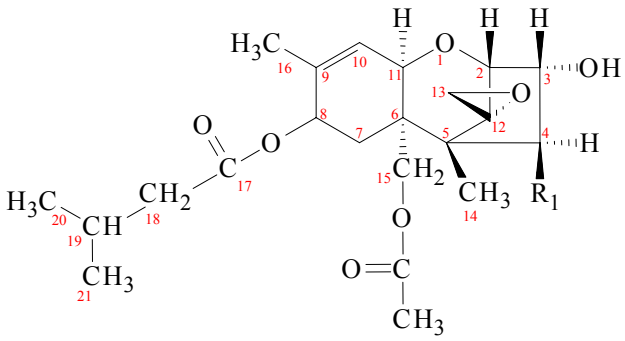

**Figure 1:** Chemical structure of T-2 toxin ($R_1$ = OAc) and HT-2 toxin ($R_1$ = OH).

T-2 toxin is the trivial name for (3α,4β,8α)-12,13-epoxytrichothec-9-ene-3,4,8,15-tetrol 4,15-diacetate 8-(3-methylbutyrate) (Chemical Abstracts Service (CAS) registry number 21259-20-1) corresponding to the molecular formula $C_{24}H_{34}O_9$ and its molecular weight is 466.5 g/mol. T-2 toxin forms white needles with a melting point of 151-152°C (Bamburg et al., 1968) and its specific rotation has been determined as $[\alpha]^{26}_D = +15°$ (c = 2.58 in 95 % ethanol) (Pohland et al., 1982).

T-2 toxin is readily metabolised to HT-2 toxin (and other substances) by various animals but can also be metabolised by plants and fungi (reviewed by Dohnal et al., 2008). The structure of HT-2 toxin differs from T-2 toxin only in the functional group at the C4-position. HT-2 toxin is the trivial name for (3α,4β,8α)-12,13-epoxytrichothec-9-ene-3,4,8,15-tetrol 15-acetate 8-(3-methylbutyrate) (CAS registry number 26934-87-2 ). The molecular formula of HT-2 toxin is $C_{22}H_{32}O_8$ with a molecular weight of 424.5 g/mol. The melting point is similar to that of T-2 toxin, at 151-152 °C. At room temperature HT-2 toxin forms white crystals or a pale yellow oil. The solubility of both T-2 and HT-2 toxins is good in most organic solvents (Yates et al., 1968) including methanol, ethanol, acetone, chloroform, ethyl acetate, diethyl ether and acetonitrile, but poor in water.

All trichothecenes have proved to be stable in acetonitrile and under argon atmosphere for at least 24 months, stored at temperatures up to 25 °C, but they are unstable in ethyl acetate at temperatures above freezing (Pettersson and Langseth, 2002b). The epoxide group at C12,13 position is extremely resistant to nucleophilic attack (Shepherd and Gilbert, 1988). In aqueous solution, both T-2 and HT-2 toxins are stable within a physiological pH range with estimated half-life times of 3.9 and 8.5 years, respectively (Duffy and Reid, 1993).

## 2. Legislation

Worldwide, 13 countries have reported legal maximum levels (MLs) or recommendations for T-2 and HT-2 toxins in food and/or feed products. (Table 1: food MLs and Table 2: feed MLs). Canada is the only country known thus far that has a ML (guidance level) for solely HT-2 toxin, but only for feed for cattle and poultry (100 µg/kg) (FAO, 2004). MLs for T-2 toxin in food have most frequently been set in Eastern Europe. These MLs resemble the ML in the Russian Federation (100 µg/kg) (with the

exception of Hungary). Various countries from all over the world have set MLs for T-2 toxin in feed products, ranging from 25 to 1000 µg/kg.

**Table 1:** Reported legal maximum levels (ML) for T-2 and HT-2 toxins in food products.

| Country | Product | ML (µg/kg) | Reference |
|---|---|---|---|
| Armenia | All foods | 100[(a)] | FAO (2004) |
| Belarus | Grain, flour, groats | Unknown | FAO (2004) |
| | Infant food | Not allowed | |
| Hungary | Milled products, cereal constituent of muesli | 300[(a),(b)] | EC enquiry (2011) |
| Moldova | Cereals and cereal flour | 100[(a)] | FAO (2004) |
| Norway | Cereals and cereal products | 100[(b),(c)] | EC enquiry (2011) |
| | Cereals and cereal products for infants and young children | 50[(b),(c)] | EC enquiry (2011) |
| Russian federation | Barley | 100[(a)] | FAO (2004) |
| Ukraine | Grains, flour, wheat middlings, bread products; all seeds to be used for immediate human consumption and for processing into products for human consumption | 100[(a)] | FAO (2004) |

EC: European Commission; FAO: Food and Agriculture Organization.
(a): T-2; (b): T-2 and HT-2 toxins, provisions explicitly confirmed; (c): guideline levels.

In the European Union (EU) tolerances in food for specific contaminants shall be established if this is necessary to protect public health (Article 2 of Council Regulation (EEC) No 315/93 of February 1993 laying down Community procedures for contaminants in food[9]). MLs are laid down in Regulation (EC) No 1881/2006 of 19 December 2006,[10] in which legal levels for T-2 and HT-2 toxins in unprocessed cereals and cereal products for human consumption are envisaged but are not established yet. To ensure agricultural productivity and sustainability, and animal and public health, animal welfare and the environment MLs for undesirable substances in feed are laid down in EU Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002[11]. Data on the presence of T-2 and HT-2 toxins in feed products have been scarce. That is why the EC recommended EU Member States to gather reliable data on year to year variation in occurrence of T-2 and HT-2 toxins (and other *Fusarium* toxins) (Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 toxins and fumonisins in products intended for animal feeding (2006/576/EC)[12]) in order to be able in the near future to establish MLs in feed. Thus, specific EU harmonised MLs for T-2 and HT-2 toxins in food and feed products have not been established yet.

[9] OJ L 37, 13.2.1993, pp. 1-3
[10] OJ L 364, 20.12.2006, pp. 5-17.
[11] OJ L 140, 30.5.2002, pp. 10-21.
[12] OJ L 229, 23.8.2006, pp. 7-9.

**Table 2:** Reported legal maximum levels (ML) for T-2 toxin and HT-2 toxin in feed products.

| Country | Product | ML (µg/kg) | Reference |
|---|---|---|---|
| Canada | Feed for swine and poultry | 1000[(a)] | FAO (2004) |
| | Feed for cattle and poultry | 100[(b)] | |
| China | Feed for pigs and poultry | 1000[(a)] | National Standard Bureau, China GB 21693-2008 (2008) |
| Croatia | Complete and supplemental feed for pigs, poultry and calves | 500[(c)] | Sokolovic et al. (2008) |
| Iran | Complete feed for sheep, goats and beef cattle | 100[(a)] | FAO (2004) |
| | Complete feed for calf, lamb, kid, dairy sheep, goats, cattle | 25[(a)] | |
| Israel | All grains | 100[(a)] | FAO (2004) |
| Norway | Compound feed for pigs and horses | 200[(c)] | Norwegian Food Safety Authority (2007)[13]; EC enquiry (2011) |
| | Compound feed for calves, lambs, kids and poultry | 600[(c)] | |
| | Compound feed for other food producing animals | 2000[(c)] | |
| Serbia and Montenegro | Compound and complementary feed mixtures for chickens, piglets and calves | 500[(a)] | Ministry of Agriculture (2010) |
| | Compound and complementary feed mixtures for pigs and poultry | 1000[(a)] | |
| | Feed mixtures for chickens, piglets and calves | 300[(d)] | |
| | Feed mixtures for saws, cows and poultry | 600[(d)] | |
| Ukraine | Combined feed for egg-layers and broilers | 200[(a)] | FAO (2004) |
| | Combined feed for calves and older cattle fed for beef | 250[(a)] | |

(a): T-2 toxin; (b): HT-2 toxin; (c): T-2 and HT-2 toxins; (d): total trichothecenes.

## 3. Analysis

### 3.1. Sampling and storage

Prior to the analysis for T-2 and HT-2 toxins, a representative sample must be provided, as this influences the reliability of the analytical data generated. Due to the possible inhomogeneous distribution of trichothecenes in lots (of grains), sampling may contribute to the variability in analytical results. In the Commission Regulation (EC) No 401/2006 of 23 February 2006[14] methods of sampling for the official control of the levels of mycotoxins are laid down. In Annex I of this Regulation general provisions for sampling are stated in part A, and specific provisions for the sampling of cereals and cereal products in part B. After taking a sample, it is stored under appropriate conditions (dry, preferably frozen) until analysis.

### 3.2. Determination of T-2 and HT-2 toxins

Analytical procedures for type A trichothecenes may be fully quantitative, semi-quantitative or qualitative. Some of the methods in use for the analysis of cereals and cereal products have been recently discussed in an extensive review by Meneely et al. (2010). Usually these methods determine T-2 and HT-2 toxins at the same time, individually or together as a sum.

[13] www.mattilsynet.no, http://www.mattilsynet.no/mattilsynet/multimedia/archive/00064/Anbefalte_grenseverd_64407a.pdf
[14] OJ L 70, 9.3.2006, pp. 12-70.

The increasing need for reliable and accurate testing methods for the sensitive determination of T-2 and HT-2 toxins has led to two major trends. There has been a shift from classical thin layer chromatographic (TLC) and gas chromatographic (GC) techniques to highly sophisticated methods based on liquid chromatography (LC) coupled with multiple-stage mass spectrometry ($MS^n$). Recently, mostly immunochemically based, rapid methods have been developed for the screening for these type A trichothecenes. There is a trend towards easy-to-use purification techniques with less requirement for extensive clean-up procedures.

### 3.2.1. Analyte isolation

For the extraction of T-2 and HT-2 toxins from food and feed, including grains and grain-based products, mostly organic solvent/water mixtures are used such as methanol/water and acetonitrile/water. The resulting extract is usually further processed to remove impurities/interfering materials and often concentrated to make determination of toxins at low concentrations possible. For T-2 and HT-2 toxins, clean-up procedures may involve the use of various types of solid phase extraction (SPE) columns including multifunctional columns (Haeubl et al., 2007; Cano-Sancho et al., 2010) and/or immunoaffinity (IA) columns (Majerus et al., 2008; Lattanzio et al., 2009). For SPE columns various packings are now commercially available, and may contain silica, charcoal, Florisil®, C8, C18 and aluminium oxide (Meneely et al., 2010).

### 3.2.2. Chromatographic methods

Chromatographic methods have been developed for the accurate quantification and identification of type A trichothecenes in various matrices including food and feed as well as samples of human and animal origin. GC has largely been the method of choice, in combination with flame ionisation detection (FID), electron capture detection (ECD) and mass spectrometry (MS) detection. While these methods provide sensitive and accurate results, the polar compounds require derivatization prior to GC separation, which is often a lengthy exercise (Schothorst and Jekel, 2001; Cervero et al., 2007). A variety of chemicals have been used for derivatization of type A trichothecenes and the choice depends on the method of detection employed (Kotal et al., 1999; Eskola et al., 2001; Majerus et al., 2008). Schothorst and Jekel (2001) as well as Leblanc et al. (2005) applied trimethylsilylation for type A trichothecenes and reported durations from 15 minutes to 2 hours for the derivatization procedure. Another common approach for derivatization of type A trichothecenes is fluoroacetylation. Derivatising agents that have been used include anhydrides of trifluoroacetic acid (Kotal et al., 1999), heptafluorobutyric acid (Scott and Trucksess, 1997) and pentafluoropropionic acid (Majerus et al., 2008; Cano-Sancho et al., 2010; Ibañez-Vea at al., 2011). The number of new publications on the use of GC methods for the analysis of T-2 and HT-2 toxins, however, is relatively small. GC methods showed higher variations in an intercomparison study on trichothecene determination as compared to LC methods (Pettersson and Langseth, 2002a). The major reason for the observed discrepancies was due to adsorption of derivatised type A trichothecenes to active sites of the GC injector and the upper part of the capillary column. This effect was more pronounced in the absence of matrix, which led to lower signals for pure calibrants compared to the analyte response in the presence of matrix. Typical limits of detection (LODs) for GC-methods for the analysis of T-2 and HT-2 toxins are given in Table 3.

In addition, high performance thin layer chromatography (HPTLC) has been used not only for screening but also for quantification of T-2 toxin, using ultra violet (UV)-fluorescence (Dawlatana et al. 1999). However, the reported LOD was relatively high (39.2 µg T-2 toxin/kg).

Within the last decade, high performance-liquid chromatography (HPLC) has become the most frequently used method for the determination of type A trichothecenes. In contrast to type B trichothecenes type A trichothecenes lack an exploitable chromophore, with an UV maximum slightly below 200 nm in ethanol (Pohland et al., 1982). Therefore, UV detection is not the method of choice

for determination of type A trichothecenes, following HPLC separation, as it is only applicable for relatively high toxin concentrations (Medina et al., 2010). Instead, fluorescence detection (FLD) and MS detection are used. The use of HPLC coupled with FLD has been applied to the determination of T-2 and HT-2 toxins and involves pre-column derivatization of the compounds using 1-anthroylnitrile (Visconti et al., 2005; Trebstein et al., 2008), coumarin-3-carbonyl chloride, 1-naphthoyl chloride, 2-naphthoyl chloride or pyrene-1-carbonyl cyanide (Lippolis et al., 2008). Typical LODs for the LC-methods for the analysis of T-2 and HT-2 toxins are given in Table 3.

LC-tandem mass spectrometric (LC-MS/MS) (also referred to as triple quadrupole MS) methods for the simultaneous determination of multiple groups of mycotoxins, including T-2 and HT-2 toxins, in a variety of different foods and feeds have become very popular during the last few years. The vast majority of these methods employ LC-MS/MS which are employed solely in the selected reaction monitoring mode (e.g. Berthiller et al., 2005; Biselli and Hummert, 2005; Cavaliere et al., 2005; Kloetzel et al., 2005; Sulyok et al., 2006; Gentili et al., 2007; Sulyok et al., 2007; Lattanzio et al., 2008; Spanjer et al., 2008; Santini et al., 2009; Capriotti et al., 2010; Desmarchelier et al., 2010; Martos et al., 2010; Monbaliu et al., 2010; Romero-Gonzalez et al., 2010). Recently also high resolution LC-MS was employed for the quantification of T-2 and HT-2 toxins (Zachariasova et al., 2010). Usually electrospray ionization (ESI) is performed, but some methods (often those with a limited number of measured analytes besides T-2 and HT-2 toxins) rely on atmospheric pressure chemical ionization (Berthiller et al., 2005; Lattanzio et al., 2008; Santini et al., 2009; Pascale et al., 2011; Škrbić et al., 2011) or even photoionisation (Capriotti et al., 2010). While some methods use both positive and negative polarities (either with two consecutive LC runs or fast polarity switching), T-2 and HT-2 toxins are almost exclusively measured in positive ion mode. Beside the formation of $[M+H]^+$ ions, very often adducts of the type A trichothecenes are monitored. To minimise the abundance of $[M+Na]^+$ ions, which hardly fragment, often ammonium salts are included as modifiers in the LC mobile phase. Fragmentation of the resulting $[M+NH_4]^+$ ions yields methods that are more sensitive. The LODs for the LC-MS/MS methods are in the low or even sub-µg/kg-range, with T-2 toxin being detectable at lower levels than HT-2 toxin (Table 3).

An inherent problem with all MS methods is signal suppression or even signal enhancement owing to matrix effects. Possible strategies to cope with these effects include selective clean-up, sample extract dilution, usage of matrix matched standards, standard addition to each sample at multiple levels or internal standards. For the latter, a stable isotope dilution assay for the quantification of T-2 and HT-2 toxins has been described using chemically synthesised ($^{13}C_4$) T-2 toxin and ($^{13}C_2$) HT-2 toxin (Asam and Rychlik, 2006). Furthermore, an LC-MS/MS stable isotope dilution method using uniformly $^{13}C$-labelled T-2 toxin was developed and in-house validated for maize and oats (Haeubl et al., 2007). The method based on the use of isotopically labelled standards of T-2 and HT-2 toxins was employed in the study concerning the distribution of these two toxins in milling fractions of durum wheat (Pascale et al., 2011. While strong matrix enhancement effects were observed for these two matrices, the internal standard successfully compensated for these effects, resulting in overall recoveries of 99 % for oats and of 100 % for maize even without prior clean-up.

Several analytical methods applying LC or GC have been validated in-house for applicability for food, beverages and feedstuffs within the last few years. Examples of such methods are given in Table 3. As can be seen from this Table, the LODs and limits of quantification (LOQs) of the methods for T-2 and HT-2 toxins strongly depend on the commodity, the clean-up, separation and end-detection method, and on the instrumentation used.

**Table 3:** Examples of in-house validated chromatographic methods for the determination of T-2 and HT-2 toxins.

| Matrix | Extraction | Clean-up | Detection | Analyte | LOD (µg/kg) | LOQ (µg/kg) | Recovery (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| wheat, rice, oats | MeOH-$H_2O$ | Immunoaffinity column | GC-ECD (PFPA) | T-2, HT-2 | 1.7-2.3 | 5.4-6.6 | 71-116 | Majerus et al. (2008) |
| cereal based food including beer | ACN-$H_2O$ | Reverse phase (C18), immunoaffinity, multifunctional columns | GC-ECD (PFPA), GC-MS, LC-DAD | DON, T-2, HT-2 | not reported | 0.1-1.4 | 70-130 | Cano-Sancho et al. (2011) |
| wheat, maize, barley | MeOH-$H_2O$ | Immunoaffinity column | HPLC-FLD (1-AN) | T-2, HT-2 | 3-5 | not reported | 70-103 | Visconti et al. (2005) |
| oats, muesli, infant food, breakfast cereals | MeOH-$H_2O$ | Immunoaffinity column | HPLC-FLD (1-AN) | T-2, HT-2 | not reported | 8 | 74-120 | Trebstein et al. (2008) |
| wheat, maize | $(CH_3)_2CO$-AcOH-$H_2O$ | None | LC-APPI-MS/MS | Multitoxin method including T-2, HT-2 | 5-7 | 9-11 | not reported | Capriotti et al. (2010) |
| tea and herbal infusions | EtOAc-HCOOH | $NH_2$-SPE | UHPLC-MS/MS | Multitoxin method including T-2, HT-2 | 3.4-12 | 6.8-24 | 97-106 | Monbaliu et al. (2010) |
| beer, wine | HF-LPME | None | UHPLC-MS/MS | T-2, OTA | not reported | < 0.1 | 87-105 | Romero-Gonzales et al. (2010) |
| beer | addition of ACN | None | UHPLC-orbitrapMS | Multitoxin method including T-2, HT-2 | not reported | 1.5-6 | 88-119 | Zachariasova et al. (2010) |
| maize, oats | ACN-$H_2O$ | Multifunctional columns | LC-APCI-MS/MS | T-2 | not reported | 2-4 | 99-100 | Haeubl et al. (2007) |
| maize, wheat, oats | PBS-buffer | Immunoaffinity column | LC-APCI-MS/MS | T-2, HT-2 | 0.4-0.5 | not reported | 61-97 | Lattanzio et al. (2009) |
| cereals, soya, maize gluten | QuEChERS ASE | None | LC-ESI-MS/MS | Multitoxin method including T-2, HT-2 | not reported | 5-125 | 65-117 | Desmarchelier et al. (2010) |

MeOH: methanol; $H_2O$: water; ACN: acetonitrile; $(CH_3)_2CO$: acetone; AcOH: acetic acid; EtOAc: ethyl acetate; HCOOH: formic acid; HF-LPME: hollow fiber liquid-phase micro extraction; PBS: phosphate buffered saline; QuEChERS: quick, easy, cheap, effective, rugged and safe; ACE: accelerated solvent extraction; GC: gas chromatograph; ECD: electron capture detector; PFPA: pentafluoropropionic anhydride; HPLC: high performance-liquid chromatography; FLD: fluorescence detector; 1-AN: 1-anthroylnitrile; MS: mass spectrometer; LC: liquid chromatograph; DAD: diode-array detector; APPI: atmospheric pressure photoionisation; MS/MS: tandem mass spectrometer; UHPLC: ultra high performance-liquid chromatograph; APCI: atmospheric pressure chemical ionisation; ESI: electrospray ionisation; T-2: T-2 toxin; HT-2: HT-2 toxin; DON: deoxynivalenol; OTA: ochratoxin A.

### 3.2.3. Immunochemical methods

Immunochemical methods to determine T-2 and HT-2 toxins are usually employed as screening methods. They include enzyme-linked immunosorbent assays (ELISA), lateral flow devices, dipstick tests and more recently biosensor assays. A fundamental requirement for an immunoassay is the specificity of the antibody used.

The immunochemical methods in use for T-2 and HT-2 toxins are mainly based on competitive ELISA techniques which are usually accommodated in microtiter plates. In this format the toxin present in the test portion competes with an enzyme-labelled toxin for antibody binding sites. One of the limiting factors of this format is the fact that structurally related toxins may lead to cross reactivities which may lead to overestimation of the toxin content. However, this cross reactivity can also be utilised to determine the sum of T-2 and HT-2 toxins with a single antibody which shows equal affinity to T-2 and HT-2 toxins (Baumgartner et al., 2010). In general, the results obtained with ELISAs can be associated with a greater measurement uncertainty compared to data produced by chromatographic methods. This is particularly pronounced for the analysis of complex matrices such as foodstuffs and compound feed. The results generated by ELISAs generally provide low LODs (Table 4) and can be generated fast and at relatively low cost.

Competitive ELISA containing a generic antibody against type A trichothecenes was developed as a tool for determination of T-2 and its metabolites in urine of rats and cynomolgus monkey (Lee et al., 1990). This kit was successfully used for assessment of T-2 exposure in both investigated animal species. Recently, some EIA and ELISA kits dedicated for T-2 determination in various commodities have become available on the market (see Table 4). Only a few are suitable, in principle, to determine the sum of T-2 and HT-2 toxins and none of assays has been validated for animal tissue and biological fluids.

LFD tests have been developed as easy-to-use immunochemical techniques for the detection of T-2 toxin (Molinelli et al., 2008). The principle is much the same as ELISA, however in contrast, these assays are qualitative and give a simple yes/no result in relation to the presence of a particular contaminant. In addition, they are extremely rapid to perform and may be used in field conditions. Several commercial companies have exploited this method, and currently multiplex dipstick assays for various *Fusarium* toxins including T-2 and HT-2 toxins are under development.[15]

Biosensors are becoming more popular in many industrial sectors including the food sector. They are composed of a biological (often antibody-antigen) recognition element connected to a transducer or sensing device. Both optical and electrochemical sensor-based methods have recently been developed for the determination of type A trichothecenes in cereals and maize-based baby food.[16] Optical biosensors based on the principle of surface plasmon resonance (SPR) have shown excellent in-house performance characteristics (Meneely et al., 2010, 2011a), and offer possibilities for high throughput analyses. The enzyme-linked-immunomagnetic-electrochemical array (ELIME-array) for T-2 and HT-2 toxin detection in food samples is based on the use of magnetic beads and screen-printed electrodes (Piermarini et al., 2007; Romanazzo et al., 2009). Advantages of electrochemical measurements over that of spectrophotometric ones include the possibility of increased speed, miniaturisation and multiplexing, whereas low cost of instrumentation, the possibility of *in situ* analysis and the insensitivity to turbid samples are other assets of this technique (Ricci et al., 2007).

[15] www.CONffIDENCE.com
[16] http://www.biocop.org/

**Table 4:** Characteristics of immunochemical test kits for T-2 and HT-2 toxins (from Meneely et al. (2011b), modified).

| Analytes | Matrices | Format | Extraction | Antibody cross reactivity | LOD (µg/kg) | Remarks |
|---|---|---|---|---|---|---|
| T-2 | Cereals, silage | EIA | ACN-$H_2O$ (84/16, v/v) | T-2: 100 %<br>Acetyl T-2: 12.3 %<br>HT-2: 3.4 %<br>Iso T-2: 2.5 % | 30-55 | |
| T-2 | Cereals, feed | EIA | MeOH-$H_2O$ (70/30, v/v) | T-2: 100 %<br>Acetyl T-2: 114 %<br>HT-2: 7 %<br>Iso T-2: 2 % | < 5 | |
| T-2 | Maize, maize seedlings, mixed feed | EIA | MeOH-$H_2O$ (70/30, v/v) | T-2: 100 %<br>Acetyl T-2: 114 %<br>HT-2: 7 %<br>Iso T-2: 2 % | < 20 | AOAC performance tested |
| T-2 | Grain, cereals | EIA | Not reported | Not reported | 35 | |
| T-2 | Maize and derived products | ELISA | MeOH-$H_2O$ | T-2: 100 %<br>HT-2: 38 %<br>T-2 Triol: 1.6 % | 25 | |
| T-2 and HT-2 | Barley, maize, oats, rye, soy, wheat | ELISA | MeOH-$H_2O$ (70/30, v/v) | T-2: 100 %<br>HT-2: 100% | not reported; LOQ: 25 | |

T-2: T-2 toxin; HT-2: HT-2 toxin; EIA: enzyme immunoassay: ELISA: enzyme-linked immunosorbent assays; MeOH: methanol; H2O: water; ACN: acetonitrile; v/v: volume/volume; LOD: limit of detection; LOQ: limit of quantification; AOAC: Association of Official Analytical Chemists.

### 3.2.4. Other approaches

Novel methodology for T-2 and HT-2 toxin determination is based on the transcriptional apparatus of a human carcinoma cell line, which provides a sensitive biological sensor of type A trichothecenes (Lancova et al., 2009). The transcriptional responses have been exploited to develop practical DNA microchip assays for the detection of type A trichothecenes at µg/kg level.[16] Transcriptomic methods require more trained personnel and currently substantially more time than rapid tests for type A trichothecenes. A key advantage of this novel test is that it represents an effect-driven bioassay that exploits the regulation of biologically relevant genes in a toxicologically relevant target system (human epithelial cells). In-house validation of the method showed an LOD for T-2 toxin in cereal-based baby food < 10 µg/kg.[16] Further optimisation is needed and it will take several more years to determine whether the transcriptomics test may be suitable for reliable analysis of T-2 and HT-2 toxins.

## 3.3. Analytical quality assurance: performance criteria, reference materials and proficiency testing

In Annex II of the Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs,[17] criteria for methods of analysis are laid down. Performance criteria for methods of analysis of T-2 and HT-2 toxins used in the official control as mentioned in this Regulation are presented in Tables 5 and 6.

[17] OJ L 70, 9.3.2006, pp. 12-34.

**Table 5:** Performance criteria for T-2 toxin.

| Level (µg/kg) | $RSD_r$ (%) | $RSD_R$ (%) | Recovery (%) |
|---|---|---|---|
| 50-250 | ≤ 40 | ≤ 60 | 60 to 130 |
| > 250 | ≤ 30 | ≤ 50 | 60 to 130 |

$RSD_r$: relative standard deviation under repeatability conditions; $RSD_R$ relative standard deviation under reproducibility conditions

**Table 6:** Performance criteria for HT-2 toxin.

| Level (µg/kg) | $RSD_r$ (%) | $RSD_R$ (%) | Recovery (%) |
|---|---|---|---|
| 100-200 | ≤ 40 | ≤ 60 | 60 to 130 |
| > 200 | ≤ 30 | ≤ 50 | 60 to 130 |

$RSD_r$: relative standard deviation under repeatability conditions; $RSD_R$ relative standard deviation under reproducibility conditions

The quality of analytical results regarding accuracy, precision and comparability is essentially linked to the use of reference materials and certified reference materials (CRMs). So far, no CRMs are available for T-2 and/or HT-2 toxin. However, a CRM for T-2 and HT-2 toxin in oat flakes (ERM code: ERM-BC720) is currently being produced by the Federal Institute for Materials Research and Testing (BAM) (Köppen et al., 2011). A few commercial diagnostic companies offer T-2 toxin and HT-2 toxin as non-certified calibrants. T-2 toxin is also available as fully $^{13}C$-labelled standard (Haeubl et al., 2007; Lattanzio et al., 2009). In 2009, a proficiency test to determine T-2 and HT-2 toxins in cereal products was conducted by the EU Reference Laboratory (EU-RL) for Mycotoxins[18] with 29 European National Reference Laboratories (NRLs) for Mycotoxins and one laboratory from a candidate country (Stroka et al., 2009). Out of the 30 laboratories, 21 showed satisfactory analytical results for T-2 toxin and 15 laboratories for HT-2 toxin.

### 3.4. Conclusions

The analytical methods for the determination of T-2 toxin and HT-2 toxin described above have been applied to the analysis of various matrices including cereals, food and feed as well as samples of human and animal origin. Accurate quantification of T-2 and HT-2 toxins is mostly carried out by LC coupled with $MS^n$ often within a multianalyte approach. However, complex matrices and the resulting signal suppression effects as observed particularly in ESI-MS methods owing to matrix effects, may require the careful optimisation of the clean-up, the usage of matrix matched standards, or e.g. the use of isotope labelled internal standards. For rapid screening several immunochemical methods (mostly ELISAs) have become available and are sold as commercial test kits. Whereas these methods work fast, cross reactivity with other trichothecenes can have an impact on their accuracy. None of the chromatographic or immunochemical methods has been formally validated in inter-laboratory validation studies, and there are no certified reference materials available for T-2 and HT-2 toxins.

## 4. Occurrence of T-2 and HT-2 toxins in food and feed

T-2 and HT-2 toxins occur mainly in cereal grains. By far the major sources of human and animal exposure to T-2 and HT-2 toxins are products of plant origin. There is no evidence for an accumulation of these toxins in specific tissues of animals fed with feed contaminated with T-2 and HT-2 toxins, and any significant human exposure from consumption of animal products is unlikely (see also 7.4.7.). The occurrence on T-2 and HT-2 toxins (grain-based) feed often also indicate

[18] European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (EC-JRC-IRMM), http://www.irmm.jrc.be/

potential occurrence in (grain-based) food. The destination of raw cereal grains, whether they are for food or feed use, is often unclear.

## 4.1. Previously reported occurrence data

Data on food and feed reported and discussed in Sections 4.1.1., 4.1.2. and 4.1.3. were obtained largely from the literature published since 2001. Most data on food from before 2001 were summarised earlier by the FAO/WHO (2001) and the SCF (2001). Recently European occurrence data have been reviewed by van der Fels-Klerx and Stratakou (2010). The information presented below reflects examples of contamination of food and feed with T-2 and HT-2 toxins. Some of these data may also have been reported (but not necessarily) in Section 4.2.

### 4.1.1. Occurrence of T-2 and HT-2 toxins in grains

#### 4.1.1.1. T-2 toxin on unprocessed grains

The occurrence of T-2 toxin in wheat, barley and oats in the United Kingdom (UK) from the period of 2001-2005 was reported by Edwards (2009a,b,c). Samples were taken just after harvest. The numbers of samples taken were 1,624 for wheat, 446 for barley and 458 for oats (no samples in 2001). At the level of LOD (10 µg/kg), T-2 toxin was found in 16 % of the wheat samples and 12 % of the barley samples. For both wheat and barley, the mean and median concentrations of all samples were < LOD. In oats, T-2 toxin was detected in 84 % of the samples, with frequently high concentrations. The mean, median and maximum concentrations in oats were 84 µg/kg, 140 µg/kg and 2406 µg/kg, respectively.

Also in the UK, a four-year study (2004-2007) investigated the incidence and concentrations of T-2 toxin in wheat, oats and maize at the intake level of the UK mills. The total number of samples taken was 60 for wheat (all UK), 27 for oats (21 from UK/Ireland and six from Scandinavian countries) and 86 for maize (56 from France and 30 from Argentina) (Scudamore et al., 2009). Of wheat, only 3 out of 60 samples were positive for T-2 toxin (LOD 10 µg/kg), with a maximum level of 13 µg/kg. The T-2 toxin levels in the 21 oat samples cultivated in the UK/Ireland were 20-49 µg/kg (n = 5), 50-499 µg/kg (n = 14), 500-999 µg/kg (n = 1) and 1610 µg/kg (n = 1). The T-2 toxin concentrations in each of the 6 samples from Scandinavia were between 5-499 µg/kg. Of the 56 samples of French maize, T-2 toxin was found in 22 samples, with their levels ranging 10-19 µg/kg (n = 12), 20-49 µg/kg (n = 8) and 50-499 µg/kg (n = 2).

More recent Norwegian data on T-2 toxin in 301 organically and 301 conventionally cultivated barley, oats and wheat samples from 2002-2004 were reported by Bernhoft et al. (2010). Organic and conventional cereal samples were collected from the farms in the same neighbourhood at the same time. In addition, the parallel conventional and the organic sample were the same cereal species and had the same matureness of threshing. Thus organic and conventional samples were collected as comparable pairs, in which climate and soil conditions were nearly the same. For barley, oats and wheat, 108, 101 and 92 sample pairs (conventional – organic), respectively were collected. T-2 toxin was not found in barley and wheat samples, while in oats samples T-2 toxin concentrations were reported to be significantly lower in organic than in conventional oats. The mean and median concentrations were 30 and < 30 µg/kg, respectively in organic oats, while the respective concentrations in conventional oats were 43 and < 30 µg/kg (LOD 30 µg/kg).

In Lithuania 36 organically cultivated different cereal cultivars were sampled in 2005-2006 and analysed for the presence of T-2 toxin (Suproniene et al., 2010). The cereal cultivars comprised winter and spring barley (n = 11) and wheat (n = 13), oats (n = 5) and winter triticale (n = 2). For the spring cereals, the mean concentrations were 17.3 µg/kg (2005) and 18.1 µg/kg (2006), and the maximum values 45.9 µg/kg (2005) and 50.2 µg/kg (2006). For the winter cereals the mean was 7.9 µg/kg

(2005) and 11.1 µg/kg (2006), and the maximum values 9.3 µg/kg (2005) and 23.6 µg/kg (2006) (LOD 7.5 µg/kg).

In the Lithuanian study carried out one year later, 60 winter and 65 spring cereal samples were collected from 2006 and 2007 harvests (Mankevičienė et al., 2011). T-2 toxin was present in 56 % of the 2006 winter cereal (wheat, rye and triticale) samples (n = 32) and the concentrations were in the range of 7.5 (LOD)-14.2 µg/kg. All the spring cereal samples (wheat and barley) from the year 2006 (n = 32) were contaminated with T-2 toxin, with concentrations ranging from 8.4 to 133.2 µg/kg. The winter wheat and rye samples (n = 28) from the 2007 harvest had 93 % of T-2 toxin positive samples. The concentration range was 7.5 (LOD)-20.2 µg/kg. Like in 2006 also in 2007 all the spring wheat and barley samples (n = 33) were contaminated with T-2 toxin. The levels were 17.0-102.8 µg/kg of T-2 toxin. It was concluded that the spring barley samples contained higher T-2 toxins levels irrespective of the year.

In the recent study of Schwake-Anduschus et al. (2010) T-2 toxin was determined in oats cultivated in Germany in 2007 (n = 8). The T-2 toxin concentrations varied from 14 to 214 µg/kg (LOD $\leq$ 3 µg/kg).

Malachova et al. (2010) reported that T-2 toxin was detected in 50 % of the different barley cultivars (n = 148) harvested in 2005-2008 in the Czech Republic. The mean concentration was 30 µg T-2 toxin/kg (LOQ 5 µg/kg). In Serbia, 54 wheat samples were collected from the harvest of 2007. The T-2 toxin concentrations in all the samples were < LOD of 0.3 µg/kg (Škrbić et al., 2011).

In the recent Spanish study, 44 barley samples were collected from the 2007 harvest for the analysis of T-2 toxin (Ibáñez-Vea et al., 2011). Of all the samples collected 11 % were between LOD (0.4 µg/kg) and LOQ (20 µg/kg). The reported maximum concentration 22.6 µg/kg was only slightly higher than the LOQ.

Pettersson et al. (2011) reported the results of a survey of T-2 toxin in oat and oat products from European oat mills in 2005-2009. Eleven oat mills from the UK (6), Germany (2), Finland (1), Poland (1) and Ireland (1) represented by CEEREAL (European Breakfast Cereal Association), participated in the study. A total of 243 raw oat samples, 529 oat flakes samples and 105 oat meal samples were taken for analysis. In most samples, T-2 toxin and HT-2 toxin occurred together and the incidence of T-2 toxin (LOD 5 µg/kg) was 73 % for raw oats (mean concentration 32 µg/kg), 24 % for oat flakes (mean concentration 5 µg/kg) and 17 % for oat meal (mean concentration 4 µg/kg).

#### 4.1.1.2. HT-2 toxin on unprocessed grains

In 2001-2005, the occurrence of HT-2 toxin in wheat, barley and oats in the UK was studied (Edwards 2009a,b,c). HT-2 toxin was detected in 31 and 36 % of the wheat and barley samples, respectively (LOD 10 µg/kg). The concentrations were usually low, with mean and median concentrations of all samples $\leq$ 10 µg/kg. For oats, 92 % of the samples contained HT-2 toxin (LOD 10 µg/kg). The HT-2 toxin concentrations were frequently high. The mean and median concentrations of all the samples were 430 and 151 µg/kg, respectively (the maximum concentration 7584 µg/kg).

A four-year study by Scudamore et al. (2009) on HT-2 toxin levels in wheat, oats and maize at the intake level of the mills in the UK, did not find HT-2 toxin in 48 out of 60 wheat samples (LOD 10 µg/kg). Ten of the resulting 12 samples contained HT-2 toxin in the range of 10-19 µg/kg, and the two remaining samples 20 and 49 µg/kg of HT-2 toxin. Of the 21 samples of oats collected in UK/Ireland, 14 samples contained 50-499 µg/kg of HT-2 toxin and four samples 500-999 µg/kg of HT-2 toxin (maximum concentration 3570 µg/kg). For the six oat samples from Scandinavian countries, the HT-2 toxin levels were 50-499 µg/kg for four samples and 500-999 µg/kg for two samples (maximum concentration 730 µg/kg). HT-2 toxin was not found in 22 out of the 56 maize

samples from France. The concentrations of HT-2 toxin in the resulting 34 positive samples were 10-19 µg/kg (n = 15), 20-49 µg/kg (n = 14) and 50-499 µg/kg (n = 5).

Recently in Norway, a total of 301 organically and 301 conventionally cultivated barley, oats and wheat samples collected in 2002-2004 were analysed for HT-2 toxin (Bernhoft et al., 2010). Organic and conventional cereal samples were collected from the farms in the same neighbourhood at the same time. In addition, the parallel conventional and the organic sample were the same cereal species and had the same matureness of threshing. Thus organic and conventional samples were collected as comparable pairs, in which climate and soil conditions were close to identical. For barley, oats and wheat, 108, 101 and 92 sample pairs (conventional – organic), respectively were collected. HT-2 toxin was found in barley and oats samples but not in wheat. For oats and barley HT-2 toxin concentrations were reported to be significantly lower in organic than in conventional oats and barely samples. The mean and median concentrations were 80 and < 20 µg/kg, respectively in organic oats, and < 20 and < 20 µg/kg, respectively in organic barley. For conventional oats, the mean and median concentrations were 117 and 62 µg/kg, respectively and for conventional barley 21 and < 20 µg/kg, respectively (LOD 20 µg/kg).

In the study of Schwake-Anduschus et al. (2010) HT-2 toxin was determined in oats cultivated in Germany in 2007 (n = 8). The concentration was < LOD in one sample while in the other samples the concentrations varied from 81 to 758 µg/kg (LOD ≤ 5 µg/kg).

In the Polish study from 1997, HT-2 toxin was found in 24 % of the 99 oat samples tested, with a mean level of 21 µg/kg (range 10-47 µg/kg, LOD 10 µg/kg) (Perkowski and Basiński, 2002). In a later Polish study (Perkowski et al., 2007), HT-2 toxin was found in seven of the 32 wheat samples from 2003 (LOD 4 µg/kg). One of the 12 organic wheat samples was positive for HT-2 toxin while 6 out of the 20 conventional wheat samples were positive. HT-2 toxin concentrations in the seven positive samples were low (4-66 µg/kg).

Malachova et al. (2010) reported that HT-2 toxin was detected in 62 % of the different barley cultivars (n = 148) harvested in 2005-2008 in Czech Republic. The mean and maximum levels were 110 and 716 µg/kg, respectively (LOQ 10 µg/kg). In Serbia 54 wheat samples were collected from the harvest of 2007 (Škrbić et al., 2011). The incidence of 6 % for HT-2 toxins in the total number of the samples and the concentrations of 128-129 µg/kg in the 3 positive samples were reported.

In total 44 barley samples collected from the 2007 harvest were analysed for HT-2 toxin in the recent Spanish study of Ibáñez-Vea et al. (2011). Of all the samples collected 23 % were positive for HT-2 toxin having estimated mean concentration of 7.8 µg/kg between LOD (2.0 µg/kg) and LOQ (20 µg/kg). The reported maximum concentration (16.4 µg/kg) was lower than the LOQ.

Pettersson et al. (2011) reported the results of a survey of HT-2 toxin in oat and oat products from European oat mills in 2005-2009. Eleven oat mills from the UK (6), Germany (2), Finland (1), Poland (1) and Ireland (1) represented by CEEREAL (European Breakfast Cereal Association), participated in the study. A total of 243 raw oat samples, 529 oat flakes samples and 105 oat meal samples were taken for analysis. In most samples T-2 and HT-2 toxin occurred together and the incidence of HT-2 toxin (LOD 5 µg/kg) was 91 % for raw oats (mean concentration 62 µg/kg), 68 % for oat flakes (mean concentration 12 µg/kg) and 34 % for oat meal (mean concentration 7 µg/kg).

### 4.1.2. Occurrence of T-2 and HT-2 toxins in food

#### 4.1.2.1. T-2 toxin in food products

In Germany, a total of 289 samples of wheat products (n = 130), rye products (n = 61) and oat products (n = 98) were collected from grain-milling factories and wholesale places in Bavaria

(Gottschalk et al., 2009). The collected food products included kernels, flour, semolina, bran and flakes. A small number of these samples (n = 18) were oat-based (n = 13) or wheat-based (n = 5) infant foods. Of the 98 oat product samples, 17 were of feed quality (not differentiated in the results). All the samples were German origin from the 2005 and 2006 harvests (Gottschalk et al., 2009). The grain samples were fully processed (including cleaning and de-hulling steps) and suitable for direct human consumption. In total 85 % of all wheat product samples (n = 130), 87 % of all rye product samples (n = 61) and 100 % of all oat product samples (n = 98) contained T-2 toxin (LOD < 0.7 µg/kg). Median concentrations were 0.11 µg/kg, 0.09 µg/kg, and 2.2 µg/kg in wheat, rye and oat product samples, respectively. The highest concentrations were reported for wheat bran (1.9 µg/kg), whole rye flour (0.8 µg/kg) and fine oat flakes (34 µg/kg). Earlier, Gottschalk et al. (2007) reported results for 70 oat samples intended for human consumption (35 from conventional and 35 from organic farming) collected at mills and at wholesale stage from the Bavarian market. The samples were German origin from the 2005 harvest and they were cleaned and de-hulled. Nine samples were oat grains, 43 oat flakes, 11 oat bran and seven oat-containing infant foods. Separate T-2 toxin concentrations were only given for the oat flakes which was the highest contaminated product. The incidence of T-2 toxin in these oat flake samples was 100 %, with the mean concentration of 6.4 µg/kg and the maximum concentration of 34 µg/kg of T-2 toxin.

In a four-year study in the UK, T-2 toxin was not found in the batches of retail products of wheat and maize (n = 186) (LOQ 10 µg/kg) (Scudamore et al., 2009). In oats (n = 27), T-2 toxin was most often found in the hulls. However, the T-2 toxin concentrations in oat flakes produced from the groats after removal of the hulls were usually < 65 µg/kg. Of all the oat samples, 16 oat flake samples were negative for T-2 toxin, eight oat flake samples contained T-2 toxin at the levels of 10-19 µg/kg, two samples 20-49 µg/kg and one sample 50-499 µg/kg.

T-2 toxin was analysed in 75 wheat-based bread and 75 pasta samples collected from the Spanish market (Gonzáles-Osnaya et al., 2011). The incidence of T-2 toxin in the bread samples was 2.6 % and in pasta samples 9.3 %, and the T-2 toxin concentrations varied between < 4.9 µg/kg (LOD) and 67.9 µg/kg for bread samples and between < 4.7 µg/kg (LOD) and 259.6 µg/kg for pasta samples (Gonzáles-Osnaya et al., 2011). In another Spanish study, over 470 food samples comprising corn flakes (n = 168), wheat flakes (n = 27), maize snacks (n = 213), pasta (n = 201), sliced bread (n = 147), bread (n = 31), sweet corn (n = 185) and beer (n = 213) from the Catalonian market were collected for the analysis of T-2 toxin in June-July 2008 (Cano-Sancho et al., 2011). Three items of each product (if available) were collected in each supermarket and pooled in order to have 72 composite samples per food group. Finally 65 composite samples of corn flakes were analysed for T-2 toxin, 27 of wheat flakes, 71 of maize snacks , 70 of pasta, 72 of sliced bread, 31 of bread, 72 of sweet corn and 71 of beer. Only 5 samples of all the composite samples for different food groups showed positive results for T-2 toxin. The maximum concentrations found were 75 µg/kg in wheat flakes, 70 µg/kg in maize snacks and 256 µg/kg in sweet corn. The LOQs varied from 42 µg/kg for wheat flakes to 135 µg/kg for sweet corn (Cano-Sancho et al., 2011).

Biselli and Hummert (2005) reported T-2 toxin results for 685 food samples of European origin including different types of food products. In nearly 40 % of the samples, T-2 toxin was detected > 0.2 µg/kg (LOD). The highest concentrations were found in maize (mean concentration 0.8 µg/kg and maximum concentration 8.4 µg/kg) and in oats or oat-based products (mean concentration 34 µg/kg and maximum concentration 266 µg/kg).

A total of 3,490 samples were collected from the eight European countries in the project of Scientific Cooperation (SCOOP) Task 3.2.10 on the occurrence of *Fusarium* toxins in foods in Europe (SCOOP, 2003).[19] Of all the samples, 20 % were positive for T-2 toxin (Schothorst and van Egmond, 2004). Reported LODs for the various food products varied significantly between reporting countries.

[19] http://ec.europa.eu/food/fs/scoop/task3210.pdf

For wheat and wheat flour samples (n = 1,417) from seven different countries (Denmark, Finland, France, Italy, Norway, Portugal and the UK), 21 % of the samples were positive for T-2 toxin (concentration range 2-160 µg/kg). The mean T-2 toxin concentrations (mean of all samples) across the countries varied between 1.7 µg/kg (UK) and 90 µg/kg (Denmark). The weighed overall mean was 15 µg/kg and the weighed mean of the positive samples was 28 µg/kg. For barley collected from Finland, France, Italy and the UK (n = 502), 3 % of the samples were positive for T-2 toxin (concentration range 1.7-280 µg/kg). The mean concentrations across the countries varied from 0.8 µg/kg (UK) to 280 µg/kg (Italy). Of the oat samples from Austria, Finland and Norway (n = 464), 16 % were contaminated with T-2 toxin (concentration range 10-550 µg/kg). The mean concentrations across the reporting countries ranged between 4.2 µg/kg (Finland) and 68 µg/kg (Austria). For T-2 toxin in rye and rye flour, 21 % of the 62 samples from Denmark, Finland and Norway gave positive results (concentration range 10-193 µg/kg). The incidence of T-2 toxin in maize was 28 % in the samples from Austria, France and Italy (n = 293), with the concentration ranging from 3 µg/kg (France) to 255 µg/kg (Austria).

#### 4.1.2.2. HT-2 toxin in food products

In a German study (Gottschalk et al., 2009), the food products from the 2005 and 2006 harvests had the HT-2 toxin incidence of 94 % in all wheat product samples (n = 130). The highest HT-2 toxin concentration of 22 µg/kg was in wheat bran. Of all the rye product samples (n = 61), 93 % contained HT-2 toxin, with the highest concentration reported for rye flour (2.6 µg/kg). Of the 98 oat samples, 99 % were contaminated with HT-2 toxin. The highest concentration of 51 µg/kg was found in fine oat flakes. In their earlier study, Gottschalk et al. (2007) reported that the HT-2 toxin incidence was 100 % for the oat flakes (n = 43; 25 conventional, 18 organic) from the 2005 harvest, with a mean concentration of 14 µg/kg and a maximum concentration of 51 µg/kg.

In the four-year study of Scudamore et al. (2009), HT-2 toxin concentrations in the retail food products in the UK were reported. Of the 146 wheat and maize product samples, only one snack sample contained HT-2 toxin (12 µg/kg). In oat flakes, 22 of the 27 samples were positive for HT-2 toxin. Nine samples contained 10-19 µg/kg, 12 samples 20-49 µg/kg and one sample 50-499 µg/kg of HT-2 toxin (LOD 5 µg/kg for all products).

In Spain, over 470 food samples comprising corn flakes (n = 168), wheat flakes (n = 27), maize snacks (n = 213), pasta (n = 201), sliced bread (n = 147), bread (n = 31), sweet corn (n = 185) and beer (n = 213) from the Catalonian market were collected in June-July 2008 (Cano-Sancho et al., 2011). Three items of each product (if available) were collected in each supermarket and pooled in order to have 72 composite samples per food group. Finally 65 composite samples of corn flakes were analysed for HT-2 toxin, 27 of wheat flakes, 71 of maize snacks, 70 of pasta, 72 of sliced bread, 31 of bread, 72 of sweet corn and 71 of beer. For sliced bread, 11 composite samples contained HT-2 toxin with the maximum level of 75 µg/kg while for other food groups the number of HT-2 toxin positive samples were from 0 (bread and beer) to 7 (pasta). The highest concentrations were reported for maize snack with the mean value of 214 µg/kg, the median 78 µg/kg and the maximum 895 µg/kg, and for wheat flakes with the mean value of 87 µg/kg, the median 61 µg/kg and the maximum 183 µg/kg. For the remaining food products contaminated with HT-2 toxin, the mean concentrations were 41-51 µg/kg and the maximum 65-84 µg/kg. The LOQs varied from 9 µg/kg for beer to 61 µg/kg for maize snacks (Cano-Sancho et al., 2011).

A total of 3,032 food samples were obtained from six European countries (Denmark, Finland, Norway, Austria, France and the UK) in the SCOOP Task 3.2.10 project on the occurrence of *Fusarium* toxins in Europe (SCOOP, 2003). The results showed that 14 % of all the samples were contaminated with HT-2 toxin (Schothorst and Van Egmond, 2004). Reported LODs for the various food products varied significantly between reporting countries. Of the 1,213 wheat samples from Denmark, Finland, France, Norway and the UK 12 % contained HT-2 toxin (concentration range 3.3-50 µg/kg). Of the 501 barley samples from Finland, France and the UK only 5 % were positive for

HT-2 toxin (concentration range 1.7-287 µg/kg), while for the 464 oat samples from Austria, Finland and Norway the incidence of positive samples was 41 % (concentration range 10-1150 µg/kg). An HT-2 toxin incidence of 17 % was reported for the 63 rye and rye flour samples from Denmark, Finland and Norway (concentration range 10-70 µg/kg). The results for maize samples (n = 261) from France and Austria showed that the HT-2 toxin incidence was 24 % (concentrations 120 µg/kg (Austria) and 3 µg/kg (France)).

### 4.1.3. Occurrence of T-2 and HT-2 toxins in feed

#### 4.1.3.1. The occurrence of T-2 and HT-2 toxins in feedingstuffs

As reported above, T-2 and HT-2 toxins occur predominantly in cereal crops. Cereal grains are widely used as feeds for livestock in the EU, and almost all (> 95 %) are grown in the EU. According to data published by FEFAC (2009), more than 71 million tonnes of cereals and cereal co-products were used in manufactured compound feeds, accounting for 48 % of all feed materials used. The other major categories of feeds used are oilseed cakes and meals (28 %) and co-products from the food and feed industries (12 %).

In addition to incorporation in compound feeds, cereals are frequently used in on-farm mixes or as single ingredients, particularly to supplement forages for ruminant livestock. Therefore, the total amount of cereal grains fed to livestock will be considerably greater than that reported for compound feed production. However, there are no data on the total amount of cereals used as feed, either by type (wheat, barley etc) or by livestock species (cattle, pigs, poultry etc).

For ruminant livestock (cattle, sheep, goats), forages are usually the major or sole feed consumed, and may be fed either in their fresh state or following ensiling. Almost all of the maize (*Zea mais*) grown for livestock feeding is ensiled, while the practice of ensiling whole-crop cereals is increasing. The maize is normally harvested when the dry matter of the plant is 25-40 %, while whole-crop cereals are normally harvested when the grain is at the 'cheesy-dough' stage, and the dry matter content of the whole crop above harvest height is 36-40 %. Although there are reports of T-2 toxins being found in silages (Eckard et al., 2011), the effects of ensiling on the survival of T-2 toxin and HT-2 toxin in forages is poorly described (Binder et al., 2007). A study by Fuchs et al. (2003) showed that while none of the T-2 toxin in a maize crop survived ensiling, about half of the HT-2 toxin in the crop was detected in the silage.

#### 4.1.3.2. T-2 toxin in feed products

T-2 toxin was reported to concentrate to a level of > 100 µg/kg in oat by-products resulting from the de-hulling process of oats (Scudamore et al., 2009). Only one sample out of the 27 was < 100 µg/kg, while two samples contained T-2 toxin with concentrations > 1000 µg/kg with one sample up to 6120 µg/kg.

Garaleviciene et al. (2002) reported the incidences and concentrations of T-2 toxin in Lithuanian cereals intended for animal consumption (n = 40) and in mixed feeds for swine and poultry (n = 52). The cereal samples included 23 winter wheat, 12 summer barley and 5 oat samples and they were collected shortly after harvest in July - September 1999 from the local factory. T-2 toxin was found in three oat samples, but not in the wheat and barley samples (LOD 50 µg/kg). The mean concentration of the three positive samples was 526 µg/kg (maximum concentration 1454 µg/kg). Of all the mixed feed samples, 17 % were contaminated with T-2 toxin with a mean concentration of 598 µg/kg (maximum concentration 3852 µg/kg). The concentrations found in mixed feed for pigs were higher than in mixed feed for poultry.

Binder et al. (2007) reported T-2 toxin incidences and concentrations in cereals (5 barley, 83 wheat, 26 oats and 18 maize samples) intended for feed production and in finished feed (n = 54) in Europe. The samples were taken directly at farms and feed production factories between October 2003 and December 2005. The samples were grouped based on the region of origin, i.e. Northern, Central, and Southern Europe. Of the samples from Northern Europe, 40 % were contaminated with T-2 toxin (maximum concentration 1776 µg/kg and median concentration 102 µg/kg). Samples from Finland had a high incidence and high levels of T-2 toxin. The Central European samples had T-2 toxin in 24 % of the samples, with a median concentration of 112 µg/kg. Of the samples collected from Southern European and the Mediterranean region (n = 123) 19 % contained T-2 toxin. The concentrations found were low < 60 µg/kg (median concentration 38 µg/kg). Overall Binder et al. (2007) reported for all the samples collected in Europe that 1 out of 18 maize samples was positive for T-2 toxin, 18 out of 83 wheat samples were positive (mean concentration 187 µg/kg), 1 out of 5 barley samples was positive and 21 out of 26 oat samples were positive (mean concentration 418 µg/kg). Seven out of all 54 finished feed samples contained T-2 toxin with the mean concentration of 219 µg/kg.

In total 62 samples of commercial horse feed preparations including cereal mixtures and grains (maize, oats and barley) were collected from the German market (Liesener et al., 2010). All samples contained T-2 toxin in the range of 0.3-91 µg/kg (median 7 µg/kg) (LOD 0.1 µg/kg).

In the recent study of Monbaliu et al. (2010) three different feed materials (maize, wheat and sow feed) were collected in 2008 and analysed for the presence of T-2 toxin (n = 82). In total 29 wheat samples were collected from the Czech Republic (n = 8), Denmark (n = 14) and Hungary (n = 7). A total of 34 maize samples were obtained from Czech Republic (n = 8), Spain (n = 14) and Portugal (n = 12). Four saw feed samples, one wheat sample and 14 maize samples were obtained in Belgium during the monitoring program. Out of all the 82 feed samples only 7 samples contained T-2 toxin, with a mean level of 28.9 µg/kg (minimum 10 and maximum 112 µg/kg) (LOD or LOQ not specified).

Driehuis et al. (2008) examined 140 maize silages, 120 grass silages and 30 wheat silages produced in the Netherlands between 2002 and 2004, and reported that none of the silages contained T-2 toxin. Similar negative results for T-2 toxin in maize silage (n = 5) were reported by Schollenberger et al. (2006).

#### 4.1.3.3. HT-2 toxin in feed products

HT-2 toxin was reported to concentrate to a level of > 100 µg/kg in oat by-products resulting from the de-hulling process of oats (Scudamore et al., 2009). Only one of the 27 samples investigated contained < 100 µg/kg, eight samples contained 100-999 µg/kg and 15 samples contained 1000-4999 µg/kg. In three samples the HT-2 toxin level exceeded 5000 µg/kg (maximum concentration 23580 µg/kg).

In a Lithuanian study of Garaleviciene et al. (2002), HT-2 toxin was detected in summer barley (n = 12) and oats (n = 5) but not in winter wheat (n = 23), all intended for animal consumption. Of the barley samples, 83 % were positive for HT-2 toxin. The mean concentration of the positive samples was 19 µg/kg (maximum concentration 54 µg/kg). All the oat samples were positive for HT-2 toxin, with a mean concentration of 66 µg/kg (maximum concentration 146 µg/kg). Of the 52 mixed feed samples for poultry and pigs, 13 % (7 samples) contained HT-2 toxin. The mean concentration for all the positive samples was 98 µg/kg (maximum concentration 126 µg/kg). HT-2 toxin was found in one out of 25 mixed feed samples for pigs (126 µg/kg) and in six out of 27 samples of mixed feed for poultry (mean concentration 70 µg/kg).

In the study of Monbaliu et al. (2010) three different feed materials (maize, wheat and sow feed) were collected in 2008 and analysed for the presence of HT-2 toxin (n = 82). In total 29 wheat samples

were collected from the Czech Republic (n = 8), Denmark (n = 14) and Hungary (n = 7). A total of 34 maize samples were obtained from Czech Republic (n = 8), Spain (n = 14) and Portugal (n = 12). Four saw feed samples, one wheat sample and 14 maize samples were obtained in Belgium during the monitoring program. Out of all the 82 feed samples only 7 samples contained HT-2 toxin at the mean level of 47 µg/kg (minimum 22 and maximum 116 µg/kg) (LOD or LOQ not specified).

Driehuis et al. (2008) examined 140 maize silages, 120 grass silages and 30 wheat silages produced in the Netherlands between 2002 and 2004, and reported that none of the silages contained HT-2 toxin. Schollenberger et al. (2006) however reported HT-2 in five samples of maize silage investigated (maximum concentration 26 µg/kg).

### 4.1.4. Co-occurrence of T-2 and HT-2 toxins

A relationship between T-2 and HT-2 toxins has been investigated in several studies and in general it has been found to be strong. This relationship is expected because T-2 toxin is metabolised to HT-2 toxin.

In 1999, Langseth and Rundberget (1999) reported for Norwegian barley, oats and wheat a strong correlation between the concentration of T-2 toxin and HT-2 toxin, being 0.73 for samples containing T-2 toxin. Also Gottschalk et al. (2009) reported T-2 toxin and HT-2 toxin concentrations being highly correlated in wheat, rye and oat samples ($r^2$-values $\geq$ 0.76). Comparable results were reported in the UK for oats, the relationship between T-2 and HT-2 toxins was found to be highly significant ($p < 0.001$) (Edwards, 2009a) but for barley and wheat only a weak relationship between T-2 and HT-2 toxins was found (Edwards, 2009b,c). This contradictory finding was possibly due to a much higher incidence and concentrations of T-2 toxin and HT-2 toxin in oats than in barley and wheat (Edwards, 2009c).

The concentration ratios calculated between the concentration of HT-2 toxin and the concentration of T-2 toxin in different cereals are variable (approximately from 2 to 7) (Langseth and Rundberget, 1999; Scudamore et al., 2007; Gottschalk et al., 2009, Schwake-Anduschus et al., 2010). Langseth and Rundberget (1999) reported that for Norwegian barley, oats and wheat samples, the concentration of T-2 toxin was on average 57 % (range 10-200 %) of the concentration of HT-2 toxin. A much higher concentration difference was reported by Gottschalk et al. (2009) for wheat, the HT-2 toxin concentrations were approximately seven times higher than the T-2 toxin concentrations. For oats the HT-2 toxin concentrations were almost twice as high as the T-2 toxin concentrations. The concentrations in rye were too low for making the comparison (Gottschalk et al. 2009). An average ratio of 3.5 (median 3.5 and standard deviation 23 %) for the concentration ratios of HT-2 toxin to T-2 toxin in raw oat (n = 12) was reported by Scudamore et al. (2007). Comparable results for oats were reported by Schwake-Anduschus et al. (2010).

No relationship between the T-2 toxin and HT-2 toxin was found in a two-year survey on beers produced in Europe (Cantrell, 2008). Only a weak correlation between T-2 toxin and HT-2 toxin in samples contaminated with > 20 µg/kg (the sum of T-2 and HT-2 toxins, n = 43) was observed in the German food market survey (Usleber, 2008).

For horse feed preparations including cereal mixtures and grains (maize, oats and barley), the overall ratio of the concentration of T-2 toxin and that of HT-2 toxin was reported to be approximately 1:2.2. However, this ratio had a high variability (Liesener et al., 2010).

## 4.2. Current occurrence results in food and feed

### 4.2.1. Data collection summary

The Dietary and Chemical Monitoring Unit (DCM) (former Data Collection and Exposure Unit, DATEX) call for data on T-2 and HT-2 toxins in food and feed[20] was launched in July 2010. European national food authorities and similar bodies, research institutions, academia, food and feed business operators and any other stakeholders were invited to submit analytical data on T-2 and HT-2 toxins in food and feed by November 2010. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a).

Data were received from national food authorities or similar bodies, research institutions and associations of food and feed business operators. They covered food, feed but also unprocessed grains of undefined end-use. Data reported were on samples collected from 2001 to 2010 with the vast majority (95 %) collected after 2004. As the LODs of the analytical methods for T-2 and HT-2 toxins have become lower within the last 5 years and older results may not reflect the current concentrations of T-2 and HT-2 toxins, only data on samples collected from 2005 onwards were used in this assessment. The year 2010 was not a complete sampling year, as the closing date of the call for data on T-2 and HT-2 toxins was November 2010. Data on samples collected before 2005 are stored in the EFSA database and might be used in the future for other evaluations.

Analytical results were reported for the individual T-2 and HT-2 toxins or as the sum of the two. In total 17,683 results for T-2 toxin and 16,536 for HT-2 toxin were reported from 2005 to 2010 for food, feed and unprocessed grains. The sum of concentrations of T-2 and HT-2 toxins was calculated for each sample where information was available (n = 16,463), and together with the 4,056 data reported as the sum of the two toxins, a set of 20,519 observations was available as sum.[21] Based on the classification provided and other information available, separate data sets were extracted for each of the three categories: food, feed and unprocessed grains. The distribution of data in the three categories is detailed in Figure 2. Data were obtained on samples collected in 22 European countries (Figure 3). For a limited data set, the only information on the country of sampling was that the country was a member of the European Union (Figure 3). It should be noted that the sampling country is not necessarily the same as the country that submitted the data to EFSA, nor the country of origin.

To ensure the quality of data included in the assessment, several data cleaning and validation steps were applied. Analytical results with incomplete or incorrect description of the relevant variables (e.g. parameter type, food classification, result value, LOD or LOQ) were not included in the data sets used in this assessment. The data sets were checked for duplicates (same samples transmitted twice or repeated analysis of the same sample) and all duplicates were excluded.

[20] http://www.efsa.europa.eu/en/dataclosed/call/datex100729.htm
[21] In this opinion the 'sum of T-2 and HT-2 toxins' means the sum of concentrations of T-2 and HT-2 toxins.

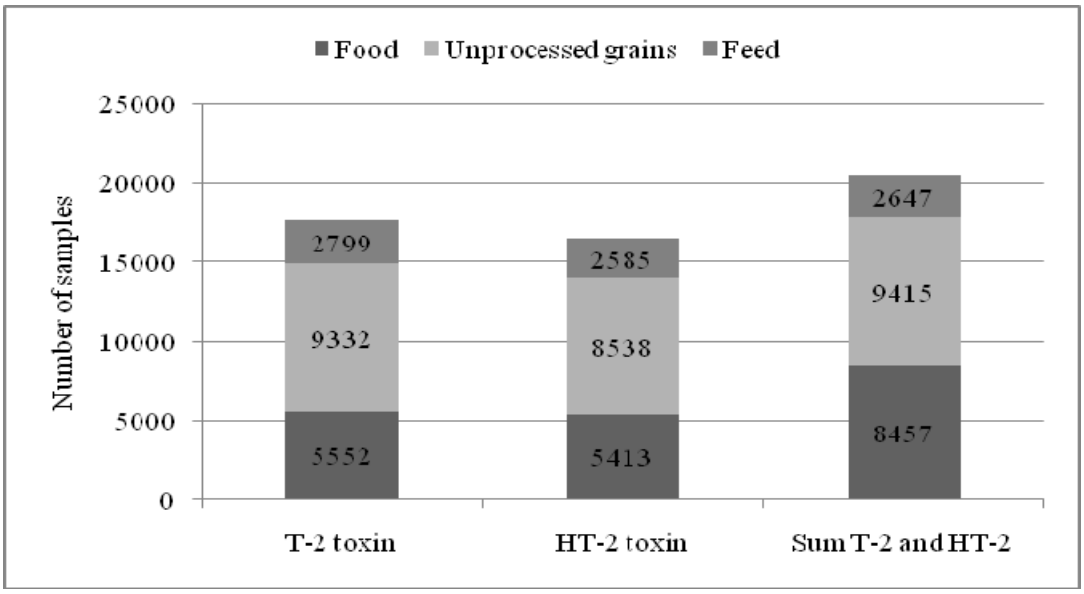


**Figure 2:** Number of analytical results for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins in food, unprocessed grains and feed collected from 2005 onwards. Number of results on the sum of the two toxins refers to results reported as a sum or a sum calculated by matching the results of the individual toxins reported for the same samples.

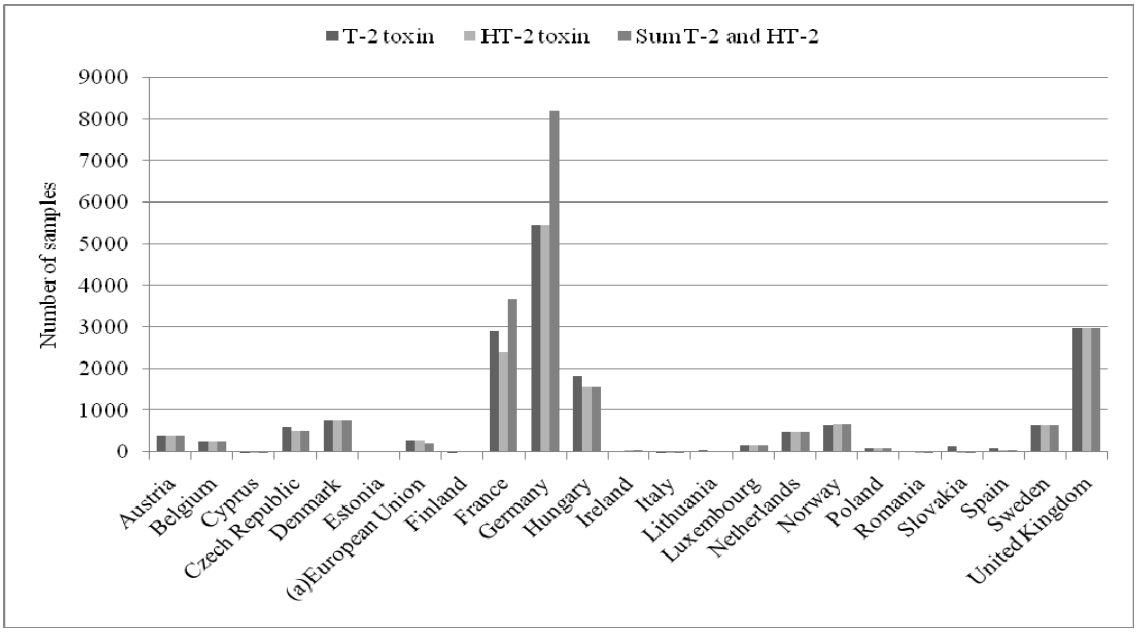


**Figure 3:** Distribution of results for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins collected from the European countries. Results were reported for food, unprocessed grains and feed. (a): Name of the EU Member State was not available.

### 4.2.2. Data collection on food

In a preliminary evaluation it was noted that the detection capabilities of the methods used for the determination of the T-2 and HT-2 toxins were not always optimal, resulting in high LODs and LOQs (up to 100 µg/kg for HT-2 toxin and 450 µg/kg for T-2 toxin). This resulted in a large number of left-censored results (results below LOD or LOQ). It was also noted that those data would bias the outcome of the assessment. Therefore, only results obtained by methods with a sum of the LOQs for T-2 and HT-2 toxins ≤ 20 µg/kg were included in the assessment. By this approach, about 20 % of the data were not included in the data set to be used for occurrence analysis and dietary exposure assessment. The final food data set included 4,458 results for T-2 toxin, 4,204 for HT-2 toxin and 7,139 for the sum of the two toxins. The number of results for the sum of T-2 and HT-2 toxins is higher than the number of results obtained by combining the individual toxins because in 2,935 instances results were reported as a sum of T-2 and HT-2 toxins.

Data on food were obtained on samples collected in 15 European countries. The distribution of occurrence data across the European countries where food samples were collected is illustrated in Figure 4. The distribution of occurrence data over the sampling years is presented in Figure 5. A higher number of samples was available from the period 2006 to 2009. The number of results for 2005 and 2010 was limited because only two European countries reported data for 2005, and 2010 was not a complete sampling year.

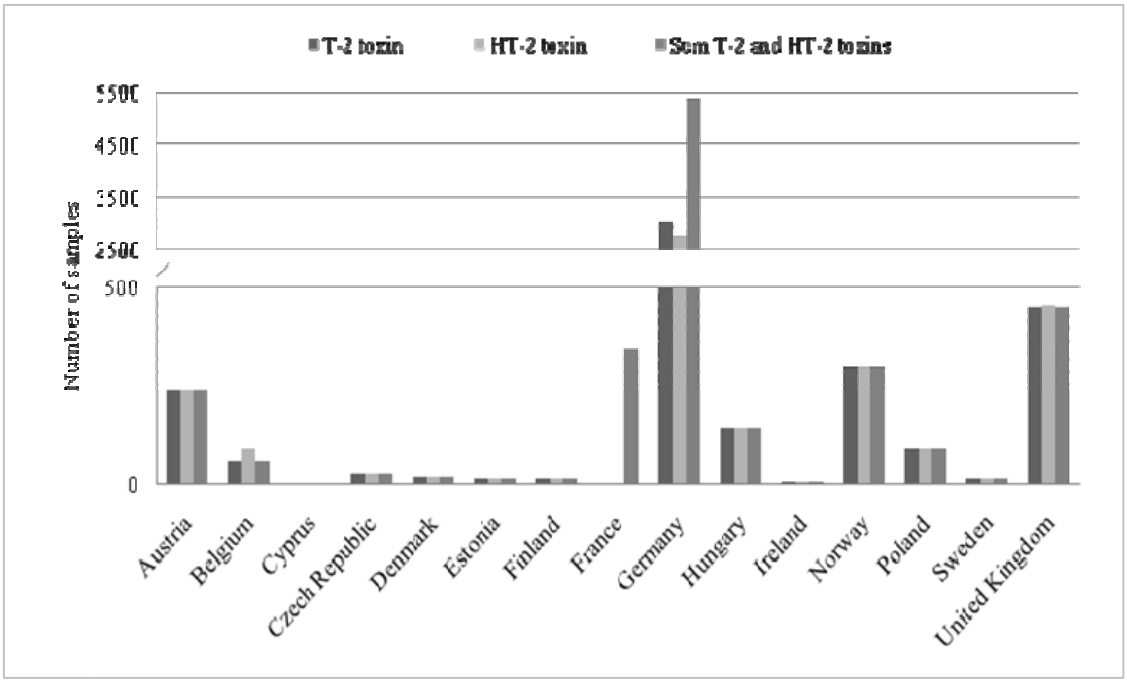


**Figure 4:** Distribution of food samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across the European countries (after excluding non-qualifying data).

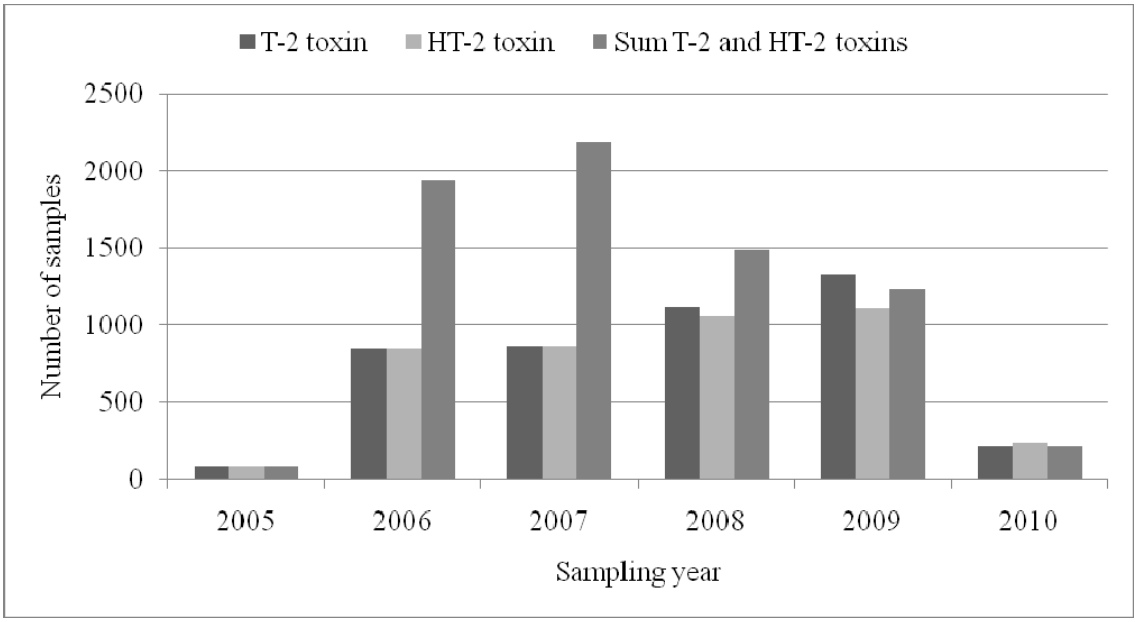


**Figure 5:** Distribution of food samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins over the sampling years (after excluding non-qualifying data).

### 4.2.3. Data collection on feed

From the data collected on feed (see Section 4.2.1.) only 97 observations were excluded because of the high LOQs (above 100 µg/kg for the sum of LOQs of T-2 toxin and HT-2 toxin). Most of the data on feed were reported by Hungary (54 %) and Denmark (26 %) (Figure 6). The distribution of occurrence data over the sampling years is presented in Figure 7. A higher number of samples was available from the period 2006 to 2009. The number of results for 2005 and 2010 was limited because only three European countries reported data for 2005 and 2006, and 2010 was not a complete sampling year.

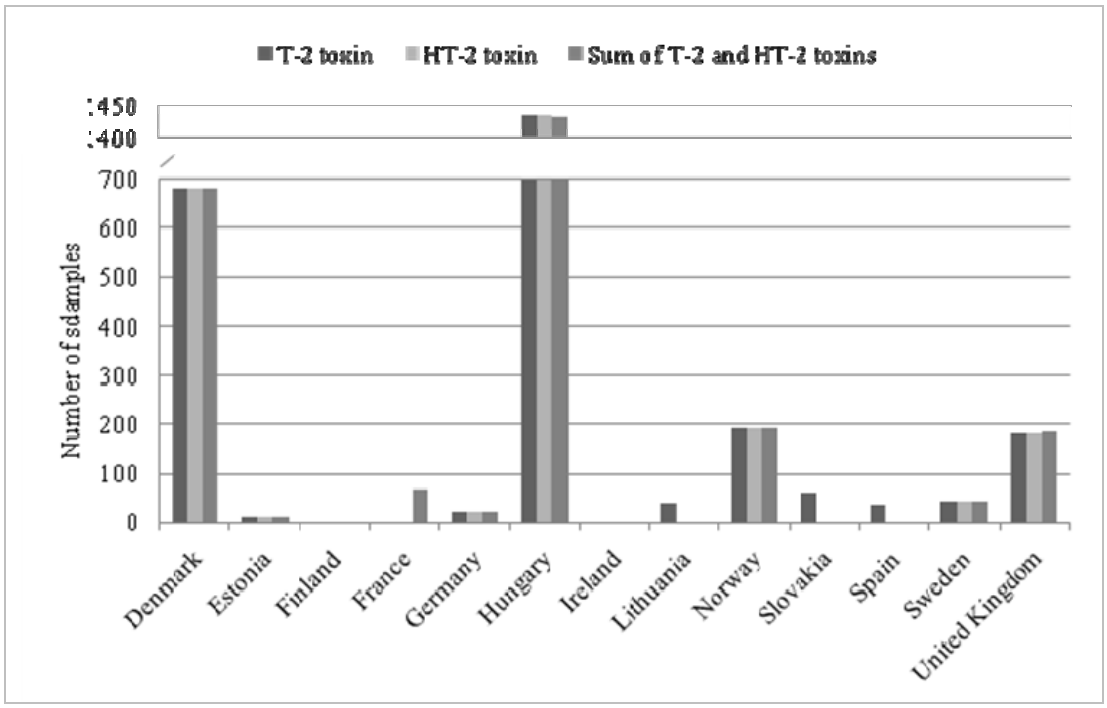


**Figure 6:** Distribution of feed samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across the European countries (after excluding non-qualifying data).

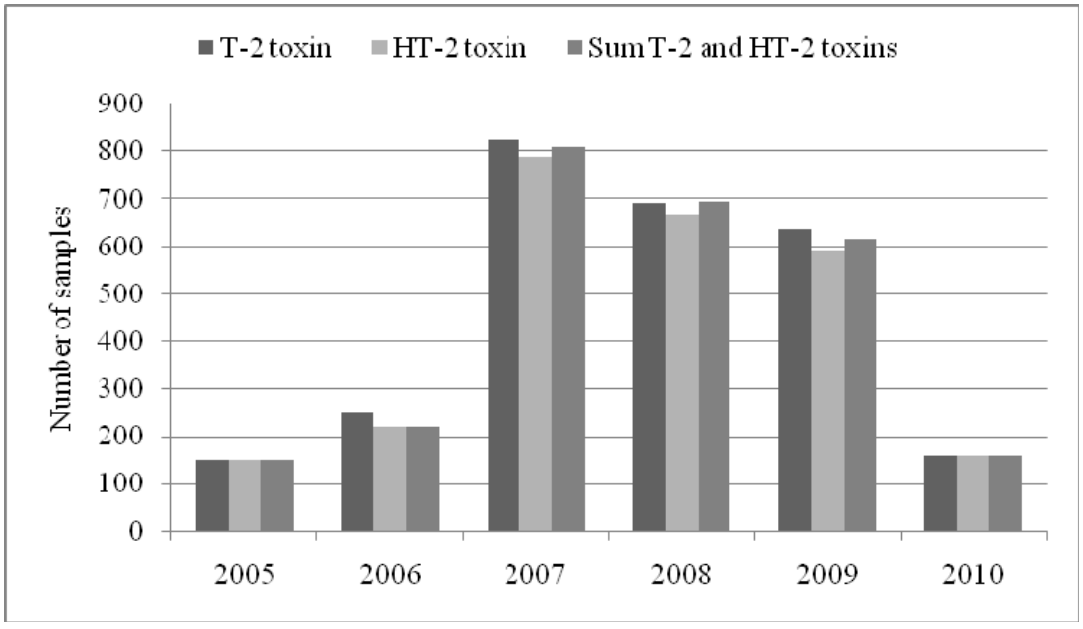


**Figure 7:** Distribution of feed samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins over the sampling years (after excluding non-qualifying data).

### 4.2.4. Data collection on unprocessed grains of undefined end-use

In addition to food and feed, a significant proportion of data were reported for unprocessed grains of undefined end-use. Since the samples analysed cannot be considered either food or feed and the processing might influence the concentration of the toxins in the end-product, these data were considered separately. In total, there were 9,332 results for T-2 toxin, 8,538 for HT-2 toxin and 9,415 for the sum of the two toxins in unprocessed grains. The number of results for the sum of T-2 and HT-2 toxins is higher than the number of results obtained by combining the individual toxins (n = 8,477) because in 938 instances results were reported only as a sum of T-2 and HT-2 toxins. Data on unprocessed grains were not used in the human or animal exposure assessment thus no exclusion based on LOQs was applied to data on these commodities. The distribution of unprocessed grain samples across the European countries and over the sampling years is presented in Figure 8 and Figure 9, respectively. The largest number of reported data was for wheat (50 %) followed by oats, barley and maize (Figure 10).

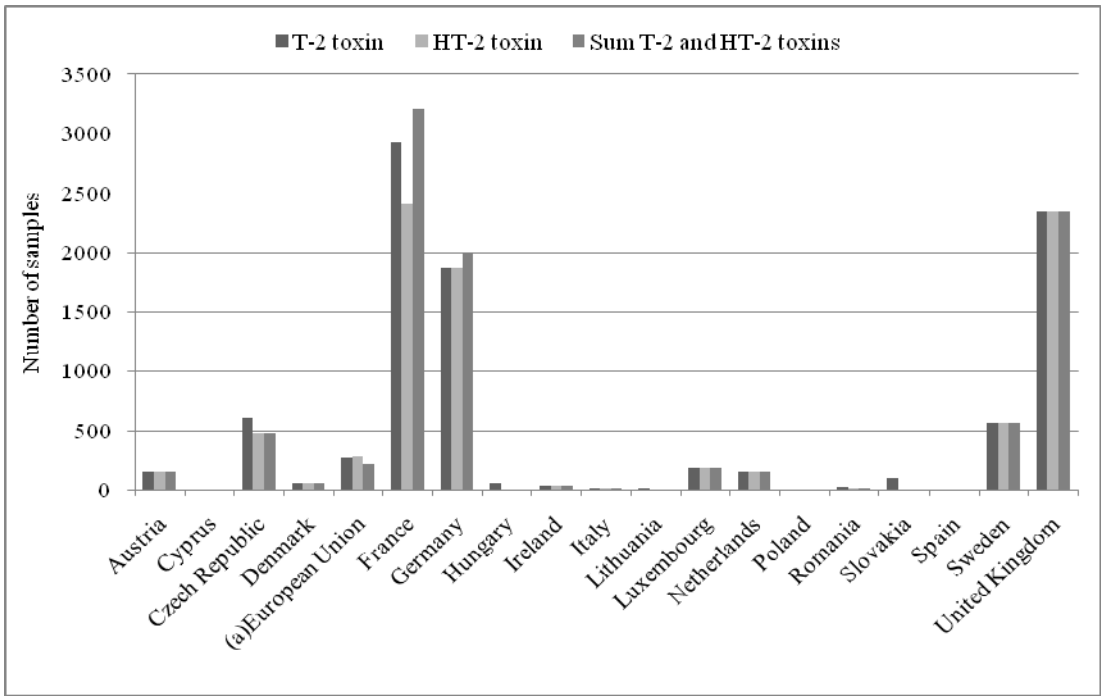


**Figure 8:** Distribution of unprocessed grain samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across the European countries. (a): Name of the EU Member State was not available.

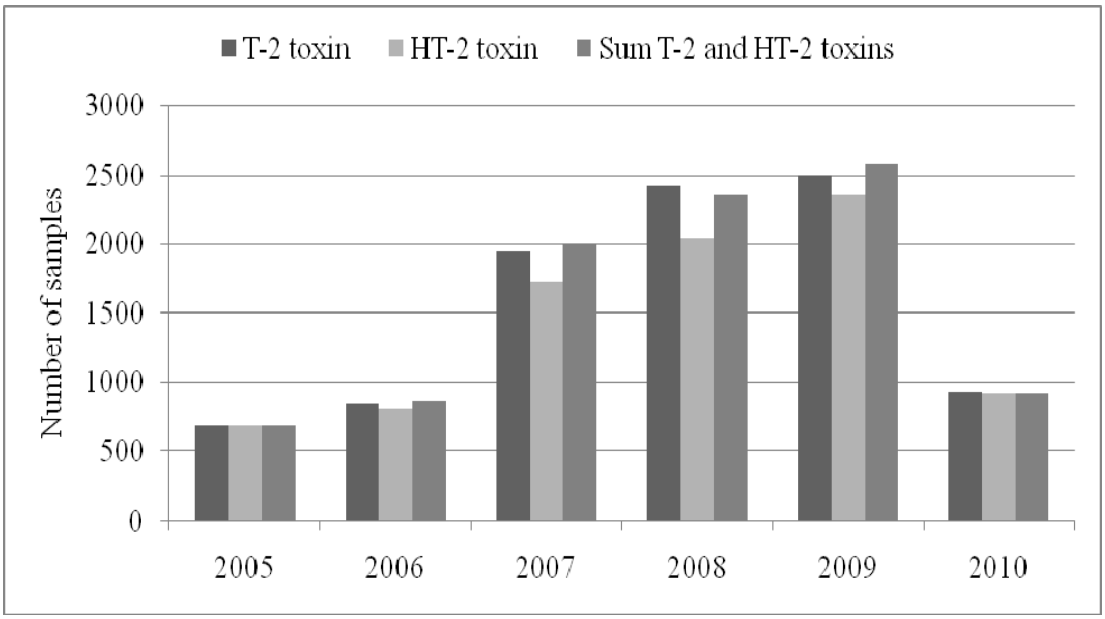


**Figure 9:** Distribution of unprocessed grain samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins over the sampling years.

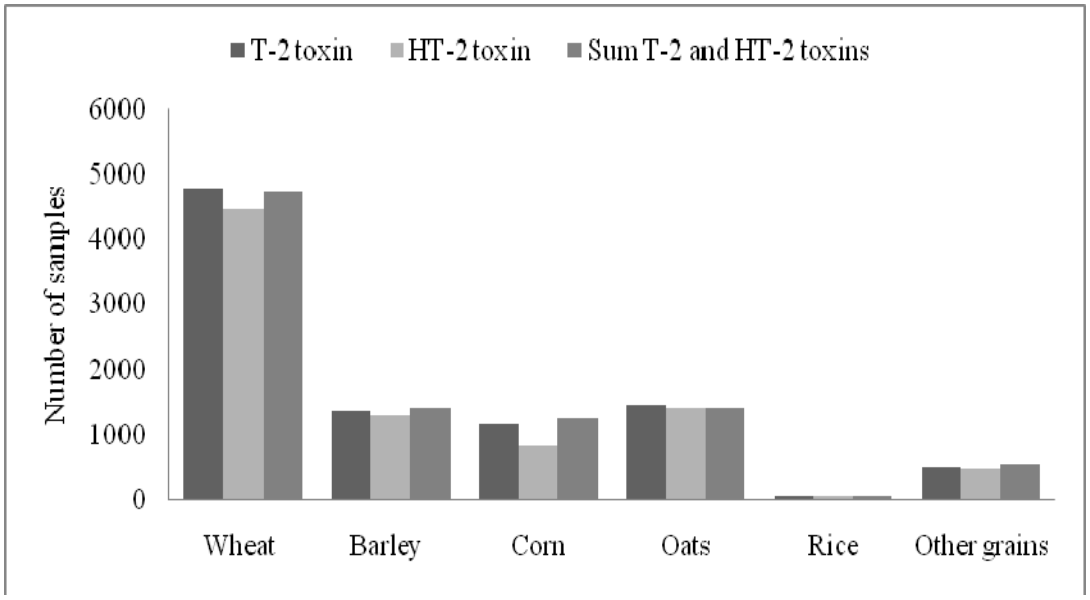


**Figure 10:** Distribution of unprocessed grain samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across grain categories.

### 4.2.5. Distribution of samples across food groups

The food samples were classified according to the FoodEx classification system (EFSA, 2011a). FoodEx is a food classification system developed by the DCM Unit in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 end-points (food names or generic food names) at the fourth level. The spread of the analytical

results for T-2 and HT-2 toxins across the several FoodEx groups prevented calculation of summary statistics at a very detailed level of the food classification system. Broad food groups with only a limited number of samples or not classifiable foods were all included in the group 'Other foods'.

The vast majority of data were on grains and grain-based foods. The groups 'Grain milling products' and 'Breakfast cereals' dominated the product coverage. The distribution of samples across the aggregated food groups is shown in Figure 11. A more detailed distribution in less aggregated food groups is presented in Appendix A, Tables A1-A3. Some food groups e.g. 'Vegetables and vegetable products' and 'Alcoholic beverages' were less represented in the data sets for T-2 toxin and HT-2 toxin, respectively, but sufficiently represented in the data set for the sum of the two toxins. Therefore, these groups will be consistently presented although only a limited number of data was available for the individual toxins.

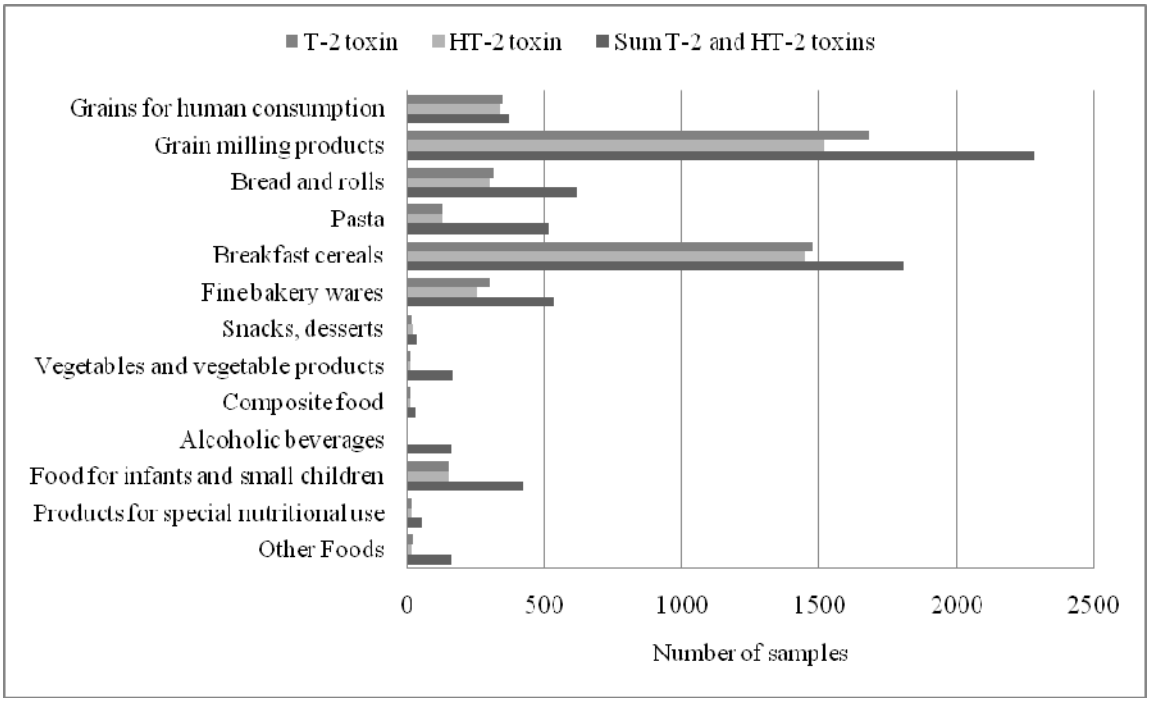


**Figure 11:** Distribution of food samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across the food groups.

### 4.2.6. Analytical methods used for food

Data on T-2 and HT-2 toxins in food were obtained by LC-MS/MS (30 %), other HPLC methods (3.3 %), ELISA (38 %) and GC methods (5.1 %). For 21 % of the food samples, the method of analysis was not reported.

LODs and LOQs were not both reported for all observations. To enable a comparison of the LOQs applied across food groups, missing LOQs (12 % of data) were estimated by multiplying the reported LODs by three. All the measurements were converted to µg/kg or µg/L.

The LOQs varied with the method applied, the food matrix and the laboratory (Figure 12). The methods for determination of T-2 and HT-2 toxins have improved in the last years, but despite this a wide range of LOQs was observed (0.02-450 µg/kg for T-2 toxin and 0.01-100 µg/kg for HT-2 toxin). Therefore, as detailed in Section 4.2.2., only results obtained by methods with a sum of the LOQs for T-2 and HT-2 toxins ≤ 20 µg/kg were included in the assessment.

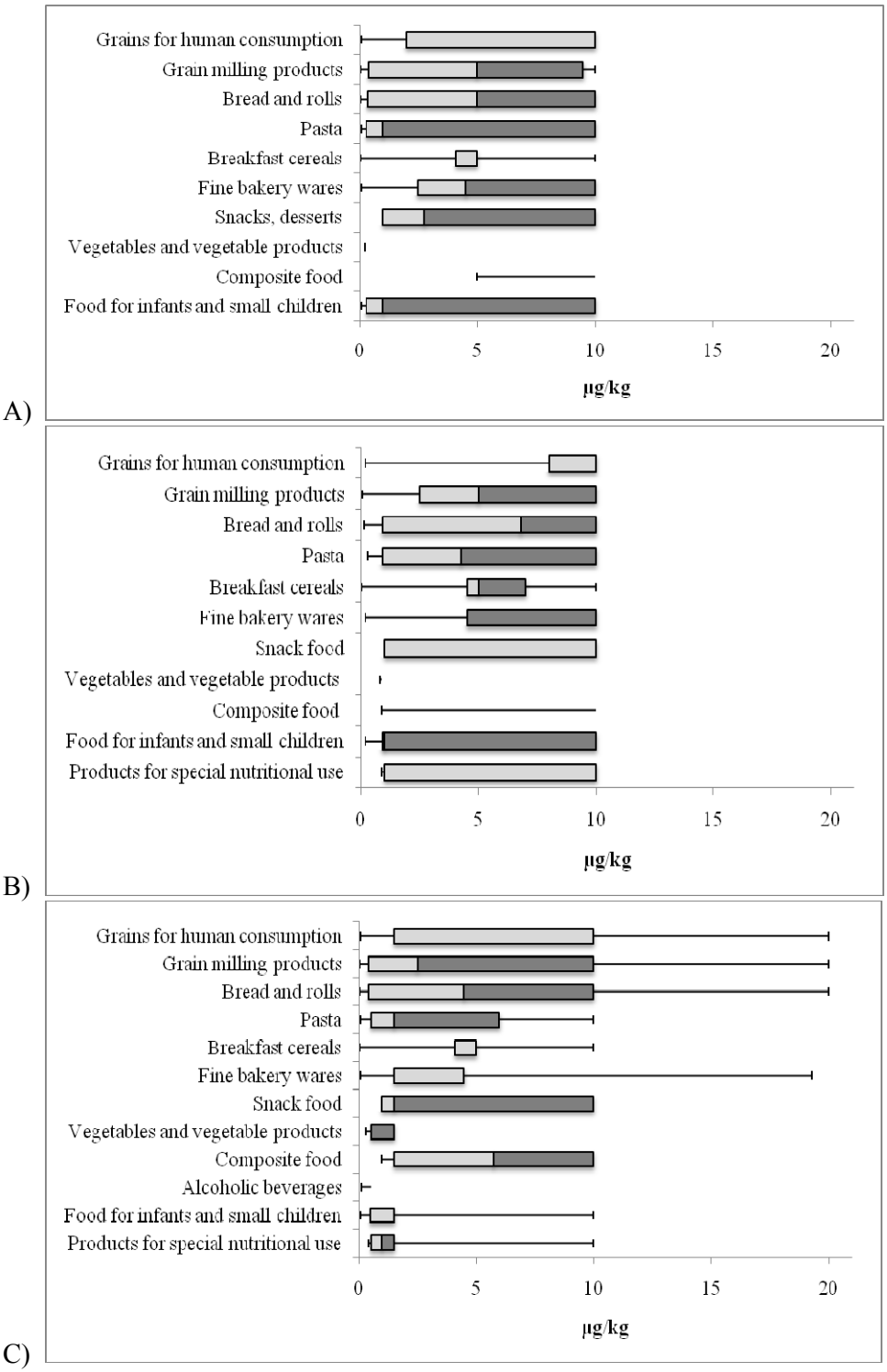


**Figure 12:** Distribution of the limits of quantification (LOQ) for A) T-2 toxin, B) HT-2 toxin and C) sum of T-2 and HT-2 toxins across all food samples included in the assessment (Box-plot: whiskers at minimum and maximum, box at 25$^{th}$ percentile and 75$^{th}$ percentile with line at 50$^{th}$ percentile).

The left-censored data accounted for 64 % of T-2 toxin results, 61 % for HT-2 toxin and 48 % for the sum of the two toxins. The proportion of left-censored data for the sum of T-2 and HT-2 toxins refers

to samples where the results for both toxins where below LOD/LOQ. (Figure 13). In general, the proportion of left-censored results varied between 30 and 70 % across the food groups.

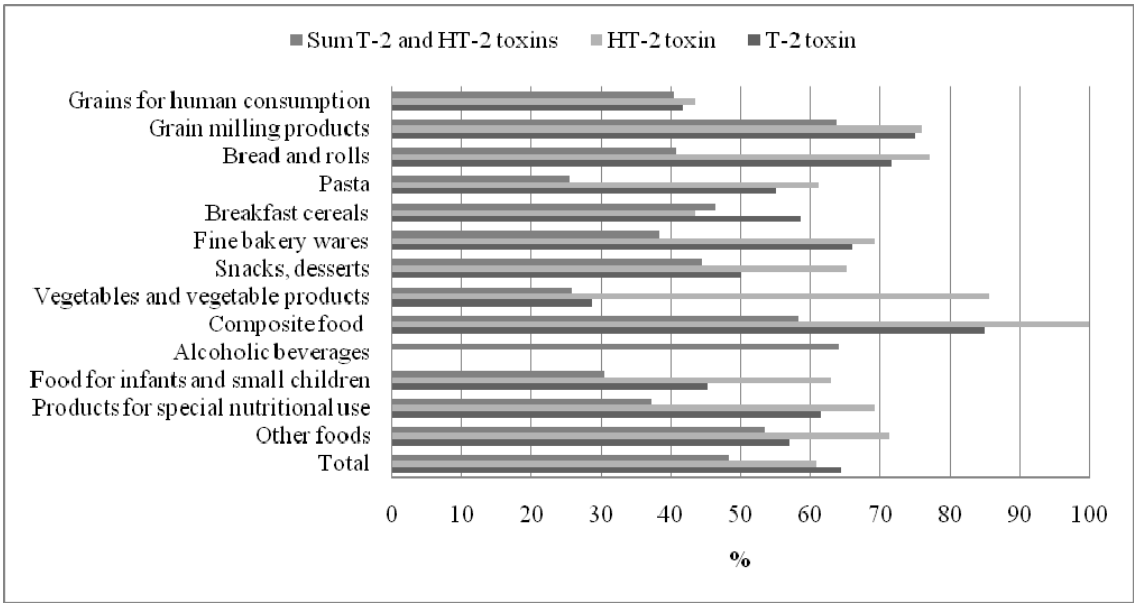


**Figure 13:** Percentage of left-censored results in the food groups.

### 4.2.7. Occurrence data on food

In the analysis of T-2 and HT-2 toxin occurrence data the non-detects (left-censored data) were treated by the substitution method as recommended in the "Principles and Methods for the Risk Assessment of Chemicals in Food" (WHO, 2009). The same method is indicated in the EFSA scientific report "Management of left-censored data in dietary exposure assessment of chemical substances" (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower-bound (LB) and upper-bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or LOQ (< LOQ). The UB is obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

The analytical results were transmitted by the data providers as either corrected or not corrected for recovery. Where results were not corrected by the data provider a correction has been applied by using the reported recovery rate. Where recovery was not available, no correction has been applied.

An overview on the number of samples and toxin concentration for T-2 toxin, HT-2 toxin and for the sum of them, in aggregated food groups, is given in Tables 7 to 9. A more detailed list on occurrence analysis per individual toxin and for the sum of them at level of food subgroups as considered in the exposure assessment is given in Tables A1 to A3 in Appendix A. However, in the following paragraphs the discussion on occurrence data refer to the sum of T-2 and HT-2 toxins only unless specified separately for T-2 toxin or HT-2 toxin. For consistency, the occurrence data are presented by the same list of food groups, although in some cases the number of samples for the individual toxins was limited.

The vast majority of data received for both toxins were on grains and grain-based products. The food group 'Grains for human consumption' consists of samples (n = 368 for the sum of T-2 and HT-2 toxins) covering only processed grains for human consumption. The highest mean concentration for the sum of T-2 and HT-2 toxins was observed in oats (outer husk removed) (LB mean = 31 µg/kg; UB mean = 34 µg/kg) followed by wheat grain (LB mean = 14 µg/kg; UB mean = 15 µg/kg), barley (LB mean = 10 µg/kg; UB mean = 13 µg/kg) and rye (LB mean = 4.2 µg/kg; UB mean = 9.5 µg/kg). Rice had the lowest contamination frequency and the lowest mean concentration (LB mean = 0.56 µg/kg; UB mean = 2.9 µg/kg) (Table 7).

'Grain milling products' was the dominant food group in all three data sets (n = 2,281 for the sum of T-2 and HT-2 toxins). Oat milling products showed the highest mean concentration (LB mean = 10 µg/kg; UB mean = 11 µg/kg) followed by maize milling products (LB mean = 5.8 µg/kg; UB mean = 8.2 µg/kg), wheat milling products (LB mean = 1.7 µg/kg; UB mean = 7.6 µg/kg) and rye milling products (LB mean = 1.1 µg/kg; UB mean = 4.3 µg/kg). Spelt milling products were less contaminated (LB mean = 0.69 µg/kg; UB mean = 3.3 µg/kg).

The 'Bread and rolls' food group (n = 617) was less contaminated compared to 'Grain milling products' (LB mean = 1.0 µg/kg; UB mean = 3.4 µg/kg). Among the different subgroups of 'Bread and rolls', 'Multigrain bread and rolls' had the highest mean concentration (LB mean = 2.6 µg/kg; UB mean = 3.6 µg/kg). The group 'Pasta' (n = 513) was also contaminated with low levels (LB mean = 1.7 µg/kg; UB mean = 2.5 µg/kg). In the 'Breakfast cereals' group (n = 1,808), the highest concentration was observed in the subgroup 'Cereal flakes', in particular in its subgroup 'Oat flakes' (LB mean = 14 µg/kg; UB mean = 15 µg/kg). 'Muesli' had also a relatively high contamination frequency (88 %) and mean concentration (LB mean = 5.6 µg/kg; UB mean = 6.2 µg/kg). The food group 'Snack food' contained only a limited number of samples (n = 36) and mainly maize-based foods. Their mean concentration was in the region of 5 µg sum of T-2 and HT-2 toxin/kg.

In the group 'Vegetables and vegetable products' (n = 167), only a limited number of data was available for soya, oilseeds and other vegetable. This group includes also data on edible fungi (dried). The highest mean concentration was found in dried edible fungi (LB mean = 9.5 µg/kg; UB mean = 9.5 µg/kg) which corresponds to an approximate 10-fold lower concentration in fresh fungi assuming a 90 % water content. The other subgroups had a mean concentration in the range of 1.1 to 1.6 µg/kg (both LB and UB).

The group 'Composite food' contained a limited number of samples (n = 55), all cereal-based dishes. The concentrations found were relatively low with a maximum measured value of 4.4 µg/kg. 'Alcoholic beverages' group included beer and beer-like beverages (n = 59) and wine (n = 97). T-2 and HT-2 toxins were found in 95 % of the beer samples at low levels (both LB mean and UB mean = 0.82 µg/L) and in none of the wine samples.

The group 'Food for infants and small children' contained mostly cereal-based food (n = 390). Their contamination frequency was relatively high (71 %) but the mean concentration was in the range of 2.7 µg/kg (LB) and 3.5 µg/kg (UB). 'Products for special nutrition' (n = 51) included fine bakery products and breakfast cereals for diabetics. Their mean concentrations of the sum of T-2 and HT-2 toxins were similar to the one found in the respective food groups for general population.

The mean concentrations for individual T-2 and HT-2 toxins across the food groups followed generally the same pattern as for the sum of T-2 and HT-2 toxins.

**Table 7:** Concentrations (µg/kg) of the sum of T-2 and HT-2 toxins across food groups.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| Grains for human consumption | 368 | 40 % | LB | 13 | 8.0 | 20 | 48 | 124 |
| | | | UB | 16 | 12 | 20 | 48 | 124 |
| Grain milling products | 2281 | 64 % | LB | 2.9 | 0.0 | 1.3 | 20 | 204 |
| | | | UB | 7.3 | 5.0 | 10 | 20 | 204 |
| Bread and rolls | 617 | 41 % | LB | 1.0 | 0.70 | 1.4 | 3.3 | 27 |
| | | | UB | 3.4 | 1.5 | 4.0 | 10 | 27 |
| Pasta | 513 | 26 % | LB | 1.7 | 1.1 | 2.1 | 5.0 | 17 |
| | | | UB | 2.5 | 1.5 | 2.8 | 10 | 17 |
| Breakfast cereals | 1808 | 46 % | LB | 9.7 | 4.8 | 13 | 36 | 197 |
| | | | UB | 11 | 6.5 | 14 | 36 | 197 |
| Fine bakery wares | 531 | 38 % | LB | 1.9 | 0.9 | 2.0 | 7.0 | 66 |
| | | | UB | 3.7 | 2.4 | 4.0 | 11 | 66 |
| Snack food | 36 | 44 % | LB | 4.9 | 0.74 | 4.5 | 20 | 20 |
| | | | UB | 5.6 | 2.0 | 5.2 | 20 | 20 |
| Vegetables and vegetable products | 167 | 26 % | LB | 2.2 | 1.0 | 2.0 | 6.3 | 45 |
| | | | UB | 2.3 | 1.0 | 2.0 | 6.3 | 45 |
| Composite food | 55 | 58 % | LB | 0.65 | 0.0 | 1.3 | 2.7 | 4.4 |
| | | | UB | 3.7 | 1.3 | 10 | 10 | 10[(c)] |
| Alcoholic beverages | 156 | 64 % | LB | 0.31 | 0.0 | 0.57 | 1.5 | 2 |
| | | | UB | 0.42 | 0.18 | 0.57 | 1.5 | 2.4[(c)] |
| Food for infants and small children | 423 | 31 % | LB | 2.6 | 0.9 | 3.2 | 10 | 31 |
| | | | UB | 3.4 | 1.4 | 4.2 | 12 | 33[(c)] |
| Products for special nutritional use | 51 | 37 % | LB | 1.7 | 0.8 | 2.9 | 5.1 | 12 |
| | | | UB | 2.9 | 1.4 | 4.4 | 10 | 17[(c)] |
| Other foods[(b)] | 133 | 53 % | LB | 1.7 | 0.0 | 1.1 | 15 | 22 |
| | | | UB | 2.1 | 0.3 | 1.3 | 16 | 22 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): Not considered in the dietary exposure assessment; (c): value represent the left-censoring limit.

**Table 8:** T-2 toxin concentrations (µg/kg) across food groups.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| Grains for human consumption | 345 | 42 % | LB | 5.0 | 1.3 | 10 | 12 | 41 |
| | | | UB | 6.0 | 5.0 | 10 | 12 | 41 |
| Grain milling products | 1679 | 75 % | LB | 1.1 | 0.0 | 0.06 | 7.0 | 81 |
| | | | UB | 3.0 | 2.0 | 5.0 | 10 | 81 |
| Bread and rolls | 318 | 72 % | LB | 0.21 | 0.0 | 0.1 | 0.9 | 4.5 |
| | | | UB | 2.2 | 2.0 | 4.4 | 5.0 | 10[(c)] |
| Pasta | 129 | 55 % | LB | 0.43 | 0.0 | 0.38 | 1.4 | 12 |
| | | | UB | 1.8 | 0.6 | 3.0 | 5.0 | 12 |
| Breakfast cereals | 1475 | 59 % | LB | 2.7 | 0.0 | 4.2 | 13 | 64 |
| | | | UB | 3.5 | 1.3 | 5.0 | 13 | 64 |
| Fine bakery wares | 297 | 66 % | LB | 0.78 | 0.0 | 0.52 | 3.3 | 24 |
| | | | UB | 2.8 | 2.0 | 4.6 | 10 | 24 |
| Snacks food | 18 | 50 % | LB | 4.3 | 1.3 | 10 | 10 | 10 |
| | | | UB | 4.9 | 2.3 | 10 | 10 | 10 |
| Vegetables and vegetable products | 7 | 29 % | LB | 0.13 | -[(d)] | -[(d)] | -[(d)] | 0.31 |
| | | | UB | 0.16 | -[(d)] | -[(d)] | -[(d)] | 0.31 |
| Composite food | 20 | 85 % | LB | 0.13 | 0.0 | 0.0 | 1.3 | 1.7 |
| | | | UB | 4.3 | 5.0 | 5.0 | 5.0 | 5.0[(c)] |
| Food for infants and small children | 150 | 45 % | LB | 0.84 | 0.1 | 0.8 | 5.2 | 7.7 |
| | | | UB | 1.8 | 0.65 | 2.5 | 7.7 | 10[(c)] |
| Products for special nutritional use | 13 | 62 % | LB | 0.32 | -[(d)] | -[(d)] | -[(d)] | 2.4 |
| | | | UB | 2.0 | -[(d)] | -[(d)] | -[(d)] | 5.0[(c)] |
| Other foods[(b)] | 7 | 57 % | LB | 4.6 | -[(d)] | -[(d)] | -[(d)] | 12 |
| | | | UB | 7.4 | -[(d)] | -[(d)] | -[(d)] | 12 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): Not considered in the dietary exposure assessment; (c): value represent the left-censoring limit; (d): not calculated where all data were left-censored or the number of data was very limited.

**Table 9:** HT-2 toxin concentrations (µg/kg) across food groups.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| Grains for human consumption | 336 | 43 % | LB | 9.3 | 8.3 | 10 | 37 | 100 |
| | | | UB | 11 | 10 | 10 | 37 | 100 |
| Grain milling products | 1517 | 76 % | LB | 2.1 | 0.0 | 0.22 | 12 | 147 |
| | | | UB | 4.9 | 3.0 | 6.0 | 12 | 147 |
| Bread and rolls | 298 | 77 % | LB | 0.44 | 0.0 | 0.10 | 2.5 | 11 |
| | | | UB | 2.9 | 3.0 | 5.0 | 8.0 | 11 |
| Pasta | 129 | 61 % | LB | 1.2 | 0.0 | 1.5 | 5.9 | 15 |
| | | | UB | 2.9 | 2.8 | 5.0 | 6.9 | 15 |
| Breakfast cereals | 1450 | 43 % | LB | 8.0 | 3.7 | 11 | 31 | 159 |
| | | | UB | 8.9 | 5.0 | 11 | 31 | 159 |
| Fine bakery wares | 256 | 69 % | LB | 1.5 | 0.0 | 1.7 | 5.7 | 58 |
| | | | UB | 3.7 | 2.0 | 5.0 | 10 | 58 |
| Snack food | 23 | 65 % | LB | 3.1 | 0.0 | 10 | 10 | 10 |
| | | | UB | 5.8 | 10 | 10 | 10 | 10 |
| Vegetables and vegetable products | 7 | 86 % | LB | 0.13 | -[(d)] | -[(d)] | -[(d)] | 0.88 |
| | | | UB | 0.4 | -[(d)] | -[(d)] | -[(d)] | 0.88 |
| Composite food | 19 | 100 % | LB | 0.0 | -[(d)] | -[(d)] | -[(d)] | 0.0 |
| | | | UB | 4.3 | -[(d)] | -[(d)] | -[(d)] | 5.0[(c)] |
| Food for infants and small children | 149 | 63 % | LB | 2.5 | 0.0 | 2.6 | 17 | 25 |
| | | | UB | 3.8 | 2.0 | 5.1 | 17 | 25 |
| Products for special nutritional use | 13 | 69 % | LB | 1.1 | -[(d)] | -[(d)] | -[(d)] | 12 |
| | | | UB | 3.7 | -[(d)] | -[(d)] | -[(d)] | 12 |
| Other foods[(b)] | 7 | 71 % | LB | 2.9 | -[(d)] | -[(d)] | -[(d)] | 10 |
| | | | UB | 6.4 | -[(d)] | -[(d)] | -[(d)] | 10 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): Not considered in the dietary exposure assessment; (c): value represent the left-censoring limit; (d): not calculated where all data were left-censored or the number of data was very limited.

### 4.2.8. Occurrence data on unprocessed grains of unknown end-use

The category 'Unprocessed grains' comprised grains of undefined end-use. Since the end use of the grains at harvest is not established and normally grains for human and animal consumption undergo several processing steps before being used it was considered appropriate to evaluate them separately. The left-censored data in the group 'Unprocessed grains' were handled by the substitution method as described for food in Section 4.2.7. Results below the LOD or LOQ accounted for 61 % for T-2 toxin, 53 % for HT-2 toxin and 61 for the sum of T-2 and HT-2 toxins. The proportion of left-censored results in the data set for the sum of T-2 and HT-2 toxins refers to samples where both toxins where not detected. High concentrations were reported in oat grains (sum of the two toxins: LB mean = 234 µg/kg; UB mean = 236 µg/kg) followed by barley and maize, wheat and rice (Table 10).

**Table 10:** Concentrations of T-2 toxin, HT-2 toxin and the sum of the T-2 and HT-2 toxins (µg/kg) in unprocessed grains of unknown end-use.

| Commodity | N[(a)] | LC | Concentration (µg/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LB/UB | Mean | P50 | P75 | P95 | Maximu |
| **T-2 toxin** | | | | | | | | |
| Wheat | 4799 | 76 % | LB | 1.7 | 0.0 | 0.0 | 5.5 | 345 |
| | | | UB | 8.0 | 5.0 | 5.0 | 37 | 345 |
| Barley | 1370 | 49 % | LB | 6.2 | 1.0 | 5.2 | 29 | 288 |
| | | | UB | 9.2 | 5.0 | 7.0 | 35 | 288 |
| Maize | 1166 | 50 % | LB | 5.9 | 0.27 | 5.0 | 20 | 730 |
| | | | UB | 16 | 5.0 | 25 | 50 | 730 |
| Oats | 1453 | 26 % | LB | 68 | 18 | 65 | 302 | 2321 |
| | | | UB | 69 | 19 | 65 | 302 | 2321 |
| Rice | 43 | 95 % | LB | 0.37 | 0.0 | 0.0 | 0.0 | 9.0 |
| | | | UB | 5.1 | 5.0 | 5.0 | 5.0 | 9.0 |
| Other grains | 482 | 89 % | LB | 0.41 | 0.0 | 0.0 | 0.6 | 138 |
| | | | UB | 5.2 | 5.0 | 5.0 | 25 | 138 |
| **HT-2 toxin** | | | | | | | | |
| Wheat | 4471 | 70 % | LB | 3.7 | 0.0 | 5.0 | 17 | 820 |
| | | | UB | 8.8 | 5.0 | 10 | 37 | 820 |
| Barley | 1307 | 41 % | LB | 20 | 5.0 | 22 | 86 | 602 |
| | | | UB | 22 | 6.3 | 22 | 86 | 602 |
| Maize | 828 | 34 % | LB | 11 | 5.0 | 5.0 | 50 | 321 |
| | | | UB | 14 | 5.0 | 10 | 51 | 321 |
| Oats | 1412 | 15 % | LB | 168 | 40 | 157 | 722 | 6480 |
| | | | UB | 169 | 40 | 157 | 722 | 6480 |
| Rice | 43 | 42 % | LB | 7.0 | 6.0 | 12 | 19 | 38 |
| | | | UB | 9.1 | 6.0 | 12 | 19 | 38 |
| Other grains | 465 | 84 % | LB | 1.7 | 0.0 | 0.0 | 6.6 | 361 |
| | | | UB | 6.6 | 5.0 | 5.0 | 20 | 361 |
| **Sum of T-2 and HT-2 toxins** | | | | | | | | |
| Wheat | 4738 | 77 % | LB | 4.9 | 0.0 | 6.0 | 22 | 1165 |
| | | | UB | 15 | 10 | 15 | 50 | 1165 |
| Barley | 1412 | 49 % | LB | 26 | 10 | 28 | 112 | 839 |
| | | | UB | 31 | 13 | 32 | 112 | 839 |
| Maize | 1249 | 52 % | LB | 13 | 0.0 | 10 | 64 | 750 |
| | | | UB | 24 | 10 | 22 | 95 | 750 |
| Oats | 1422 | 26 % | LB | 234 | 58 | 224 | 981 | 8399 |
| | | | UB | 236 | 60 | 225 | 981 | 8399 |
| Rice | 43 | 95 % | LB | 7.3 | 6.0 | 12 | 19 | 47 |
| | | | UB | 14 | 11 | 17 | 24 | 47 |
| Other grains | 533 | 80 % | LB | 2.0 | 0.0 | 0.54 | 6.6 | 499 |
| | | | UB | 9.9 | 10 | 10 | 25 | 499 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b);

A direct comparison between the mean concentrations in grains for human consumption and unprocessed grains was not feasible due to the limited number of observations on grains for human consumption. Therefore the comparison was made between unprocessed grains and the corresponding milling products. Consistently higher concentrations were observed in unprocessed grains (Figure 14). This suggests that processing results in lower T-2 toxin and HT-2 toxin concentrations in grain milling products.

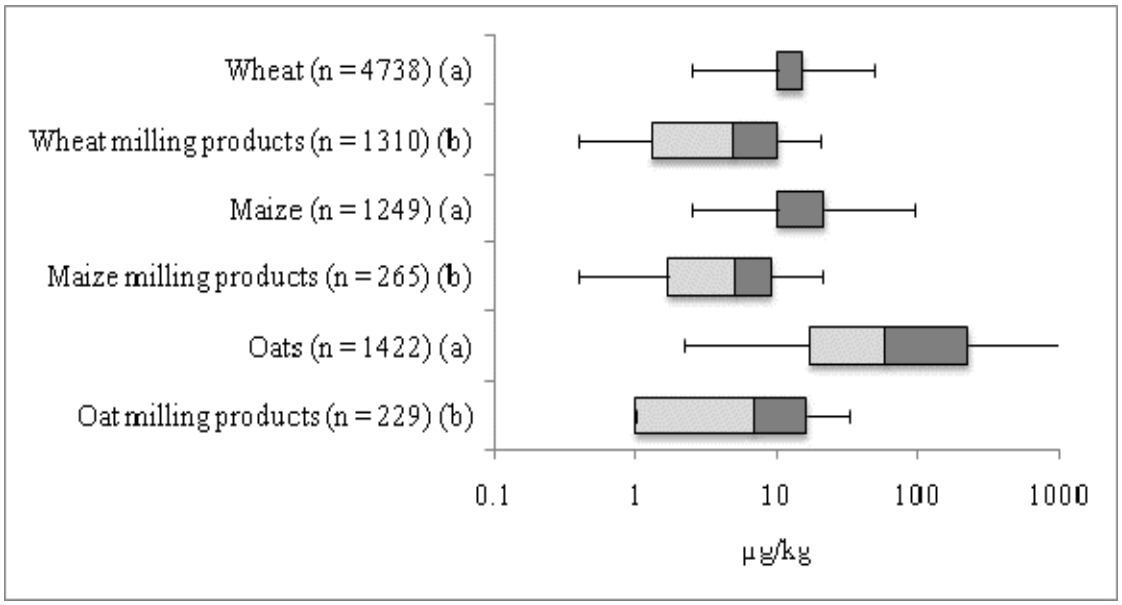


**Figure 14:** Comparison of the sum of T-2 and HT-2 toxin concentrations (µg/kg) in unprocessed grains (a) and in grain milling products (b). (Box-plot on logarithmic scale: whiskers at 5$^{th}$ percentile and 95$^{th}$ percentile, box at 25$^{th}$ percentile and 75$^{th}$ percentile with line at 50$^{th}$ percentile). For Wheat (a) and maize (a) the 25$^{th}$ and the 50$^{th}$ percentiles are equal.

### 4.2.9. Comparison of the occurrence of T-2 and HT-2 toxins in foods from organic and conventional farming

A direct comparison of the occurrence of T-2 toxin and HT-2 toxin in foods from organic farming and conventional farming was not possible because of the very limited number of samples for which the conventional farming method was specified. A sufficient number of samples with clear specification of the farming method was available in the data set on unprocessed grains. The comparison of the concentration of the sum of T-2 and HT-2 toxins is presented in Table 11 In the tested commodity groups, the concentration of the sum of T-2 and HT-2 toxins was consistently lower in products of organic farming. However, since the number of samples of the organic commodities was smaller than for the conventional ones and the sampling-countries and sampling years were not the same, this result should be viewed with caution.

**Table 11:** The sum of T-2 and HT-2 toxin concentrations (µg/kg) in unprocessed grains of organic farming (a) and conventional farming (b).

| Commodity | N[(a)] | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|
| | | Mean | P50 | P75 | P95 | Maximum |
| Wheat (a) | 185 | 7.5 | 6.3 | 10 | 20 | 30 |
| Wheat (b) | 1341 | 11 | 10 | 10 | 26 | 127 |
| Barley (a) | 57 | 7.2 | 2.9 | 10 | 25 | 50 |
| Barley (b) | 477 | 21 | 10 | 10 | 66 | 342 |
| Oats (a) | 90 | 77 | 8.9 | 26 | 410 | 2109 |
| Oats (b) | 542 | 426 | 131 | 448 | 1931 | 8399 |

N: number of samples; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b).

### 4.2.10. Comparison of occurrence of T-2 toxin and HT-2 toxin in foods over the sampling years

An important factor for the development of *Fusarium* spp. and the production of *Fusarium* toxins are the climatic conditions, mainly low temperature and high humidity. As these conditions can vary between the years, it is expected that *Fusarium* toxins may occur with higher frequency and at higher concentrations in the years when the climatic conditions are favourable. It seemed therefore interesting to evaluate the contamination frequency and concentration of T-2 and HT-2 toxins in foods over the years 2005 to 2010. An important constraint in this exercise is the overlapping of at least two harvests in one sampling year. As most grains are harvested in summer they will predominantly enter into the food chain in the second half of the harvest year and in the first half of the following year. In addition, grains might be stored and used after more than one year. An exercise comparing the occurrence of T-2 and HT-2 toxins over the sampling years was done but given the aforementioned limitations no clear variation over years could be observed (results not shown in this scientific opinion).

### 4.2.11. Classification of occurrence data on feed

Feed was classified according to the catalogue of feed materials specified in the Commission Regulation (EU) No 242/2010 of 19 March 2010 creating the Catalogue of feed materials.[22] Compound feedingstuffs were classified in groups according to the species/production categories for which the feed is intended. Results were reported either on a fresh weight or on 88 % dry matter. With the exception of maize silage all other groups contained feeds with dry matter content in range of 85-90 %. It was therefore considered that conversion to a common basis (88 % dry matter) was not necessary, as this would have only a negligible impact. Results on maize silage were all expressed on 88 % dry matter.

### 4.2.12. Distribution of samples across feed categories

The vast majority of samples were in the groups 'Cereals grains, their products and by-products' and 'Compound feedingstuffs' (Figure 15). Fewer results were available for 'Oil seeds, oil fruits, their products and by-products' and for 'Forages and roughage (including maize silage)'. A more detailed distribution of the samples in feed sub-groups is presented in Appendix B, Tables B1 to B3.

[22] OJ L 77, 24.3.2010, p. 17.

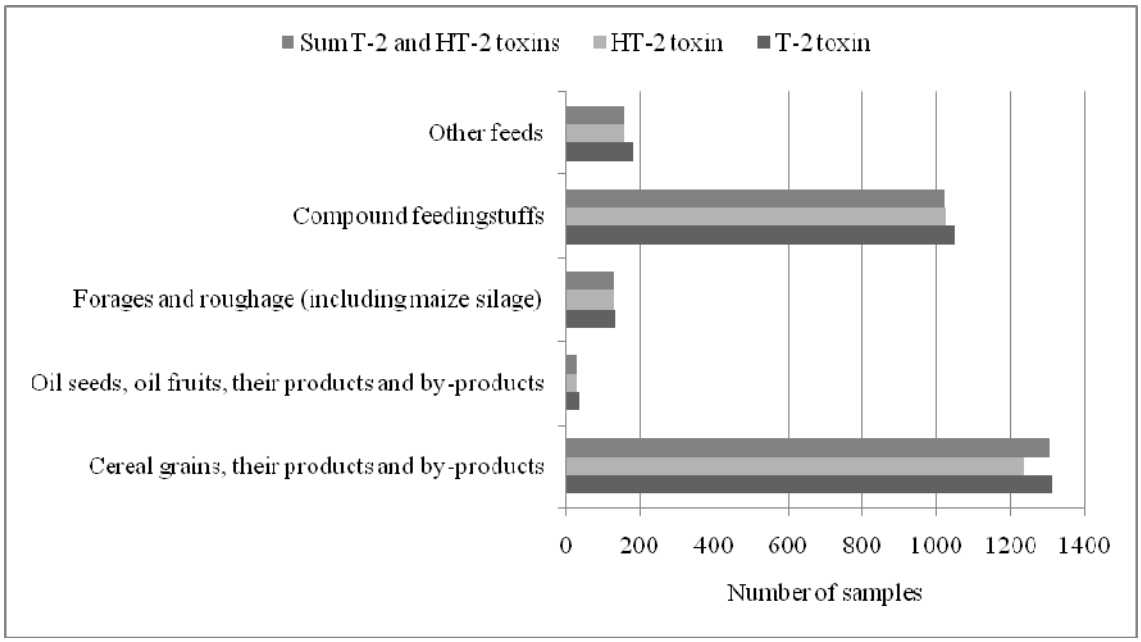


**Figure 15:** Distribution of feed samples for T-2 toxin, HT-2 toxin and the sum of T-2 and HT-2 toxins across the feed groups.

### 4.2.13. Analytical methods used for feed

The most common analytical methods reported for the analysis of T-2 and HT-2 toxins in feed was LC-MS (54 %) followed by LC-MS/MS (34 %), ELISA (2.6 %), HPLC with standard detection (0.9 %), GC-ECD (0.7 %) and GC-MS (0.2 %). For 7.7 % of results the analytical method was not provided. Similarly to food, where the LOQs were not available they were estimated by multiplying the LODs by three. The LOQs varied with the method applied, the feed matrix and the laboratory. However, most of the LOQs were in the range of 10 to 20 µg/kg (Figure 16).

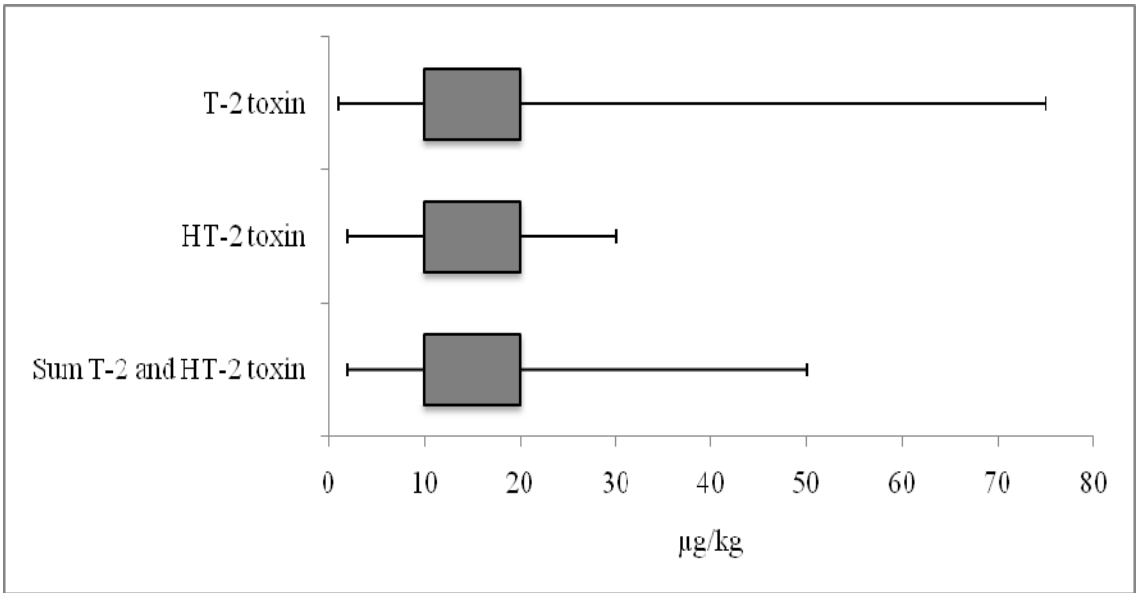


**Figure 16:** Distribution of the limits of quantification (LOQ) for T-2 toxin, HT-2 toxin and sum of T-2 and HT-2 toxins across all feed samples included in the assessment (Box-plot: whiskers at minimum and maximum, box at 25$^{th}$ percentile and 75$^{th}$ percentile with line at 50$^{th}$ percentile). Note that 25$^{th}$ percentile and 50$^{th}$ percentile were both equal to 10 µg/kg.

### 4.2.14. Occurrence data on feed by feed group

The left-censored data in feed were handled by the substitution method as described for food in Section 4.2.7. Results below the LOD or LOQ accounted for 77 % for T-2 toxin and 65 % for HT-2 toxin and 63 % for the sum of T-2 and HT-2 toxins. The proportion of left censored results in the data set for the sum of T-2 and HT-2 toxins refers to samples where the results for both toxins where below LOD/LOQ.

Among feed groups, T-2 and HT-2 toxins (as the sum of them) were most frequently found in oats and oats middlings (87 % and 98 %, respectively) with maximum values up to 3061 µg/kg and 1711 µg/kg, respectively. The mean concentrations were also the highest in oat middlings (LB and UB means = 300 µg/kg) and in oats (LB mean = 152 µg/kg; UB mean = 170 µg/kg). Barley, maize and maize products were less contaminated (LB and UB mean concentration in the range of 20 to 50 µg/kg), but maximum levels of 600-800 µg/kg were recorded in these feedstuffs. Among wheat and wheat products, the highest mean concentrations were found in wheat gluten (LB and UB means = 177 µg/kg) and in wheat middlings (LB mean = 46 µg/kg; UB mean = 52 µg/kg). In all the other feed groups T-2 and HT-2 toxins were found only occasionally. It should be noted that for oats, oat middlings and wheat gluten the 75$^{th}$ percentile value for the sum of T-2 and HT-2 toxins was in the range of 200 to 423 µg/kg while for all other feed groups it was below 50 µg/kg.

The mean concentrations for individual T-2 and HT-2 toxins across the feed groups followed generally the same pattern as for the sum of T-2 and HT-2 toxins. A detailed presentation of the contamination frequency and concentrations found in feed is given in Appendix B Tables B1to B3.

#### 4.2.15. The ratio of concentrations of T-2 toxin and HT-2 toxin in food, feed and unprocessed grains

To evaluate the ratio of concentrations of T-2 toxin and HT-2 toxin, a subset of samples where both toxins were detected was selected for food (n = 1,234), feed (n = 741) and unprocessed grains (n = 3,260). The ratio between the concentration of T-2 toxin and the concentration of HT-2 toxin was calculated for each sample and the distribution of the individual results was evaluated. For both food and unprocessed grains, the median of the ratios was 0.5 suggesting that HT-2 toxin occurs at twice as high concentrations as T-2 toxin. In feed, the median of the ratios between T-2 toxin and HT-2 toxin was slightly higher (0.65). A similar relationship for the concentrations of HT-2 toxin and T-2 toxin in cereals has been previously reported in the literature (see Section 4.1.4.). The current results suggest that in general HT-2 toxin represents approximately 2/3 of the sum of T-2 toxin and HT-2 toxin. However, a large variation in the concentration ratios between the individual samples was observed. The variation in ratios obtained for the ratio of T-2 and HT-2 toxin might, to a degree, be influenced by the analytical methods used. As feed products are composed of a variety of different ingredients, the uncertainty associated with the analytical result of such complex matrices could be significantly higher than anticipated. This is particularly true for not sufficiently characterised immunoassays where the cross reactivities of the employed antibodies with other trichothecenes can influence the result as well.

### 4.3. Food and feed processing

The extent to which cereals are processed depends on the cereal type and the final feed/food product. The effects of processing on cereals and cereal products in ways common to the food and feed industry have been investigated in various studies. In general, processing substantially reduces T-2 and HT-2 toxin concentrations in products for human consumption but may increase levels in feed products. This is because mechanical cleaning of cereals (de-hulling) may lead to by-products (for the feed industry) in which T-2 and HT-2 toxins concentrate significantly. This may result in (much) higher concentrations of T-2 and HT-2 toxins in these materials than in the cereals before cleaning. Food processing has been previously addressed by the JECFA (FAO/WHO, 2001). The more recent studies are discussed below.

#### 4.3.1. Food processing

##### 4.3.1.1. Cleaning and sorting

The effects of cleaning and sorting appear to have been mainly carried out on oats. Pettersson et al. (2008) reported that sorting and sieving to remove debris and small kernels markedly reduced T-2 and HT-2 toxin concentrations in the kernels intended for further processing as compared to the harvested oats, as did removal of the husk. At initial T-2 and HT-2 toxin levels of 200 µg/kg or higher in the harvested oats, normal cleaning and dehulling during mill processing can reduce these levels by 80-95 %, but the reduction is lower at lower initial toxin levels. The levels of T-2 and HT-2 toxins could be further reduced by removing discoloured kernels from the dehulled kernel fractions, since discoloured kernels had up to 10 times higher T-2 and HT-2 toxin concentrations than the kernels with normal colour. Recently Schwake-Anduschus et al. (2010) reported that cleaning of the raw oats did not lead to significant reductions of T-2 and HT-2 toxin concentrations while de-hulling resulted in a reduction of over 90 %. The initial concentrations were 14-214 µg T-2 toxin/kg (LOD $\leq$ 3 µg/kg and < LOD-758 µg HT-2 toxin/kg (LOD $\leq$ 5 µg/kg).

Comparable findings were reported by Scudamore et al. (2007), investigating the fate of T-2 and HT-2 toxins in oats. There were mean reductions of 75-98 % for T-2 toxin and 92-99 % for HT-2 toxin in oat flakes compared with the original grains. The reduction was the highest in the most highly contaminated samples. At the same time T-2 and HT-2 toxins were concentrated into the residual by-

product, where the increase from the raw oats averaged by a factor of 4.6 (range 1.6-10.3) for T-2 toxin and 4.2 (range 1.7-7.9) for HT-2 toxin. The resulting concentrations in the by-product reached more than 6000 µg/kg for T-2 toxin and 20000 µg/kg for HT-2 toxin (Scudamore et al., 2007). Similar results have been reported by Pettersson (2008, 2009) and Hietaniemi et al. (2009).

#### 4.3.1.2. Rolling and milling

Scudamore et al. (2009) reported separate T-2 and HT-2 toxin values for the different wheat fractions (wheat, flour, germ and bran) after milling 22 consignments of wheat in large commercial mills (1-100 tonnes). All the 22 flour samples were negative for both T-2 and HT-2 toxins (LOQs 10 µg/kg). T-2 toxin could be determined in only two of the 22 intact wheat samples (levels of 10 µg/kg and 12 µg/kg), but 12 of the 21 germ samples and 16 of the 22 bran samples contained T-2 toxin levels up to a maximum value of 34 µg/kg and 77 µg/kg, respectively. These results suggest a concentration factor of two to three in the germ and about six in the bran. Similarly, HT-2 toxin occurred in two intact samples (LOQ 10 µg/kg) but nine of the germ samples and six of the bran samples were positive up to a maximum of 95 µg/kg (Scudamore et al., 2009).

In a four-year study undertaken in the UK, little information was obtained on the fate of HT-2 toxin as a result of milling and food processing of wheat and maize (total of 146 samples). Manufacturing retail products from wheat and maize resulted in one snack product in which HT-2 toxin was detected at the level of 12 µg/kg; HT-2 toxin was undetected in the other products (see Section 4.1.2. for initial levels). In oat flakes, five of the 27 samples were negative for HT-2 toxin. Of the resulting 22 samples, nine contained 10-19 µg HT-2 toxin/kg, 12 samples contained 20-49 µg HT-2 toxin/kg and the remaining one sample contained 55 µg/kg (the oat flake production method was not reported) (Scudamore et al., 2009). Similar differences between the different grain fractions for levels of T-2 and HT-2 toxins in maize (Schollenberger et al., 2008) and wheat (Lancova et al., 2008a; Pascale et al., 2011) have been reported.

In a study on the effects of milling on the fate of trichothecenes, four wheat batches were sampled at different processing steps. Initial levels of both T-2 and HT-2 toxins in the wheat were low: T-2 toxin content was < 5 µg/kg and HT-2 toxin content was < 22 µg/kg. The two toxins showed to concentrate in the waste fractions, i.e., screenings and outer layers of the wheat bran, obtained during cleaning of wheat (Lancova et al., 2008a).

#### 4.3.1.3. Cooking and baking

Stability of T-2 toxin under baking conditions was studied by Beyer et al. (2009). The formation of degradation products of T-2 toxin was investigated in a baking experiment of spiked wheat flour (200 °C for 1 hour). The concentrations of the degradation products were found to be 10-20 % of the spiked T-2 toxin concentrations. Schwake-Anduschus et al. (2010) reported that cooking and baking appeared to have only little effect on T-2 and HT-2 toxin concentrations when naturally contaminated cleaned and ground raw oats were used in the experiments. However, great variations in T-2 toxin and HT-2 toxin contents in the porridge after cooking oats with water were observed (the temperature was first gradually increased from 30 °C up to 96 °C and finally the porridge was cooked for 1 minute at 96 °C). The variations were concluded likely to be due to the porridge making procedure. Similarly, variations in T-2 and HT-2 toxin contents were found when bread made with 20 % oats and 80 % wheat meal was baked at 220 °C for 40 minutes. Again the variations were probably due to the baking process (Schwake-Anduschus et al., 2010).

#### 4.3.1.4. Malting process

Producing malt from raw barley grain includes steeping, germinating and drying the grain. The fate of HT-2 toxin during the malting process was investigated by Lancova et al. (2008b). Two batches of

barley grains containing HT-2 toxin at the level of < LOQ of 10 µg/kg (naturally infected) and 11 µg/kg (artificially infected in the field with *Fusarium* spp.) were used. The LOD was 1 µg HT-2 toxin/kg. HT-2 toxin levels remained < LOQ during the steeping process. However, during the following steps of germination and drying, HT-2 toxin levels increased to 17 µg/kg in the naturally contaminated batches and to 22 µg/kg in the artificially infected batches. As a result, HT-2 toxin level was 2.1 times higher in the final malt product compared to the initial barley grains. The HT-2 toxin concentrations in the by-products resulting from removing the rootlets in order to obtain the final barley malt were 1061 µg/kg for the naturally and 493 µg/kg for the artificially infected batches. This by-product may be further used for animal feeding or as 'healthy' food supplement. The HT-2 toxin concentration did not change during the process of brewing beer from malt (Lancova et al., 2008b). Comparable results of the effect of brewing beer on T-2 and HT-2 toxin levels present in malt were reported by Cantrell (2008).

The fate of T-2 and HT-2 toxins during the malting process was also studied by Fournier (2009). The 100 % contamination with T-2 and HT-2 toxins of the starting barley was 0 % in the steeped barley, and at mid and end germination. However, in the final malt, HT-2 toxin was present in about 25 % and T-2 toxin in about 5 % of the samples. HT-2 toxin was present in 78 % and T-2 toxin in 15 % of the barley samples collected from the different harvests while in the produced malt HT-2 toxin was only present in 35 % and T-2 toxin in 4 % of the malt samples. The overall reduction during the malting process was 40 %. The elimination of T-2 and HT-2 toxins during the steeping step was about 80-100 %, and the resynthesis of T-2 and HT-2 toxins occurring during germination and kilning steps varied between 0 % and 50 %. Overall, a large variation in the resynthesis of T-2 and HT-2 toxins during the malting process was observed.

Levels of T-2 and HT-2 toxins in malt have reported to be lower than in the initial grains, but the relationship between the T-2 and HT-2 toxins in the original barley and the resulting malt is not constant (Slaiding 2008, 2009; Fournier, 2009).

### 4.3.2. Feed processing

Cereals intended for use as livestock feeds may undergo a number of processes, including cleaning, sorting, drying and rolling/grinding and/or extrusion before being fed to livestock.

#### 4.3.2.1. Cereal grains

Cereals intended for use as livestock feeds may be subject to cleaning and sorting, drying and rolling/grinding before being fed. As described above (Section 4.3.1.1), cleaning and sorting can result in a reduction in levels of T-2 and HT-2 toxins in the grain. Grains that are heavily infected with *Fusarium* become shrivelled and are lower in weight than healthy grains, and these can be separated by physical processes using gravity separators (Tkachuk et al., 1991). However, *Fusarium* infected grains can be indistinguishable from healthy grains and not removed in this way. Scott et al. (1984) suggested that routine grain cleaning could lead to, at best, a small (< 20 %) reduction in T-2 toxin and HT-2 toxin levels. In practice, unless there are obvious indications of contamination – either with trichothecene or other contaminants such as weed seeds - cereal grains intended for use directly as livestock feed are not routinely processed in this way.

Depending on the moisture content of the grains at harvest and the length of storage period, cereals may be dried using hot air. The temperature of air used to dry the grains, and the temperature of the grains themselves, will vary according to the equipment being used, the ambient temperatures and humidity, and the intended end moisture content. For long-term storage, a moisture content of approximately 12 % is generally recommended. In order to achieve this, air temperatures of up to 125-130 °C may be used, resulting in grain temperatures of up to 45 °C for wheat, and slightly lower for other cereal grains. However, T-2 and HT-2 toxins are stable at these temperatures (Schwake-

Anduschus et al., 2010). Drying temperatures higher than this are likely to result in deterioration in grain quality, particularly the protein fraction.

After storage and drying, cereals grains may be fed in their fresh state or after processing. The form and extent of processing will depend on the type of grain and the species of animal to which the grains are intended to be fed. While whole grains may be fed to some livestock (e.g. barley or oats to sheep and goats), others require the grains to be rolled, milled, extruded or flaked. These processes involve the application of pressure (e.g. rolling, extruding) and/or heat (e.g. cooking, flaking). As with most mycotoxins, T-2 and HT-2 toxins are stable and survive these processes.

In addition to physical processing, alkalis may be applied to whole cereal grains used as feeds for ruminant livestock. The alkalis have the effect of making the outer husk more digestible, therefore removing the need for physical processing. Trichothecenes containing an ester group are hydrolysed to their respective parent alcohols when treated with alkali, and a dilute solution sodium hydroxide or ammonium hydroxide has been shown to hydrolyse T-2 toxin to T-2 tetraol.[23] Oldham et al. (1980) suggested that T-2 toxin was 10 times more toxic than its hydroxylated derivative, suggesting that the application of an alkali to cereal grains may reduce the risks associated with the contamination with these trichothecenes.

#### 4.3.2.2. Cereal by-products

By-products of cereal processing are widely used as livestock feeds. The EC recently produced a Catalogue of Feed Materials[22] that listed over 80 cereal by-products used as animal feeds. These include by-products from the major cereals (wheat, barley, oats and maize) used in the manufacture of foods for human consumption, as well as in the production of alcohol. As discussed above (Section 4.3.1.1.), concentrations of T-2 and HT-2 toxins tend to be higher in these materials than in the grains from which they originate, and therefore pose an increased risk of exposure. However, in many countries in Europe, feed compounders operate to quality assurance standards that include a requirement for routine analysis for mycotoxins in both raw materials and finished product.[24] Such quality assurance schemes, where implemented, clearly reduce the risk of exposure to T-2 and HT-2 toxins by farm livestock.

#### 4.3.2.3. Compound feeds

As reported above (Section 4.1.3.1), a wide range of feed materials are used in the manufacture of compound feeds for livestock. Compound feeds generally consist of a mixture of various raw materials and additives formulated to meet the specific nutritional requirements of the livestock to which they are fed, and the finished product may be in the form of meal or pellets of varying sizes. They may be complete feeds that provide all of the daily requirements of nutrients, or individual feeds that provide part of the ration (e.g. protein and energy). In 2008, more that 130 million tonnes of compound feed were manufactured in the 27 EU Member States (FEFAC, 2009). The main categories of production were for pigs (35 %) poultry (30 %) and cattle (27 %).

During compound feed manufacture the raw materials may be subjected to a number of the processes described above, while the manufacture of the pelleted compounds is likely to involve the application of heat and pressure. The temperature achieved will depend on many factors, including the types of ingredients used in the formulation, the amount of moisture added and the equipment used, but pellets generally leave the die at temperatures ranging 60-95 °C (Thomas et al., 1997). As reported above, T-2 and HT-2 toxins are stable at these temperatures (Schwake-Anduschus et al., 2010).

[23] http://services.leatherheadfood.com/mycotoxins/item.asp?sectionid=1&mytype=basic&number=8&fsid=13
[24] e.g. UFAS (UK), GMP+ (Netherlands), GMP Animal Feed (Belgium) and QS (Germany).

#### 4.3.3. Conclusions

In general, the following effects of various processes, common in the food and feed industry, are observed for T-2 and HT-2 toxins: cleaning, sorting and sieving, and in particular de-hulling of grains lead to a marked overall reduction of up to 98 % in the concentrations of *Fusarium* toxins, including T-2 and HT-2 toxins in the final product such as oat flakes compared with the original grains. During grain milling, T-2 and HT-2 toxins are not destroyed but unevenly redistributed between the fractions. The toxins are mostly attached to the outer hull of the grain, and therefore occur at much higher concentrations in bran and germ fractions than in the whole meal flour. The de-hulling process may lead to by-products for the feed industry in which T-2 and HT-2 toxins significantly accumulate. During baking and cooking T-2 and HT-2 toxins are relatively stable compounds for which some degradation has been reported. However, the study results are variable and inconclusive. Malting leads to reduction in T-2 toxin and HT-2 toxin levels. As a result, T-2 and HT-2 toxin concentrations are substantially lower in malt than in the original barley, although the ratio varies considerably. Manufacturing of pelleted feedstuffs does not have an effect on the concentrations of T-2 and HT-2 toxins.

## 5. Food and feed consumption

### 5.1. Food consumption

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer. This included food consumption data concerning infants (2 surveys from 2 countries), toddlers (8 surveys from 8 countries), children (17 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries) for a total of 32 different dietary surveys carried out in 22 different countries. Surveys on children were mainly obtained through the Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., 2011). Thus, not all countries provided consumption information for all age groups, while in some cases the same country provided more than one consumption survey.

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected by using different methodologies and thus they are not suitable for direct country-to-country comparison.

#### 5.1.1. EFSA's Comprehensive European Food Consumption Database

The CONTAM Panel considered that only repeated exposure to T-2 and HT-2 toxins has to be assessed. Therefore, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b) dietary surveys with only one day per subject were not considered for the calculation of chronic dietary exposure, as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies although the protocol prescribed more reporting days per individual were also excluded. Thus, for the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries as follows:

1. Infants: 2 countries; 2 dietary surveys
2. Toddlers: 7 countries; 9 dietary surveys
3. Other children: 13 countries; 17 dietary surveys
4. Adolescents: 10 countries; 12 dietary surveys

5. Adults: 14 countries; 15 dietary surveys
6. Elderly: 7 countries; 7 dietary surveys
7. Very elderly: 6 countries; 6 dietary surveys

Within the dietary studies, subjects were classified in different age classes as defined below:

1. Infants: < 12 months old
2. Toddlers: ≥ 12 months to < 36 months old
3. Other children: ≥ 36 months to < 10 years old
4. Adolescents: ≥ 10 years to < 18 years old
5. Adults: ≥ 18 years to < 65 years old
6. Elderly: ≥ 65 years to < 75 years old
7. Very elderly: ≥ 75 years old

In particular, results from consumption surveys from 13 different European countries for children gathered by means of the EFSA Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., 2011) were incorporated in the database. Consumption records were codified according to the FoodEx classification system, which has been developed by the DCM Unit in 2009 (EFSA, 2011a).

The dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes are presented in Table 12. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011b).

**Table 12:** Dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes.

| Country | Dietary survey[a] | Abbreviation[b] | Number of subjects | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | Infants | Toddlers | Other children | Adolescents | Adults | Elderly | Very elderly |
| Belgium | Diet National 2004 | BE/1 | 3118 | | | | 584 | 1304 | 518 | 712 |
| | Regional Flanders | BE/2 | 661 | | 36 [c] | 625 | | | | |
| Bulgaria | NUTRICHILD | BG | 1721 | 860 | 428 | 433 | | | | |
| Cyprus | Childhealth | CY | 303 | | | | 303 | | | |
| Czech Republic | SISP04 | CZ | 2353 | | | 389 | 298 | 1666 | | |
| Denmark | Danish_Dietary_Survey | DK | 4120 | | | 490 | 479 | 2822 | 309 | 20[c] |
| Finland | DIPP | FI/1 | 1430 | | 497 | 933 | | | | |
| | FINDIET_2007 | FI/2 | 2038 | | | | | 1575 | 463 | |
| | STRIP | FI/3 | 250 | | | 250 | | | | |
| France | INCA2 | FR | 4079 | | | 482 | 973 | 2276 | 264 | 84 |
| Germany | DONALD_2006 | DE/1 | 303 | | 92 | 211 | | | | |
| | DONALD_2007 | DE/2 | 311 | | 85 | 226 | | | | |
| | DONALD_2008 | DE/3 | 307 | | 84 | 223 | | | | |
| | National_Nutrition_Survey_I | DE/4 | 13926 | | | | 1011 | 10419 | 2006 | 490 |
| Greece | Regional_Crete | GR | 839 | | | 839 | | | | |
| Hungary | National_Repr_Surv | HU | 1360 | | | | | 1074 | 206 | 80 |
| Ireland | NSIFCS | IE | 958 | | | | | 958 | | |
| Italy | INRAN_SCAI_2005_06 | IT | 3323 | 16[c] | 36[c] | 193 | 247 | 2313 | 290 | 228 |
| Latvia | EFSA_TEST | LT | 1965 | | | 189 | 470 | 1306 | | |
| Netherlands | DNFCS_2003 | NL/1 | 750 | | | | | 750 | | |
| | VCP_kids | NL/2 | 1279 | | 322 | 957 | | | | |
| Spain | AESAN | ES/1 | 410 | | | | | 410 | | |
| | AESAN_FIAB | ES/2 | 1067 | | | | 86 | 981 | | |
| | NUT_INK05 | ES/3 | 1050 | | | 399 | 651 | | | |
| | enKid | ES/4 | 382 | | 17[c] | 156 | 209 | | | |
| Sweden | Riksmaten_1997_98 | SE/1 | 1210 | | | | | 1210 | | |
| | NFAn | SE/2 | 2491 | | | 1473 | 1018 | | | |
| United Kingdom | NDNS | UK | 1724 | | | | | 1724 | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom. (a): More information on the dietary surveys is given in the Guidance of EFSA "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment" (EFSA, 2011b); (b): Abbreviations to be used consistently in all tables on exposure assessment; (c): 95$^{th}$ percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and therefore for these dietary surveys/age classes the 95$^{th}$ percentile estimates will not be presented in the exposure assessment.

### 5.2. Feed consumption

In contrast to the situation for the human population (Section 5.1.), there is no comprehensive database on what or how much feed livestock in the EU consume. What follows, therefore, are general estimates of feeds consumed for each of the main categories of farm livestock and companion animals. These are based on published guidelines on nutrition and feeding (e.g. AFRC, 1993; Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2008; EFSA, 2009a; OECD, 2009; McDonald et al., 2011), and data on EU manufacture of compound feeds (FEFAC, 2009). As a result, the composition of diets for each of the major farm livestock species given in this Scientific Opinion are estimates of the CONTAM Panel, but are in agreement with common practice. These feed consumption data are used subsequently (Section 6.2) to estimate exposures to the sum of T-2 and HT-2 toxins.

A wide range of feed materials is used in livestock rations. Forages, which include grasses, legumes, root crops, whole-crop cereals and crop residues, are consumed mainly by ruminants (cattle, sheep and goats), horses and rabbits. They maybe fed fresh and *in situ*, or conserved (e.g. as hay or silage). Non-forage feeds[25] include cereal grains and their by-products, vegetable oilseed meals, and by-products of food manufacture and production. They may be given as individual feeds or as mixtures, frequently as manufactured compound feeds. Approximately 150 million tons of compound feeds are produced in the EU each year (FEFAC, 2009). In addition to compound feeds, livestock consume cereals and legumes produced on-farm; although no official figures are available, these probably represent an equivalent volume. At least 60 % of all cereals grains produced in the EU are used in livestock feeds (FEFAC, 2009).

The amounts of feed consumed by livestock are influenced by many factors, of which the size, type and age of the animal, and the level of productivity, are particularly important. The choice of feed will be determined by the feeds available and their cost, and their suitability in meeting the nutritional needs of the animal (McDonald et al., 2011).

In summarising the feed consumption of livestock, it must be stressed that there is considerable variation in feeding systems throughout Europe and that the examples given do not represent 'average' diets, nor do they necessarily reflect 'typical' feeding systems applicable to all production systems in the Europe. Instead they are used to estimate levels of exposure to the sum of T-2 and HT-2 toxins that might not be atypical.

#### 5.2.1. Dairy cows

The diet of cows in the EU consists of forages and compound or other non-forage feeds, supplemented with minerals, trace elements as necessary. Fresh or conserved forages typically account for between 60 and 100 % of dry matter consumed (AFRC, 1993), depending on the level of production and the quality of the forage. They are predominantly grasses and legumes, but whole-crop maize and cereals are also fed after ensiling. Forages are supplemented with commercial compounds or other non-forage feeds where the forages on their own do not provide the necessary nutrients to meet the animals' requirements. The amounts fed are adjusted according to the amount and quality of the forage available and the milk yield of the cow, and adjusted for pregnancy and live weight gain, but will typically be about 0.25-0.35 kg feed/kg milk production (Nix, 2010).

For the exposure estimations for a 650 kg dairy cow, the CONTAM Panel used a range of milk yields (30, 40 and 50 kg milk/day) and a variable compound feeding rate of 0.28, 0.30 and 0.32 kg/kg of milk (equivalent to 8.4, 12 and 16 kg/day of non-forage feed intake), respectively, as reported by Nix (2010) (see details in Appendix C, Table C1). Details on the composition of the diets used in

[25] Also frequently referred to as concentrate feeds

estimating exposure to the sum of T-2 and HT-2 toxins for dairy cows are given in Appendix C, Table C4.

Cereal grains are an important constituent of dairy cow diets. In northern Europe, barley and wheat are most commonly used. Actual amounts used can vary considerably, depending on their price and availability of other feeds, but levels of up to 30 % of total dry matter intake are not uncommon. Because of their lower energy content relative to other cereals, oats are less widely used in diets for dairy cows.

While most dairy farmers supplement forages with commercially manufactured compound feeds, a significant proportion mix other feeds such as cereals, oilseed meals and mineral/vitamin supplements on the farm. A recent report from France (AFSSA, 2009) described typical rations for dairy cows based on forages and two non-forage feeds for a range of milk yields and forages (Appendix C, Table C5). In Section 6.2 the CONTAM Panel has used these rations to illustrate the effect that both level of milk production and type of diet can have on exposure to the sum of T-2 and HT-2 toxins.

### 5.2.2. Beef cattle

There are many beef production systems in the EU, ranging from extensively reared cattle from suckler cows to intensively reared Holstein-Friesian bulls from the dairy herd. These systems aim to produce animals for slaughter from less than 8-months of age (veal production), to 12 month 'cereal beef' ('barley beef'), or for slaughtering at up to 24 months of age. For many beef cattle, forages represent the major, and often only, ingredient in their diets. The forages are the same as those used for dairy cows. Other non-forage feeds are given when forages, because of their quality or availability, do not provide sufficient nutrients to achieve intended growth rates. An exception to this is the production of 'cereal beef', in which animals are fed almost exclusively on cereal grains, usually barley, supplemented with some vegetable protein (McDonald et al., 2011). In this system of production, feed intake may be as high as 2.5 % of body weight (NRC, 2000), and therefore a 400 kg bull may consume up to 10 kg of dry matter feed per day (AFRC, 1993), of which 85 % may be rolled barley grains. Details on the composition of the diets used in estimating exposure to the sum of T-2 and HT-2 toxins for beef cattle fed a grass silage and supplemented with non-forage feed materials and for beef cattle fed cereals (cereal beef) are given in Appendix C, Table C4.

For the exposure estimates the CONTAM Panel applied the live weight of fattening (beef) cattle of 400 kg and a daily intake 3.8 kg dry matter (1.9 kg dry matter/day of non-forage feeds) for the cattle fed grass silage and non-forage feeds. For cereal beef an intake of 8.5 kg dry matter/day (7.1 kg dry matter/day of non-forage feeds) has been adopted (see details in Appendix C, Table C1).

### 5.2.3. Sheep and goats

As with cattle, good quality forage is the single most important dietary ingredient for smaller ruminants, and for many of these livestock forages may be the only feeds used after weaning (NRC, 2007a). Exceptions to this are:

- Pregnant and lactating animals: Supplementation with non-forage feeds or commercial compound feeds usually occurs in late pregnancy, up to a maximum of 2.5 kg/day, and often continues into lactation, depending on the quality and availability of forages, and the number of lambs or does produced (AFRC, 1993; NRC, 2007a). They usually consist of cereals, cereal by-products and vegetable proteins supplemented with minerals and vitamins (McDonald et al., 2011).

- Lambs and kids: Compound feeds may be given around the time of weaning to encourage the intake of solid feed. The amounts consumed are generally small, particularly if there is forage available.

- Sheep and goats reared for meat production: Their diets consist predominantly of forage, with additional feeds given to achieve levels of live weight gain required. Total daily dry matter intakes can range from 1.9-3.8 % of their body weight (Devendra and Burns, 1983), of which forages typically account for 75 % or more of total intake. In commercial practice, goats reared for meat production and with a body weight > 10 kg are often fed green fodder *ad libitum* (AFRC, 1993) supplemented with cereal grains (barley, oats or maize) and cereal by-products, plus vegetable proteins to produce a feed with about 15 % crude protein (McDonald et al., 2011).

- Milking sheep and goats: Non-lactating sheep and goats usually receive only forage feeds, with compound feeding usually commencing in late pregnancy. For an 80 kg lactating ewe, compound or other non-forage feeds may be fed at a flat rate of 1.0-1.3 kg/day for the first 60-70 days of lactation, reducing to 0.9 to 1.0 kg/day for the next 60 days, and 0.5 kg/day in late lactation (AFRC, 1993). The actual amounts depend on the quality of the forage available.

The CONTAM Panel have used a daily dry matter intake of 2.8 kg for a 80 kg lactating sheep feeding twin lambs to estimate the exposure to the sum of T-2 and HT-2 toxins (Appendix C, Table C1). Details on the composition of the diets used in estimating the exposure for lactating sheep are given in Appendix C, Table C4.

The dry matter intakes of goats reared for meat and fed *ad libitum* can be as high as 3.8 % of body weight (Devendra and Burns, 1983). The CONTAM Panel have used daily dry matter intakes of 3.3 kg for a 60 kg goat for milking (4 kg milk/day) and 1.5 kg for a 40 kg goat for fattening to estimate the exposures to the sum of T-2 and HT-2 toxins (Appendix C, Table C1). Details on the composition of the diets used in estimating the exposure for goats are given in Appendix C, Table C4.

### 5.2.4. Pigs

There is a considerable range of pig production systems and diets fed to pigs in Europe. However, the majority of diets for fattening pigs and sows consist of cereals and cereal by-products supplemented with vegetable proteins (e.g. soybean meal, peas and beans, rapeseed meal). For breeding pigs, the relative proportions of these ingredients in the diets will be different during pregnancy and lactation. Diets for breeding pigs also tend to include greater proportions of fibrous feeds such as cereal by-products and sugar beet pulp (McDonald et al., 2011).

Exposure estimates have been made for piglets (20 kg b.w.), fattening pigs (100 kg b.w.) and lactating sows (200 kg b.w.) using feed intakes proposed by EFSA (2009a) (Appendix C, Table C2). Details on the composition of the diets used in estimating the exposure for pigs are given in Appendix C, Table C6.

### 5.2.5. Poultry

Poultry have limited ability to digest fibre,[26] and therefore cereal grains form the major part of their diets. In Europe, wheat, maize and barley are most commonly used, with rye, sorghum triticale and oats used less widely. Other ingredients include cereal by-products and vegetable proteins, supplemented with minerals, trace elements and vitamins. The main vegetable proteins are by-

[26] An exception to this is geese, which can live entirely on grass and similar forage.

products of commercial vegetable oil production, particularly soybean, rapeseed, cottonseed and sunflower meals, and legumes such as peas and lupins (Leeson and Summers, 2008; McDonald et al., 2011).

The amount of feed voluntarily consumed is largely determined by the age of the bird. Under *ad libitum* feeding, daily intake increases as the birds get older, although relative to body weight it declines with age. For meat producing and egg laying birds, *ad libitum* feeding is widely practiced, but for breeding stock feed intake is frequently restricted to maintain a steady body weight (Leeson and Summers, 2008).

The CONTAM Panel applied the live weights and feed intakes reported for different poultry (broilers, laying hens and turkeys) by EFSA (2009a) and for ducks by Leeson and Summers (2008) for the exposure estimations (see Appendix C, Table C2). Details on the composition of the diets used in estimating the exposure for poultry are given in Appendix C, Table C6.

### 5.2.6. Rabbits

Commercial rabbit production takes place in at least 14 EU Member States, predominantly in Italy, France and Spain. Annual rabbit meat production in EU is about 230,000 tonnes, corresponding to 100,000,000 animals/year.[27] Young rabbits are normally kept with the mother to around 4-5 weeks old, then moved to a fattening cage, until 10-12 weeks old before slaughter at about 2 kg b.w.[27]

Rabbits are usually fed a pelleted diet of dried forages, cereals and vegetable proteins supplemented with minerals, vitamins and trace elements. Lebas and Renouf (2009) reviewed diet formulations used in experimental studies: in 58 diets, the proportions of cereals, cereal by-products (mostly wheat bran) and oilseed meals (mostly soya bean meal and sunflower seed meal) were 18-20 %, 18-20 % and 16 %, respectively. In these studies, maize was a major constituent and was included in more then one-third of all diets. In northern Europe, however, maize may be replaced by barley and wheat. The composition of a typical French rabbit compound feed is given in Appendix C, Section C2.3. Because their natural diets consist of predominantly of fibrous feeds, rabbits have developed a strategy of high feed intakes of 65-80 g/kg b.w. in order to meet their nutritional requirements (Carabano and Piquer, 1998).

For the exposure estimates the CONTAM Panel used a live weight of 2 kg, a feed intake of 75 g/kg b.w. (Appendix C, Section C1.3). A typical diet composition for rabbits is given in Appendix C, Section C2.3.

### 5.2.7. Farmed fish

Atlantic salmon is economically the most important farmed fish in Europe, although other fish species are farmed including rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. Given the wide range of species and environmental conditions for farmed fish, many different feeding strategies have been developed. However, given its predominance in EU aquaculture, feed intake and exposure to T-2 and HT-2 toxins have been estimated for salmon in this Scientific Opinion.

Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fish meals and fish oils, and although alternative sources of oil and protein (e.g. soybean meals and vegetable oils) are increasingly being used these still remain the major ingredients. Since cold-water fish do not utilize carbohydrates as energy sources as well as warm-water species, there is less use of cereals. Berntssen et al. (2010) provided details of the composition of a diet for growing Salmonids, and the CONTAM Panel used this feed formulation to estimate exposure to the sum of T-2 and HT-2

[27] http://faostat.fao.org

toxins (Appendix C, Section C2.4.), for salmon (2 kg) with a feed intake of 0.04 kg dry matter/day (EFSA, 2009a) (Appendix C, Table C2).

#### 5.2.8. Feed consumption by companion animals

##### 5.2.8.1. Dogs and cats

Almost all small companion animals derive their nutritional needs from processed pet food, and in the EU annual sales of pet food products in 2010 was approximately 8.3 million tonnes.[28] Although a wide range of ingredients is used in commercial diets, most dog and cat diets contain at least some animal protein. Other ingredients include cereals (predominantly wheat, rice or maize), cereal by-products, vegetable proteins and by-products of human food production. In commercially manufactured pet food, the cereal content may be as high as 65 %.[29] The composition of a typical commercial pet food is given in Appendix C, Section C2.5.1.

Appendix C, Table C3 gives estimates of typical daily intakes for cats and dogs of different body weight. However, the intake estimates should be regarded as being indicative only, and vary depending on breed and level of activity. In the exposure estimates for cats, the CONTAM Panel applied a live weight of 4 kg and a feed intake of 60 g/day (cereal intake 33 g/day) of standard quality pet food and the typical diet for cats in France (Appendix C, Section C2.5.1.). For the exposure estimates of dogs it used a live weight of 25 kg and a feed intake of 360 g/day (cereal intake 234 g/day) of standard quality dog food and the typical diet for dogs in France (Appendix C, Section C2.5.1.).

##### 5.2.8.2. Horses

Horses are complete herbivores. They will generally consume 2-3.5 % of their body weight in feed (dry matter) each day, of which a minimum of 50 % should be as forage (pasture or hay) (NRC, 2007b). Mature horses with minimal activity can be fed forage alone, but for growing and active horses supplementary feeding with cereal grains, cereal by-products (e.g. oats, barley, and wheat bran) and vegetable proteins is necessary. Although oats are the preferred cereal for many horse owners, other cereal grains and cereal by-products are also routinely used. The CONTAM Panel estimated the exposure for a 450 kg horse, with a daily intake of 9 kg dry matter/day, of which half is in the form of oat-based feeds (see Appendix C, Sections C1.4.2. and C2.5.2.).

## 6. Exposure assessment of T-2 and HT-2 toxins in humans and animals

### 6.1. Human exposure assessment

#### 6.1.1. Previously reported human exposure assessments

##### 6.1.1.1. JECFA report

The 56th JECFA report (FAO/WHO, 2001) summarised the dietary exposure of T-2 and HT-2 toxins based on the mean concentration of each commodity (barley, maize, oats, rice, rye and wheat), weighted by sample size and the corresponding amount consumed in the Global Environment Monitoring System (GEMS)/food European diet (WHO, 1998). A total of 175 data points representing 8,410 individual samples were collected internationally. As 147 out of the 175 were from

[28] www.Fedif.org.

[29] B.M. Paragon (2011), Personal communication. Based on statistics of 2010 of French association of pet food manufacturers (FACCO), http://www.facco.fr/.

EU countries, the GEMS/food European diet was used for exposure assessment in EU population. It was estimated that the total dietary exposure of T-2 toxin was 7.6 ng/kg b.w. per day, and the total dietary exposure of HT-2 toxin was 8.7 ng/kg b.w. per day. The mean daily total dietary exposure of both toxins in Europe was 17 ng/kg b.w. per day, below the group PMTDI of 60 ng/kg b.w. per day (Table 13).

In this report, national dietary exposure was assessed in the UK and Norway population. Norway provided median and 95$^{th}$ percentile consumption of oats, rye and wheat by eight population subgroups. It was estimated that males aged 16-29 years were the group with the highest exposure in the nation consuming around 8 (median) and 36 (95$^{th}$ percentile) ng/kg b.w. per day of T-2 toxin and 10 (median) and 53 (95$^{th}$ percentile) ng/kg b.w. per day of HT-2 toxin. The UK provided mean, median and 97.5$^{th}$ percentile consumption of grains by two population subgroups: children aged 1.5-4.5 years and adults aged 16-64 years. In adults, the dietary exposure per person was 0.40 µg per day (median) and 1.46 µg per day (95$^{th}$ percentile) for T-2 toxin, and 0.53 µg per day (median) and 2.07 µg per day (95$^{th}$ percentile) for HT-2 toxin. In children, the median dietary exposure of T-2 and HT-2 toxins from all five grains was estimated at 0.14 and 0.16 µg/person per day, respectively. The T-2 and HT-2 toxin exposure in both UK groups was below the PMTDI level.

#### 6.1.1.2. Reports from Nordic countries

The Finnish Food Safety Authority (EVIRA) assessed the exposure of T-2 and HT-2 toxins in 2008 (EVIRA, 2008). Median concentration and average (or 95$^{th}$ percentile) consumption levels based on the survey data by Agrifood Research Finland (MTT) between the year 1999-2007 were used and T-2 and HT-2 toxins were considered as a group. From the average consumption data the exposure of T-2 and HT-2 toxins was 26 ng/kg b.w. per day for both female and male adults (Intake1 in Table 13). From the 95$^{th}$ percentile consumption data, the exposure was 56 ng/kg b.w. per day for women and 60 ng/kg b.w. per day for men (Intake2 in Table 13), the latter reached the PMTDI level. The exposure assessment based on the National FINDIET data at 2007 was very close to the assessment based on MTT data (Rautala et al., 2008).

Based on individual quantitative consumption questionnaire data and the Scandinavian means of concentration, the combined daily intake for T-2 and HT-2 toxins was estimated to be 130 and 140 ng/kg b.w. per day, respectively, in Denmark and Norway (Nordic Council of Ministers, 1998). Similar levels of dietary exposure were obtained based on food balance sheet consumption data as shown in Table 13. The food balance sheet data, although potentially overestimating the intake compared to individual data, provided exposure assessment for more Nordic countries. For example, the mean and high consumer dietary exposure in the Finnish population was 140 and 280 ng/kg b.w. per day, respectively, based on intake data on food balance sheets.

#### 6.1.1.3. SCOOP task 3.2.10

The SCOOP task 3.2.10 assessed T-2 toxin exposure from eight EU Member States and HT-2 toxin from six EU Member States (SCOOP, 2003; Schothorst and van Egmond, 2004). A total of 3,490 and 3,032 food samples were analysed for T-2 and HT-2 toxins. Food consumption data from FAO Food balance sheets and food frequency questionnaires for the whole population and, where possible, for specific groups were used for the intake calculation.

Four types of intake estimate for each food commodity were calculated as:

A: mean food consumption and mean 1 occurrence
B: mean food consumption and mean 2 occurrence
C: 95$^{th}$ percentile consumption and mean 1 occurrence
D: 95$^{th}$ percentile consumption and mean 2 occurrence

Whereas 'mean-1 occurrence' considered all individual data and the negatives were estimated taking into account the LOD/LOQ value, 'mean-2 occurrence' only considered the positive individual data. For each state, the best exposure estimate (ng/kg b.w. per day) for the whole population and/or for specific groups was made by summing up exposure A (and C) of all food commodities. Dietary exposure data for T-2 and HT-2 toxins were available from eight and six EU Member States, respectively (Table 13). HT-2 and T-2 toxin exposure in most of the cases exceeded the PMTDI, and the percentage of PMTDI in adults ranged from 62-172 %, and in children 27-563 %. It is highlighted, however, that owing to low positive occurrence of 20 % and 14 % for the two toxins, the mean-1 may be strongly influenced by the high LOD/LOQ levels because ½ LOD/LOQ were taken for the calculation and hence the intake may have been overestimated.

#### 6.1.1.4. The German study

A total of 3,837 food samples from the German retail market (2006-2008) were analysed for T-2 and HT-2 toxins. A "mean" and a "bad" ($90^{th}$ percentile of occurrence) case scenario of exposure of the two toxins combined were calculated using the median food intake for a certain type of food with either the median toxins level or the $90^{th}$ percentile of the toxin level for this type of food (Curtui et al., 2008) (Table 13). Toxin exposure was the highest in the age group of 4-6 years, and it decreased with age. Dietary exposure in males was 10-25 % higher than in females. Exposure in the younger age group was not calculated because of insufficient food intake data. However, it was estimated that in infants, the worst case of dietary exposure could reach the t-TDI level of 60 ng/kg b.w. per day.

#### 6.1.1.5. The Dutch study

T-2 and HT-2 toxin dietary exposure assessment was conducted in a small number of adults (56-101 adults) in the spring and autumn in the years 1976/1978, 1984, 1995 and 2004 using a duplicate diet assessment approach (Jekel et al., 2011). At each time point, all samples were pooled. The mean exposure of T-2 and HT-2 toxins in adults was well below the t-TDI of 60 ng/kg b.w. per day, ranging from 2.5-10.7 ng/kg b.w. per day over the study years/seasons.

In spring and autumn of 2006 duplicate diets were also collected for approximately 60 children (2-6 years old) and every two samples were pooled to reduce the number of samples for analysis. The mean T-2 toxin and HT-2 toxin exposure levels were 4.8/15.6 and 5.8/12.9 ng/kg b.w. per day in the spring and autumn of 2006, respectively. The dietary exposure for children ranged from 61-93 ng/kg b.w. per day based on six samples analysed (in total 120 samples).

In summary, the previous assessments on exposure of infants and children to T-2 and HT-2 toxins (FAO/WHO 2001; SCOOP, 2003; Curtui et al., 2008; Jekel et al., 2011) indicate that the exposure (ng/kg b.w. per day) in young children are usually higher than in adults due to the high consumption of cereal based food and low body weight of children, with the exposure ranging from 5-60 ng/kg b.w. per day for T-2 toxin and 13-44 ng/kg b.w. per day for HT-2 toxin. The exposure levels in adults and elderly groups in the EU population varied largely depending on the region and the study. In Nordic countries, high exposure levels were reported, possibly due to high grain consumption and high concentrations in the grain. In the SCOOP report, the majority of the adults had high exposure although overestimation is likely, due to high LOD in the analytical methods and a large number of samples below the LOD level. No significant seasonal variation in the dietary exposure level was found. The dietary exposure level of HT-2 toxin was typically 3-fold higher than that of T-2 toxin.

**Table 13:** Previously reported human exposure assessments for T-2 and HT-2 toxins (ng/kg b.w. per day).

| Country | Population group | T-2 exposure | HT-2 exposure | Reference |
|---|---|---|---|---|
| **Norway** | Male and female 6 years | 19-47 | 31-71 | FAO/WHO (2001)[a] |
| | Male and female 10years | 16-43 | 21-62 | |
| | Male 16-29 years | 9-37 | 11-54 | |
| | Male 30-59 years | 8-28 | 9-40 | |
| | Male 60-79 years | 7-34 | 9-48 | |
| | Female16-29 years | 8-27 | 10-39 | |
| | Female 30-59 years | 7-26 | 9-37 | |
| | Female 60-79 years | 7-26 | 8-39 | |
| **EU** | All population | 8 | 9 | |
| **Austria** | Total | 43-296 | 107-369 | SCOOP (2003)[b] |
| **Denmark** | Total | 50-69 | 0 | |
| **Finland** | Adult | 18-75 | 19-79 | |
| **France** | Adult (child) | 45-47 (60-67) | 30-0 (44-0) | |
| **Italy** | Total (consumers) | 11-12 (104-110) | | |
| **Norway** | Male (female) | 5-34 (5-30) | 30-38 (26-34) | |
| **Portugal** | All population | 0 | | |
| **UK** | 10 population groups | <15 | <19 | |
| **Finland** | Female (male) | The sum of T-2 and HT-2 toxins 26-55 (26-60) | | EVIRA (2008)[c] |
| **Finland** | All population | 90-180 | 50-100 | Nordic Council of Ministers (1998)[d], Based on food balance sheet |
| **Denmark** | All population | 40-80 | 20-40 | |
| **Norway** | All population | 110-220 | 70-140 | |
| **Sweden** | All population | 90-180 | 50-100 | |
| **Denmark** | All population | 90-190 | 50-100 | Based on individual quantitative questionnaire |
| **Norway** | All population | 80-160 | 50-100 | |
| **The Netherlands** | Children (2-6 years) | | | Jekel et al. (2011) |
| | Spring 2006 | 5 | 16 | |
| | Autumn 2006 | 6 | 13 | |
| | Adults | | | |
| | Spring 2004 | 2 | 8 | |
| | Autumn 2004 | 1 | 6 | |
| **Germany** | | The sum of T-2 and HT-2 toxins | | Curtui et al. (2008)[e] |
| | Adults, mean | 6-18 | | |
| | Age 4-6 years | 13-42 | | |
| | Age 7-9 years | 8-27 | | |
| | Age 10-14 years | 7-23 | | |
| | Age 14-12 years | 7-21 | | |
| | Age 25-50 years | 6-18 | | |
| | Age > 50 years | 7-19 | | |

b.w.: body weight. Single value, where presented, is the average value. (a): FAO/WHO (2001): median - 95$^{th}$ percentile consumption; (b): SCOOP (2003): mean1-mean2; (c): EVIRA (2008): Intake1 - Intake2; (d): Nordic Council of Ministers (1998): mean of average consumer – mean of high consumer; (e) Curtui et al., (2008): mean – "bad" case (90$^{th}$ percentile occurrence).

#### 6.1.1.6. Important dietary sources of human exposure to T-2 and HT-2 toxins

Although the occurrence of T-2 and HT-2 toxins was primarily limited to oat and barley in Europe, the high consumption level of wheat made it the most important dietary source in general in the previously reported exposure assessments. The JECFA (FAO/WHO, 2001) summarised that wheat and barley are the major dietary sources for T-2 toxin in the European diet and wheat, barley and oats are the most important dietary sources for HT-2 toxin.

Wheat contributed > 50 % of the T-2 and HT-2 toxin exposure in the Finnish diet, although the contamination in oats was the highest amongst all cereals (Rautala et al., 2008). In contrast to the total EU population diet reported by the JECFA (FAO/WHO, 2001), rye was the second most important food source, contributing around 30 % of the toxins exposure; oats contributed a higher proportion of HT-2 toxin exposure than T-2 toxin exposure. Similar results were reported in Nordic countries by Nordic Council of Ministers (1998) based on food balance sheets for consumption data. Wheat was the primary source of T-2 and HT-2 toxin dietary exposure; rye was the second key dietary source of T-2 toxin dietary exposure, whilst oats were the second largest source for HT-2 toxin in Norway and Sweden.

SCOOP (2003) assessed the dietary source of T-2 and HT-2 toxins only in some population groups, due to a lack of data. Some data were based on the occurrence in raw food and some based on processed food. Wheat and wheat containing products were the major source of exposure for T-2 and HT-2 toxins. In Finland the information on food source was only available for 24-64 year adults, with T-2 toxin exposure primarily from wheat (62 % of total exposure) and rye (33 %). The dietary exposure to HT-2 in Norwegian adults is similar to the above figures. In countries such as Denmark, France and the UK, where only processed food consumption data were provided, bread was the major source of T-2 toxin exposure. Baby food contributed to a high T-2 toxin exposure in toddlers and babies groups in the UK and Norway. However, the reliability of this information is questionable because of large individual variation in consumption, lack of consideration of T-2 and HT-2 toxin level reduction during food processing and the underestimation due to recovery not being corrected, especially in the case of small numbers of samples tested.

In contrast to the above findings, a recent study in Germany (Gottschalk et al., 2009) reported that the consumption of oat flakes in young children (2-5 year old and 9 month old groups) played the major role in T-2 toxin/HT-2 toxin exposure (37/195 ng/person per day) in comparison to wheat (22/72 ng/person per day) and rye flours (7/26 ng/person per day) in the mean case scenario in 2-5 year old Bavarian children. The study highlights that children may be at risk of high exposure due to the high intake level of oat product in muesli. However, the small number of infant foods analysed in this study and lack of properly measured consumption data in these groups cannot justify a proper conclusion.

Food processing such as de-hulling, malting and brewing may significantly reduce the T-2 and HT-2 toxins concentrations as detailed in the previous sections. Subsequently, consideration should be given to the impact of food processing on the exposure level during assessment.

Edwards (2009) compared the percentage of T-2 and HT-2 toxin contamination exceeding 50 µg/kg in oat samples from four countries over a period of 14 years and the trend suggested that T-2 and HT-2 toxin have increased dramatically over the last decade. However, whether this is a recent phenomenon cannot be certain owing to lack of historical data.

### 6.1.2. Current mean and high dietary exposure to T-2 and HT-2 toxins

For calculating the chronic dietary exposure to T-2 and HT-2 toxins, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.1.1).

The mean dietary exposure (average consumption in total population) and the high dietary exposure (95th percentile food consumption in total population) to T-2 and HT-2 toxins were calculated separately for each dietary survey using consumption data recorded at the individual level. Individual food consumption data were combined with the mean occurrence values in order to provide mean and high percentile exposure estimates (95th percentile). Exposure estimates were calculated for both LB and UB scenarios. The LB and UB mean concentrations of the food groups used in the exposure calculation are presented in Appendix A Tables A1 to A3.

Minimum, median and maximum exposure estimates across dietary surveys are reported for both the sum of T-2 and HT-2 toxins (Table 14) and for the individual T-2 and HT-2 toxins (Tables D1-D2, Appendix D). Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 28 dietary surveys are presented in Tables D3 to D8 (Appendix D). In accordance with the specifications of the EFSA Guidance on the use of the Comprehensive database (EFSA, 2011b), 95th percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust and therefore they should not be considered in the risk characterisation.

The CONTAM Panel decided to use in the risk characterisation (see Section 8) the exposure estimates for the sum of T-2 and HT-2 toxins and therefore the mean and 95th percentile dietary exposures to the sum of T-2 and HT-2 toxins are discussed in more detail below.

#### 6.1.2.1. (< 12 months old)

Since only two dietary surveys reported consumption data for children younger than 1 year, the dietary exposure estimate cannot be considered as representative of the European infant population. One of the surveys did not qualify for the calculation of the 95th percentile exposure (number of subjects < 60). Taking into account these limitations, the mean dietary exposure to the sum of T-2 and HT-2 toxins were between 5.9 and 16 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile dietary exposure in the qualifying study was 19 ng/kg b.w. per day in LB and 51 ng/kg b.w. per day in UB (Table 14).

#### 6.1.2.2. Toddlers, other children and adolescents (≥ 1 to < 18 years old)

The dietary exposure to the sum of T-2 and HT-2 toxins in toddlers, other children and adolescents decreased with increasing age. This is explained by the higher intake of food per kg b.w. in younger age groups. The highest exposure was estimated in toddlers (age ≥ 12 months to < 36 months), for which mean chronic dietary exposure ranged from 12 to 43 ng/kg b.w. per day (minimum LB to maximum UB) and the 95th percentile dietary exposure ranged from 23 to 91 ng/kg b.w. per day (minimum LB to maximum UB) (Table 14).

#### 6.1.2.3. Adults (≥ 18 to < 65 years old)

In the adult population, the mean dietary exposure to the sum of T-2 and HT-2 toxins across dietary surveys ranged from 3.4 to 18 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile dietary exposure ranged from 7.2 to 39 ng/kg b.w. per day (minimum LB to maximum UB) (Table 14).

#### 6.1.2.4. Elderly and very elderly (≥ 65 years old)

The mean dietary exposure to the sum of T-2 and HT-2 in the elderly and very elderly populations was slightly lower than in adults. The mean values across the dietary surveys ranged from 2.8 to 15 ng/kg b.w. per day (minimum LB to maximum UB). The 95th percentile dietary exposure ranged from 5.3 to 26 ng/kg b.w. per day (minimum LB to maximum UB) (Table 14).

#### 6.1.2.5. Conclusions

It can be concluded that the dietary exposure to the sum of T-2 and HT-2 toxins is higher in younger consumers than in adults. In addition, there is a relatively high variation between the exposure estimates across the dietary surveys within each age class. The exposure estimates in this assessment are in the same range as those reported previously by Curtui et al. (2008), Rautala et al. (2008) and Jekel et al. (2011). Higher dietary exposure estimates were reported in earlier years (Nordic Council of Ministers, 1998; SCOOP, 2003) but this was probably due to important differences in the assessment methodologies (e.g. data obtained by methods with high LODs/LOQs, different treatment of left-censored data, use of aggregated consumption data). The dietary exposure to individual T-2 and HT-2 toxins in all age classes followed generally the same pattern as for the sum of T-2 and HT-2 toxins. A summary of the dietary exposure to the sum of T-2 and HT-2 toxins in all age classes is presented in Table 14. The summaries of the dietary exposure to the individual T-2 and HT-2 toxins in all age classes are presented in Tables D1-D2 (Appendix D).

**Table 14:** Summary statistics of the chronic dietary exposure to the sum of T-2 and HT-2 toxins (ng/kg b.w. per day) across European countries.

| Age class | Summary statistics of exposure (ng/kg b.w. per day) | | | | | |
|---|---|---|---|---|---|---|
| | Minimum | | Median | | Maximum | |
| | LB | UB | LB | UB | LB | UB |
| | **Mean dietary exposure in total population** | | | | | |
| Infants | 5.9 | 11 | -[(a)] | -[(a)] | 6.2 | 16 |
| Toddlers | 12 | 30 | 16 | 34 | 28 | 43 |
| Other children | 10 | 26 | 14 | 31 | 16 | 39 |
| Adolescents | 4.4 | 13 | 7.9 | 19 | 9.2 | 24 |
| Adults | 3.4 | 10 | 5.6 | 14 | 9.0 | 18 |
| Elderly | 3.3 | 10 | 4.2 | 13 | 5.8 | 14 |
| Very elderly | 2.8 | 10 | 4.0 | 12 | 6.4 | 15 |
| | **95th percentile dietary exposure in total population[(b)]** | | | | | |
| Infants | 19 | -[(c)] | -[(c)] | -[(c)] | -[(c)] | 51 |
| Toddlers | 23 | 48 | 33 | 62 | 65 | 91 |
| Other children | 21 | 44 | 31 | 58 | 44 | 71 |
| Adolescents | 12 | 29 | 19 | 38 | 25 | 47 |
| Adults | 7.2 | 20 | 14 | 26 | 25 | 39 |
| Elderly | 6.7 | 21 | 10 | 23 | 14 | 26 |
| Very elderly | 5.3 | 17 | 7.0 | 19 | 12 | 25 |

b.w.: body weight; LB: lower-bound; UB: upper-bound.
(a): Not calculated; estimates available only from two dietary surveys; (b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table; (c): Not calculated; estimates available only from one dietary survey.

### 6.1.3. Contributions of the individual T-2 and HT-2 toxins to the dietary exposure

Although at this time the toxic effects of T-2 toxin and its metabolite HT-2 toxin cannot be differentiated, the CONTAM Panel considered it important to evaluate the contribution of the individual toxins to the combined exposure. The evaluation was performed on the mean and 95th percentile dietary exposure estimates in all age groups obtained for the individual toxins in LB scenarios. The rationale for this approach is that in LB scenarios the left-censored data were treated

equally (replaced by zero). In UB, left-censored data were replaced by LOD or LOQ and this has a variable influence on the estimates depending on the detection capability of the analytical methods for the individual toxins (ratios between LOQs of T-2 toxin and LOQs of HT-2 toxin are not constant). The average contribution of T-2 toxin and HT-2 toxin to the combined chronic mean and 95$^{th}$ percentile dietary exposure in all age groups is presented in Figure 17. In general, T-2 toxin contributes to the combined exposure by 1/3 while HT-2 toxin accounts for 2/3 in all age classes across the dietary surveys. This observation is in line with the result found on the T-2 toxin/HT-2 toxin ratio in occurrence data in food (ratio T-2 toxin/HT-2 toxin = 1:2).

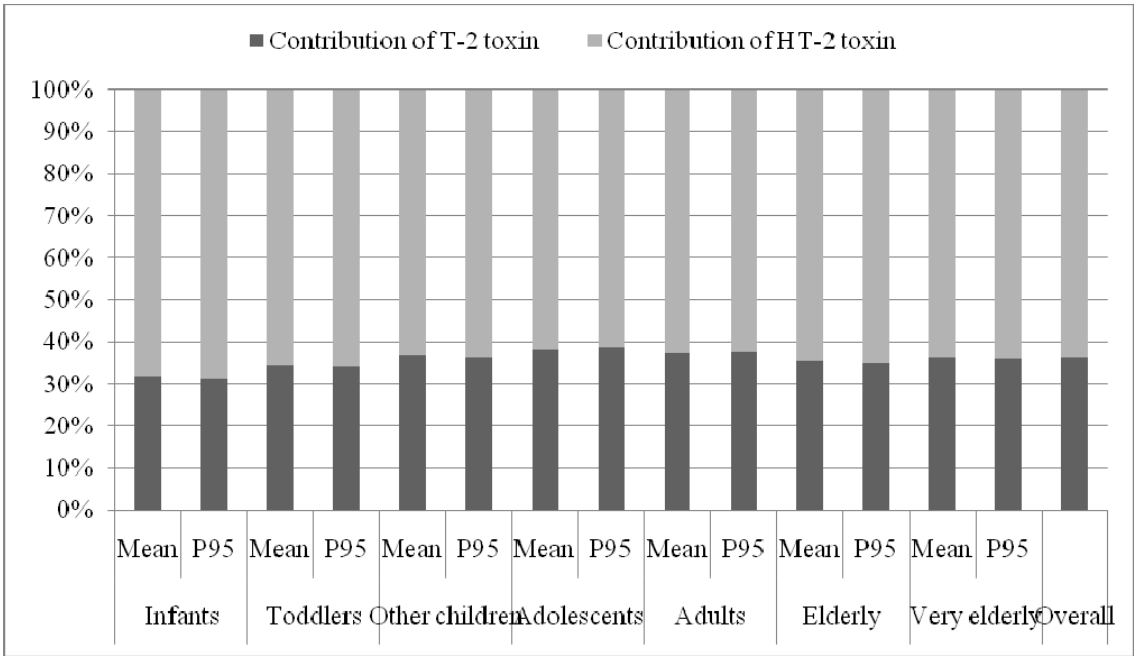


**Figure 17:** Average contribution (normalised to 100 %) of T-2 toxin and HT-2 toxin to the combined chronic mean and 95$^{th}$ percentile (P95) dietary exposure (%) in all age groups. Calculation was performed on exposure estimates obtained in lower-bound scenarios because in this case all left-censored data were treated equally (replaced by zero). As the occurrence data used to calculate the exposure estimates for T-2 toxin, HT-2 toxin and for the sum of T-2 and HT-2 toxins were not the same, the contribution of the individual toxins do not sum up to 100 %.

### 6.1.4. Contributions of different food groups to the sum of T-2 and HT-2 toxin exposure

The contribution of individual food groups to dietary exposure to the sum of T-2 and HT-2 toxins varied between the dietary surveys. This is explained by the specific food consumption patterns in the individual European countries and even in the different regions of one country. The contribution of the individual food groups to the dietary exposure to the sum of T-2 and HT-2 toxins was calculated for both LB and UB scenarios. It is to note that in two dietary surveys foods (e.g. bread, fine bakery products) were disaggregated to ingredients (flour) and therefore these studies did not qualify for calculation of the contribution of food groups to the exposure. A summary of the median values calculated from the average contribution of each food group across the dietary surveys and the range of the lowest and highest average contribution is shown in Table 15.

Grains and grain-based foods made the largest contribution to the dietary exposure to the sum of T-2 and HT-2 toxin in all age classes. Although the sum of concentrations of T-2 and HT-2 toxins in bread was relatively low, due to the high consumption bread had the highest contribution to exposure

in all age classes, except infants. Other important contributors were fine bakery wares, grain milling products and breakfast cereals. The contribution of fine bakery wares and breakfast cereals was higher in children and adolescents compared to adults. The contribution of pasta was important in the European countries with high pasta consumption. Beer was also an important contributor to the exposure in the adult population, especially in the European countries with high beer consumption. However, since only a limited number of occurrence data on beer was available this observation should be interpreted with caution. Vegetables and vegetable products had only a minor contribution in all age classes and in all dietary surveys. The contribution of composite food is less relevant as in most of the dietary surveys composite foods (dishes) were disaggregated in their main ingredients (flour, pasta, bread, vegetables etc.). The highest contribution of the sum of T-2 and HT-2 toxins from snack food was observed in adolescents and children.

In infants, the highest contributors were the foods for infants and small children mainly representing cereal-based foods. The contribution of infant food also accounted for up to 29 % in toddlers. Other contributors in infants were bread, pasta, grain milling products and fine bakery products. The contribution of breakfast cereals was very low (maximum 0.6 %).

**Table 15:** Contribution (%) of the different food groups to chronic dietary exposure to the sum of T-2 and HT-2 toxins in lower-bound and upper-bound scenarios. Median values across dietary surveys and range of the average contribution are presented.

| Food group | Median contribution across dietary surveys (Lowest average contribution – Highest average contribution) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
| **Lower-bound** | | | | | | | % | | | | | | | |
| Grains for human consumption | 3.3 | (3.1-3.5) | 3.0 | (0.0-9) | 2.2 | (0.52-9.4) | 2.1 | (0.78-9.9) | 2.6 | (0.7-11) | 2.2 | (0.54-12) | 1.9 | (0.43-6.6) |
| Grain milling products | 11 | (6.7-15) | 7.1 | (0.06-13) | 6.3 | (0.0-30) | 3.8 | (0.2-27) | 4.7 | (0.1-24) | 8.7 | (0.2-26) | 7.6 | (0.26-27) |
| Bread and rolls | 13 | (0.72-25) | 30 | (8.5-42) | 23 | (9.9-40) | 24 | (13-36) | 23 | (13-36) | 34 | (28-42) | 35 | (25-45) |
| Pasta | 17 | (3.4-30) | 7.0 | (3.7-34) | 7.5 | (1.4-33) | 9.7 | (1.1-27) | 4.8 | (0.1-26) | 9.9 | (1.0-31) | 9.3 | (0.9-33) |
| Breakfast cereals | 0.30 | (0.0-0.6) | 11 | (1.5-28) | 27 | (6.2-45) | 26 | (2.4-45) | 12 | (3.3-33) | 6.9 | (1.3-10) | 5.0 | (2.6-12.3) |
| Fine bakery wares | 13 | (0.0-26) | 13 | (5.9-25) | 18 | (1.6-26) | 15 | (1.8-27) | 12 | (1.5-22) | 11 | (1.8-16) | 14 | (2.2-19) |
| Snack food | 2.9 | (0.0-5.8) | 5.1 | (1.5-15.4) | 8.7 | (2.1-13) | 9.3 | (2.1-23.3) | 4.0 | (1.0-11) | 0.37 | (0-1.7) | 0.26 | (0.0-1.0) |
| Vegetables and vegetable products | 0.28 | (0.0-0.57) | 0.28 | (0.0-3.3) | 0.3 | (0.09-1.7) | 0.36 | (0.09-2) | 0.88 | (0.07-3.8) | 1.0 | (0.39-3.8) | 1.2 | (0.38-5.2) |
| Composite food | 0.0 | (0.0-0.0) | 1.0 | (0.0-2.1) | 1.8 | (0.0-19) | 1.4 | (0.0-14) | 0.6 | (0.0-11) | 0.14 | (0.0-1.1) | 0.06 | (0.0-1.0) |
| Beer | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.3)[a] | 0.10 | (0.0-1.1)[b] | 0.77 | (0.0-11) | 26 | (6.8-48) | 20 | (2.6-38) | 17 | (0.86-38) |
| Food for infants and small children | 40 | (20-59) | 20 | (3.9-43) | 0.5 | (0.0-8.6) | 0.0 | (0.0-0.32) | 0.0 | (0.0-0.14) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.09) |
| Products for special nutritional use | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.11) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.06) | 0.0 | (0.0-0.05) | 0.0 | (0.0-0.2) |
| **Upper-bound** | | | | | | | % | | | | | | | |
| Grains for human consumption | 8.0 | (5.6-10) | 4.5 | (0.0-12) | 3.4 | (0.40-10) | 4.0 | (1.7-10) | 4.3 | (1.5-9.8) | 3.5 | (1.3-7.7) | 3.0 | (1.1-5.5) |
| Grain milling products | 19 | (17-21) | 7.1 | (0.10-16) | 7.8 | (0.0-27) | 4.4 | (0.32-28) | 6.8 | (0.17-29) | 10 | (0.35-30) | 8.8 | (0.55-31) |
| Bread and rolls | 21 | (2.6-39) | 49 | (22-58) | 44.0 | (18-57) | 43 | (27-57) | 48 | (32-58) | 52 | (49-63) | 54 | (46-66) |
| Pasta | 14 | (1.9-26) | 5.2 | (2.2-21) | 5.6 | (1.2-18) | 6.3 | (0.70-13) | 3.5 | (0.08-12) | 4.6 | (0.67-14) | 4.0 | (0.63-14) |
| Breakfast cereals | 0.12 | (0.0-0.2) | 5.8 | (0.50-12) | 14.0 | (1.9-27) | 13 | (0.9-21) | 5.8 | (1.2-18) | 2.3 | (0.38-5.9) | 1.7 | (0.85-7.1) |
| Fine bakery wares | 9.6 | (0.0-19) | 12 | (7.6-19) | 16.0 | (1.7-25) | 14 | (1.9-20) | 12 | (1.8-16) | 9.2 | (2.1-16) | 9.4 | (2.8-19) |
| Snack food | 1.2 | (0.0-2.5) | 2.9 | (0.70-6.6) | 4.3 | (10-6.2) | 4.6 | (1-8.9) | 2.3 | (0.38-5.6) | 0.18 | (0-0.62) | 0.14 | (0-0.40) |
| Vegetables and vegetable products | 0.11 | (0.0-0.21) | 0.12 | (0.0-1.3) | 0.1 | (0.0-0.85) | 0.13 | (0.0-1.0) | 0.39 | (0.0-1.4) | 0.31 | (0.21-1.2) | 0.38 | (0.20-1.5) |
| Composite food | 0.0 | (0.0-0.0) | 2.7 | (0.0-5.6) | 4.7 | (0.0-44) | 3.3 | (0.0-33) | 1.2 | (0.0-25) | 0.28 | (0-1.9) | 0.11 | (0.0-1.7) |
| Beer | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.20) [a] | 0.0 | (0.0-0.60)[b] | 0.35 | (0.0-5.4) | 12 | (3.6-24) | 8.5 | (2.8-20) | 7.8 | (1.5-20) |
| Food for infants and small children | 27 | (9.7-45) | 12 | (1.6-29) | 0.2 | (0.0-4.8) | 0.0 | (0.0-0.15) | 0.0 | (0.0-0.09) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.0) |
| Products for special nutritional use | 0.0 | (0.0-0.0) | 0.0 | (0-0.08) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.0) | 0.0 | (0.0-0.10) |

(a): Contribution made exclusively by alcohol-free beer and malt drink; (b): Contribution mainly made by alcohol-free beer and malt drink.

### 6.1.5. Dietary exposure to the sum of T-2 and HT-2 toxin for specific groups

Vegetarian diets include more cereal and cereal-based products and therefore it was considered that T-2 and HT-2 toxin exposure in this consumer group could be higher. The Comprehensive Database contains only limited data on food consumption of vegetarians. Dietary surveys with at least 15 adult vegetarians in each survey were selected; dietary exposure was calculated and compared to the exposure of all subjects included in the respective dietary study. Generally, higher mean and 95$^{th}$ percentile exposures were observed in vegetarians compared to total population within the same dietary survey (Table 16). The limited data on vegetarians do not indicate significant difference in the dietary exposure to the sum of T-2 and HT-2 toxins between the vegetarians and the general population.

**Table 16:** Comparison of the chronic dietary exposure to the sum of T-2 and HT-2 toxins (ng/kg b.w. per day) between adult vegetarians and total adult population.

| Dietary survey | N Veget. | N All | ng/kg b.w. per day | | | |
|---|---|---|---|---|---|---|
| | | | Mean exposure | | 95$^{th}$ percentile exposure | |
| | | | Veget. | All | Veget. | All |
| **Lower-bound** | | | | | | |
| FI/2 | 39 | 1575 | 6.0 | 5.6 | 18[(a)] | 14 |
| FR | 15 | 2276 | 6.7 | 4.3 | 17[(a)] | 11 |
| DE/4 | 237 | 10419 | 7.1 | 6.1 | 19 | 16 |
| SE/1 | 18 | 1210 | 6.8 | 5.4 | 23[(a)] | 11 |
| UK | 77 | 1724 | 7.7 | 7.5 | 17 | 18 |
| **Upper-bound** | | | | | | |
| FI/2 | 39 | 1575 | 13 | 14 | 30[(a)] | 26 |
| FR | 15 | 2276 | 15 | 13 | 31[(a)] | 24 |
| DE/4 | 237 | 10419 | 13 | 12 | 27 | 24 |
| SE/1 | 18 | 1210 | 17 | 13 | 43[(a)] | 24 |
| UK | 77 | 1724 | 17 | 15 | 32 | 28 |

N: number of subjects in the dietary surveys; Veget.: adult vegetarians; All: total adult population; b.w.: body weight.
(a): The 95$^{th}$ percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

## 6.2. Animal exposure assessment

### 6.2.1. Estimating of the levels of the sum of T-2 and HT-2 toxins intake in feeds for farm livestock

For many livestock in Europe, feeds are supplied in the form of commercially produced blends or compound feeds, and as indicated in Section 4.2.14 some data on the T-2 and HT-2 toxin contents of compound feeds were provided by the European countries. However, the numbers of samples were generally small, and for many the product description was insufficient to identify the target species. As a result, these data have not been used to estimate exposure. Instead, the CONTAM Panel have identified example diets for a range of farm livestock and companion animals (Appendix C, Section C2) based on general principles and practices for animal feeding. Using these estimates, together with feed intakes and the mean occurrence data provided by the European countries for individual feed materials (Appendix B, Table B1), the mean LB and UB levels of the sum of T-2 and HT-2 toxins in diets for different categories of livestock have been calculated (Appendix C, Section C2). These mean LB and

UB dietary concentrations, together with estimates of feed intake described above (Section 5.2.) have been used in the exposure estimations below.

As discussed above (Section 5), a wide range of feeding systems and feeds for livestock are used in Europe, and it must be stressed that the estimated feed intakes or diet composition used in estimating exposure are not 'average' diets, nor are they an attempt to describe 'worst case' scenarios. Rather, they are intended to provide an indication of likely exposure to T-2 and HT-2 toxins across a range of feeding systems in Europe. In some situations, exposure may he higher than described below. For example, concentrations of T-2 and HT-2 toxins are generally higher in oat grains and oat by-products than other cereal grains (Section 4.2.14.), and since these are important livestock feeds, particularly in the Nordic countries, exposure to these toxins may be higher here than estimated in this Scientific Opinion.

The exposure estimates below are for adult animals only, with the exception of pigs, since they are known to be sensitive to the exposure to T-2 and HT-2 toxins in feed. Therefore, the exposure to the sum of T-2 and HT-2 toxins for piglets has also been estimated.

#### 6.2.1.1. Ruminants

##### 6.2.1.1.1. Dairy cows

Since levels of T-2 and HT-2 toxins in grass and legume-based forages are low or non-existent (see Section 4.1.3 and Appendix B, Table B1), it has been assumed that they make no contribution to exposure. However, there is some evidence of T-2 and HT-2 toxins in maize silage in the data provided by the European countries (Appendix B, Table B1), although levels are generally low. Furthermore, since maize silage is unlikely to account for more than 50 % of the total intake (AFRC, 1993; McDonald, 2011), exposure from this source is likely to be low. Table 17 provides estimates of intake by dairy cows of the sum of T-2 and HT-2 toxins from non-forage feeds, at three levels of milk production (30, 40 and 50 kg milk/day). The calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets are presented in (Appendix C, Table C4) and feed consumption in Section 5.2.1.

**Table 17:** Estimated lower-bound and upper-bound exposure by lactating dairy cows to the sum of T-2 and HT-2 toxins at three levels of milk production (30, 40 and 50 kg milk/day) (µg/day, µg/kg b.w. per day and µg/kg milk).

| | Exposure | | |
|---|---|---|---|
| | Milk yield/Non-forage feed intake (kg/day) | | |
| | **30/8.4** | **40/12** | **50/16** |
| | **µg/day** | | |
| Lower-bound | 81 | 116 | 155 |
| Upper-bound | 180 | 257 | 342 |
| | **µg/kg b.w. per day** | | |
| Lower-bound | 0.13 | 0.18 | 0.24 |
| Upper bound | 0.28 | 0.39 | 0.53 |
| | **µg/kg milk** | | |
| Lower-bound | 2.7 | 2.9 | 3.1 |
| Upper-bound | 6.0 | 6.4 | 6.8 |

b.w.: body weight.

Based on the feed intakes reported by AFSSA (2009) (Appendix C, Table C5) and the mean LB and UB concentrations for the sum of T-2 and HT-2 toxins reported in Appendix B (Table B1), estimates of

exposure were calculated for lactating dairy cows (milk yields 30, 40 and 50 kg/day) fed different forage based diets (Table 18).

**Table 18:** Estimates of lower-bound and upper-bound exposure to the sum of T-2 and HT-2 toxins by lactating dairy cows (milk yields 30, 40 and 50 kg/day) fed diets based on different forages, as µg/day, µg/kg b.w. per day and µg/kg milk (see also Appendix C, Table C5).

| | **Exposure** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Forage type** | | | | | | | | | | | |
| | **Maize silage** | | | **Grass silage** | | | **Hay** | | | **Pasture grass** | | |
| | **Milk yield (kg/day)** | | | | | | | | | | | |
| | **30** | **40** | **50** | **30** | **40** | **50** | **30** | **40** | **50** | **30** | **40** | **50** |
| | **µg/day** | | | | | | | | | | | |
| Lower-bound | 262 | 454 | 428 | 134 | 351 | 476 | 304 | 480 | 421 | 46 | 274 | 486 |
| Upper-bound | 861 | 1082 | 1053 | 164 | 421 | 570 | 362 | 573 | 515 | 54 | 324 | 576 |
| | **µg/kg b.w. per day[(a)]** | | | | | | | | | | | |
| Lower-bound | 0.4 | 0.7 | 0.66 | 0.2<br>1 | 0.5<br>4 | 0.7<br>3 | 0.4<br>7 | 0.7<br>4 | 0.6<br>5 | 0.0<br>7 | 0.4<br>2 | 0.75 |
| Upper-bound | 1.3 | 1.7 | 1.6 | 0.2<br>5 | 0.6<br>5 | 0.8<br>8 | 0.5<br>6 | 0.8<br>8 | 0.7<br>9 | 0.0<br>8 | 0.5<br>0 | 0.89 |
| | **µg/kg milk** | | | | | | | | | | | |
| Lower-bound | 8.7 | 11 | 8.6 | 4.5 | 8.8 | 9.5 | 10 | 12 | 8.4 | 1.5 | 6.8 | 9.7 |
| Upper-bound | 29 | 27 | 21 | 5.5 | 11 | 11 | 12 | 14 | 10 | 2 | 8 | 12 |

b.w.: body weight.
(a): b.w. of 650 kg.

As discussed earlier, it was assumed that the concentrations of the sum of T-2 and HT-2 toxins in grass silage, hay and grazed grass did not contribute to exposure in this estimate. Mean levels of the sum of T-2 and HT-2 toxins in maize silage (Appendix B, Table B1) were included in the calculations. This illustrates the effect that both level of milk production and type of diet can have on exposure to the sum of T-2 and HT-2 toxins. In particular, the inclusion of maize silage and/or maize grain can result in increased exposure to the sum of T-2 and HT-2 toxins (Table 18).

#### 6.2.1.1.2. Beef cattle

Based on the feed and gain data used for a 400 kg beef (fattening) cattle fed a grass silage and supplemented with non-forage feed materials (Section 5.2.2.), estimates of exposure for the sum of T-2 and HT-2 toxins are given in Table 19. The exposure to the sum of T-2 and HT-2 toxins was also estimated for the beef cattle reared on a cereal beef system (see details in Section 5.2.2). The calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets are presented in Appendix C, Table C4.

**Table 19:** Estimated lower-bound and upper bound exposure to the sum of T-2 and HT-2 toxins by 400 kg body weight fattening beef cattle reared on grass silage plus non-forage feeds system or a cereal beef system (µg/day and µg/kg b.w. per day).

| | Exposure | |
|---|---|---|
| | Grass silage + non-forage feeds | Cereal beef |
| | Non-forage feeds consumed (kg dry matter/day) | |
| | 1.9 | 7.1 |
| | µg/day | |
| Lower-bound | 10 | 154 |
| Upper-bound | 116 | 303 |
| | µg/kg b.w. per day | |
| Lower-bound | 0.020 | 0.39 |
| Upper-bound | 0.29 | 0.76 |

b.w.: body weight.

#### 6.2.1.1.3. Sheep and goats

The exposure estimates for lactating sheep, fattening goats and milking goats (milk yield 6 kg/day) in Table 20 are based on estimated feed intakes presented in Section 5.2.3. The calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets are given in Appendix C, Table C4.

**Table 20:** Estimated lower-bound and upper bound exposure to the sum of T-2 and HT-2 toxins by lactating sheep, milking goats and fattening goats (µg/day and µg/kg b.w. per day).

| | Exposure | | |
|---|---|---|---|
| | 80 kg lactating sheep | 60 kg milking goat | 40 kg fattening goat |
| | µg/day | | |
| Lower-bound | 18 | 161 | 36 |
| Upper-bound | 35 | 200 | 48 |
| | µg/kg b.w. per day | | |
| Lower-bound | 0.30 | 2.7 | 0.91 |
| Upper-bound | 0.59 | 3.3 | 1.2 |
| | µg/kg milk | | |
| Lower-bound | -(a) | 27 | - |
| Upper-bound | -(a) | 33 | - |

b.w.: body weight; -: not applicable
(a): Feeding twin lambs.

### 6.2.1.2. Pigs

Based on feed consumption data for pigs presented in Section 5.2.4 and the calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets presented in Appendix C, Table C6, estimates of exposure to the sum of T-2 and HT-2 toxins are given in Table 21.

**Table 21:** Estimated lower-bound and upper-bound exposure of pigs to the sum of T-2 and HT-2 toxins (µg/day and µg/kg b.w. per day).

| | Exposure | | |
|---|---|---|---|
| | **Piglets** | **Fattening pigs** | **Lactating sows** |
| | **µg/day** | | |
| Lower-bound | 5.0 | 28 | 59 |
| Upper-bound | 25 | 87 | 168 |
| | **µg/kg b.w. per day** | | |
| Lower-bound | 0.27 | 0.28 | 0.30 |
| Upper-bound | 1.3 | 0.87 | 0.84 |

b.w.: body weight.

#### 6.2.1.3. Poultry

For broilers (fattening chickens), laying hens, turkeys and ducks for fattening, estimated exposures to the sum of T-2 and HT-2 toxins are given in Table 22. They are based on feed consumption data presented in Section 5.2.5 and the calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets given in Appendix C, Table C6.

**Table 22:** Estimated lower-bound and upper bound exposure of poultry to the sum of T-2 and HT-2 toxins (µg/day and µg/kg b.w. per day).

| | Exposure | | | |
|---|---|---|---|---|
| | **Broilers[(a)]** | **Laying hens** | **Turkeys for fattening** | **Ducks for fattening** |
| | **µg/day** | | | |
| Lower-bound | 1.9 | 1.0 | 3.2 | 1.1 |
| Upper-bound | 3.5 | 3.1 | 11 | 3.7 |
| | **µg/kg b.w. per day** | | | |
| Lower-bound | 0.95 | 0.49 | 0.27 | 0.35 |
| Upper-bound | 1.8 | 1.6 | 0.95 | 1.2 |

b.w.: body weight.
(a): Chickens for fattening.

#### 6.2.1.4. Rabbits

Estimates of exposure for rabbits are based on a typical French compound feed formulation. The feed consumption for rabbits is presented in Section 5.2.6 and the mean LB and UB concentrations for the sum of T-2 and HT-2 toxins in individual feed materials used for exposure calculations in Appendix B, Table B1. For a 2 kg rabbit, it is estimated that the LB and UB exposure to the sum of T-2 and HT-2 toxins would be 2.0 and 3.4 µg/day, respectively. Expressed on a body weight basis, this is equivalent to 0.98 and 1.7 µg/kg b.w. per day, respectively.

#### 6.2.1.5. Farmed fish

Based on the feed consumption presented in Section 5.2.7 and the calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the example diets given in Appendix C, Table C7, it is estimated that the LB and UB exposure to the sum of T-2 and HT-2 toxins by a 2 kg salmon would be 0.19 and 0.37

µg/day, respectively. Expressed on a body weight basis this is equivalent to 0.090 and 0.19 µg/kg b.w. per day, respectively.

### 6.2.2. Estimation of the sum of T-2 and HT-2 intake in feed by companion animals

#### 6.2.2.1. Dogs and cats

Based on the intakes presented in Section 5.2.8.1., the estimated exposures to the sum of T-2 and HT-2 toxins for dogs and cats are given in Table 23. The calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets are given in Appendix C, Section C2.5.1.

**Table 23:** Estimated lower-bound and upper bound exposure of cats and dogs to the sum of T-2 and HT-2 toxins (µg/day and µg/kg b.w. per day).

| | Exposure | |
|---|---|---|
| | **Dogs** | **Cats** |
| | **µg/day** | |
| Lower bound | 5.7 | 0.81 |
| Upper bound | 9.6 | 1.4 |
| | **µg/kg b.w. per day** | |
| Lower bound | 0.23 | 0.20 |
| Upper bound | 0.38 | 0.34 |

b.w.: body weight.

#### 6.2.2.2. Horses

For a 450 kg horse undergoing moderate activity for which forages account for 50 % of intake, the LB and UB exposure to the sum of T-2 and HT-2 toxins are estimated to be 498 and 538 µg/day, respectively. Expressed on a body weight basis this is equivalent to 1.1 and 1.2 µg/kg b.w. per day, respectively. The exposure for horse was calculated using the feed consumption presented in Section 5.2.8.2 and the calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in the estimated example diets presented in Appendix C, Table C8.

## 7. Hazard identification and characterisation

### 7.1. Toxicokinetics

The toxicokinetics of T-2 and HT-2 toxins were reviewed by the JECFA (FAO/WHO, 2001) and in recent review papers of the toxicokinetics of trichothecenes (Dohnal et al., 2008; Wu et al., 2010; Li et al., 2011). No specific information on the toxicokinetics of HT-2 toxin in animals is available, other than that reported with T-2 toxin.

The JECFA (FAO/WHO, 2001) concluded in their assessment that more comparative studies of the toxicokinetics of T-2 toxin are needed in rodents, cats and pigs to clarify potential differences between species. Only very few new data on the toxicokinetics in these species have been published since the JECFA review. Wu et al. (2011) recently published a study comparing the metabolism of T-2 toxins in hepatocytes and liver microsomes from several species. The study was considered to be inconclusive by the CONTAM Panel and is not further discussed.

### 7.1.1. Experimental animals

#### 7.1.1.1. Absorption

T-2 toxin is rapidly absorbed in rodents after oral and inhalational exposure. The plasma radioactivity after oral administration of $^{3}$H-T-2 toxin to mice peaked after 30 minutes and T-2 toxin is rapidly metabolised and excreted in faeces and urine (Matsumoto et al, 1978; Doi et al., 2006; Wu et al., 2010). No information on the total bioavailability was given.

#### 7.1.1.2. Distribution

T-2 toxin itself is rapidly removed from plasma, the plasma half-life being less than 20 minutes (reviewed in FAO/WHO 2001; SCF, 2001; Wu et al., 2010). Orally administered T-2 toxin in mice and rats was rapidly distributed to liver, kidneys and other organs without any particular accumulation. T-2 toxin is also able to cross the placenta and reach fetal tissues.

#### 7.1.1.3. 7.1.1.3. Metabolism

T-2 toxin is metabolised in the intestines and by liver and other tissues. T-2 toxin is rapidly metabolised to a range of different compounds in rodents, one of the major metabolites being HT-2 toxin. Metabolic transformations of T-2 toxin include deacetylation, acetylation, hydroxylations, de-epoxidation and glucuronide conjugations (Figure 18). Carboxyesterases have been shown to be responsible for the transformations of T-2 toxin to HT-2 toxin and neosolaniol in white blood cells (Johnsen et al., 1988). The major metabolites in urine from rats given radioactively labelled T-2 toxin (0.15 or 0.6 mg/kg b.w.) by intravenous (*i.v.*), oral or dermal administration, were 3'-hydroxy-HT-2 toxin, T-2 tetraol and one unknown metabolite, and in faeces de-epoxy-T-2 tetraol, 3'-hydroxy-HT-2 toxin and 3 unknown metabolites, including one major metabolite in faeces tentatively identified as de-epoxy 3'-hydroxy-HT-2 toxin. The relative formation of the different metabolites was independent of dose, but dependent on route of exposure (Pfeiffer et al., 1988). In order to elucidate the further metabolism of 3'-hydroxy-T-2 toxin and 3'-hydroxy-HT-2 toxin found in cows and mice liver homogenates, Wistar rats were exposed to these metabolites and four new metabolites were found in the excreta; 3-de-epoxy-3'-hydroxy-HT-2 toxin, de-epoxy-3'-hydroxy-T-2 triol, de-epoxy-15-acetyl-T-2 tetraol and de-epoxy-2 tetraol (Yoshizawa et al., 1985). Most of the metabolites formed in animal tissues retain the epoxy-moiety.

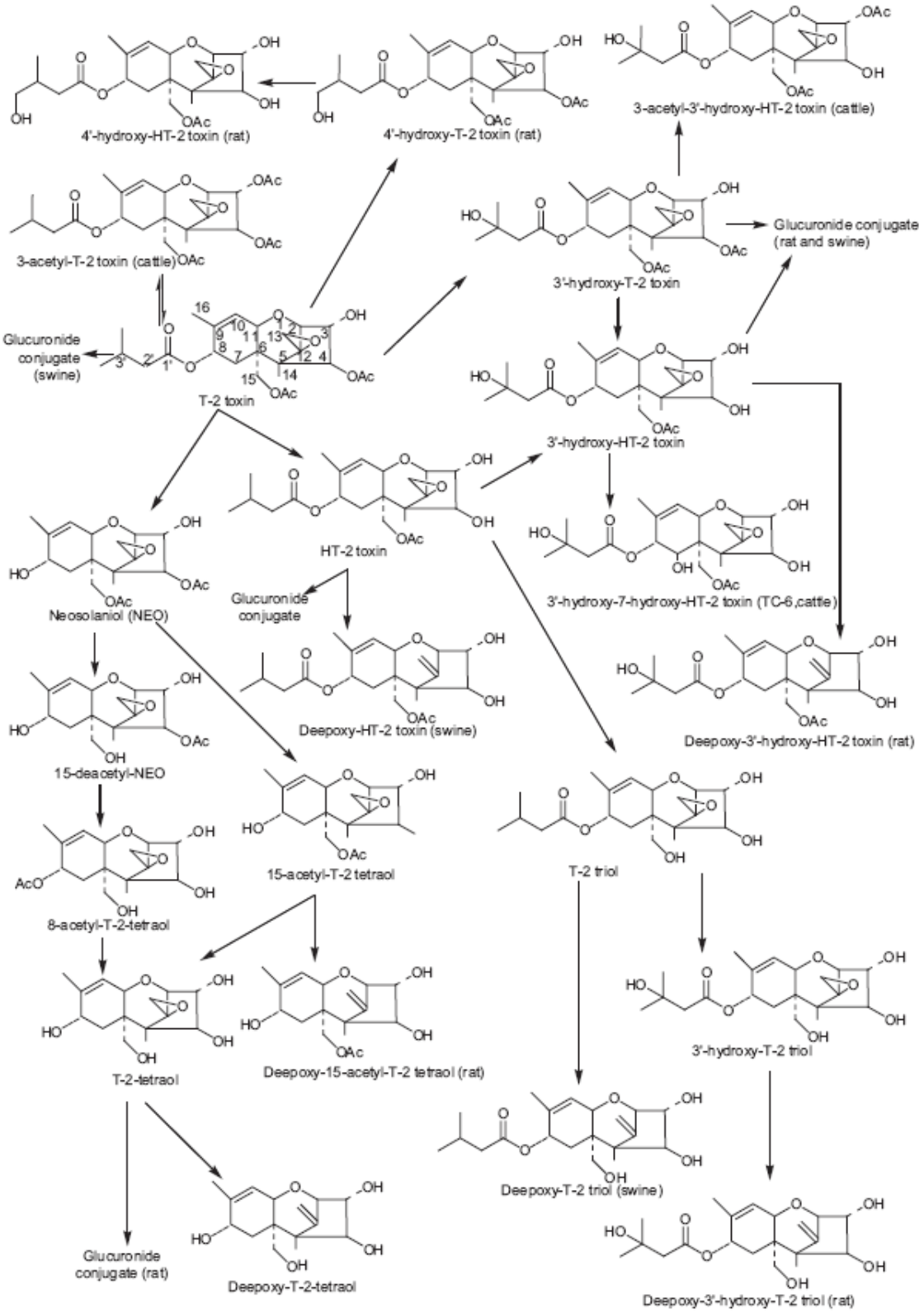


**Figure 18:** Proposed metabolic pathways of T-2 toxin in animals (from Wu et al., 2010).

However, the de-epoxidation of T-2 toxin and its metabolites is an important detoxification mechanism. The de-epoxide of 3'-hydroxy-HT-2 toxin and other de-epoxide metabolites such as de-epoxide T-2 tetraol have been found in rat faeces. De-epoxide metabolites have not been found in *in vitro* studies with liver preparates, plasma or intestines not including intestinal micro-organisms (reviewed in Wu et al., 2010). The de-epoxidation in rats may therefore occur in the hindgut and it is unclear whether the presence of the 3'-hydroxy-HT-2 de-epoxide in rat urine is a result of rats being coprophagous. In addition, T-2 toxin and all the metabolites are extensively glucuronide conjugated.

#### 7.1.1.4. Excretion

T-2 toxin and its metabolites are excreted via urine and bile, but the urine:faeces ratio depends on the species (FAO/WHO, 2001; SCF 2001; Wu et al., 2010). T-2 toxin was rapidly excreted in faeces and urine at a ratio of 4-5:1 in mice and rats. In mice 62 % of the radioactivity was recovered in the excreta within 24 hours, increasing to 69 % after 72 hours, and the pattern in rats was similar (Matsumoto et al., 1978). In guinea pigs given an intramuscular injection of 1 mg/kg b.w. of radioactively labelled T-2 toxin, 75 % of the dose was recovered in urine and faeces at a ratio of 4:1 within 5 days. The concentration peaked in urine after 24 hours (Pace et al., 1985). In rats given either 0.15 or 0.6 mg/kg b.w. of tritium-labelled T-2 toxin, > 95 % of the administered dose was recovered in faeces or urine within 72 hours (Pfeiffer et al., 1988). The excretion was similar in rats given *i.v.* injections with 0.15 mg T-2 toxin/kg b.w. However, the excretion within the same time period was considerably lower in rats given 0.6 mg T-2 toxin/kg b.w. by *i.v.* suggesting potentially saturable metabolism or elimination pathways at doses that are relevant to those used in the toxicity studies considered in this evaluation. After dermal application, < 60 % of the either dose (0.15 or 0.6 mg/kg b.w.) was eliminated in urine or faeces within 72 hours (Pfeiffer et al., 1988). Enterohepatic recycling of T-2 toxin and its glucuronide-conjugated metabolites have been found in rats given T-2 toxin intraduodenally (Coddington et al., 1989).

### 7.1.2. Humans

There is no information available on the toxicokinetics of T-2 or HT-2 toxins in humans. The main metabolic pathway *in vitro* is via deacetylation in C4, which results in the formation of HT-2 toxin. This has been found to occur in incubations with human liver microsomes (FAO/WHO, 2001) as well as in human skin samples incubated with T-2 toxin. In the latter four other minor metabolites including T-2 tetraol, were also found (Wu et al., 2010). Human erythrocytes formed both HT-2 toxin and neosolaniol whereas human white cells produced only HT-2 toxin as the primary metabolite. The enzymes responsible for hydrolysis of T-2 toxin were all identified as carboxylesterases by use of specific inhibitors (Johnsen et al. 1988). In primary cultures of normal human lung fibroblasts, HT-2 toxin was the only metabolite detected, while neosolaniol was formed as a second metabolite in primary cultures of human renal proximal tubule epithelial cells (Königs et al., 2009).

### 7.1.3. Farm animals

The toxicokinetics of T-2 toxin in farm animals were not specifically addressed in the JECFA evaluation (FAO/WHO, 2001). However, studies in farm animals were considered. Only one new study addressing the toxicokinetics in farm animals (pigs) has been come available since then.

#### 7.1.3.1. Ruminants

After 72 hours, nearly 72 % and 29 % of the $^{3}$H-T-2 toxin (156.9 mg) administered to a 375 kg lactating Jersey cow were eliminated via faeces or urine, respectively (Yoshizawa et al., 1981). Only 0.2 % was recovered in milk. Maximum levels of radioactivity were reached 44 hours after administration in faeces (equivalent to 9.2 mg T-2 toxin/kg), after 16 hours in urine (5.5 mg/kg) after 16 hours in milk (37

µg/kg) and after 8 hours in plasma (64 µg/kg) and the radioactivity in urine, milk and plasma decreased with half-lives of 12, 24 and 16 hours, respectively.

When a Holstein cow (365 kg) was given a single oral dose of T-2 toxin (200 mg) trace amounts of T-2 toxin were detected in blood up to 24 hours. In addition, HT-2 and T-2 tetraol as well as de-epoxy-T-2-tetraol were detected in blood and urine (Chatterjee et al., 1986). Further metabolites, namely 3'-hydroxy-T-2 toxin, 3'-hydroxy- HT-2 toxin and respective iso- and de-epoxy-forms were detected in urine.

Following an *i.v.* administration of 0.6 or 1.2 mg/kg of T-2 toxin to female calves (201-268 kg) the disappearance of the parent T-2 toxin followed a two-compartment open model. The mean elimination half-life was 17.4 minutes and apparent volume of distribution was 0.376 L/kg (Beasley et al., 1986). The fraction of T-2 toxin eliminated as parent compound in the urine was negligible.

The metabolic pathway of T-2 toxin in ruminants was reviewed by Wu et al. (2010). The authors summarised that 3'-hydroxy-T-2 toxin, 3-acetyl-3'-hydroxy-HT-2 toxin, 3'-hydroxy- HT-2 toxin, de-epoxy-T-2 tetraol, 3'-hydroxy-7-hydroxy-HT-2 toxin, acetyl-T-2 toxin and acetyl-HT-2 toxin are the typical metabolites in bovines. HT-2 toxin, neosolaniol, 4-deacetylneosolaniol, 3-acetyl-3'-hydroxy-HT-2 toxin, 3'-hydroxy-HT-2 toxin, de-epoxy-T-2 tetraol and 3'-hydroxy-7-hydroxy- HT-2 toxin are the main metabolites detected in urine and rumen fluid of bovines. Interconversions between acetylation and deacetylation in the metabolism of T-2 toxin can occur in bovines.

#### 7.1.3.2. Pigs

Following intra-aortal administration in swine (26-66 kg), the disappearance of the parent compound T-2 toxin followed a two-compartment open model. The mean elimination half-life was 13.8 minutes and the mean apparent specific volume of distribution was 0.366 L/kg (Beasley et al., 1986). Even though a lethal oral dose (2.4 mg/kg b.w., n = 2) was administered, no parent T-2 toxin could be detected in plasma or urine, indicating a rapid first pass metabolism. The highest concentrations were found in lymphoid organs.

The percentages of the administered $^{3}$H-T-2 toxin, 18 hours after intubating one pig (7.5 kg live weight) with 0.1 mg $^{3}$H-T-2 toxin/kg b.w. were 0.7 % in muscle, 0.43 % in liver, 0.08 % in kidney, 0.06 % in bile, 22 % in urine and 25 % in faeces. In another pig dosed (intubated) with 0.4 mg $^{3}$H-T-2 toxin/kg b.w. the percentages amounted to 0.7, 0.29, 0.08, 0.14, 18 and 0.86 % in muscle, liver, kidney, bile, urine and faeces, respectively (Robison et al., 1979).

Four hours after *i.v.* administration of $^{3}$H-T-2 toxin (0.15 mg/kg b.w.) to pigs, the largest amount of radiolabelled T-2 toxin was found in the gastrointestinal tract (15-24 % of the dose) and 4.7-5.2 % of the dose was found in the remaining tissues, 2.9-3.2 % in muscle and 0.7-1.7 % in liver (Corley et al., 1986).

In pigs, glucuronide-conjugated products were found to be the main metabolites in urine. The glucuronides included HT-2 toxin, 3'-hydroxy-T-2 toxin, 3'-hydroxy-HT-2 toxin and T-2 toxin. The major free metabolites were 3'-hydroxy-T-2 toxin and T-2 triol in urine. In total, 21 metabolites were found in tissues and the gastrointestinal tract of pigs. De-epoxy metabolites were also identified in pigs.

#### 7.1.3.3. Poultry

For poultry T-2 toxin metabolism and toxicokinetics were recently reviewed by Dohnal et al. (2008), AFSSA (2009), Wu et al. (2010), and Li et al. (2011). T-2 toxin is usually metabolised and eliminated after ingestion, yielding more than 20 metabolites. The major metabolic reactions are hydrolysis, hydroxylation, de-epoxidation and conjugation. The most typical metabolites of T-2 toxin are HT-2 toxin (hydrolysis), T-2 triol, T-2 tetraol, neosolaniol, 3'-hydroxy HT-2 toxin, 3'-hydroxy T-2 toxin,

3'-hydroxy T-2 triol, dihydroxy HT-2 toxin, de-epoxy-3'-hydroxy T-2 toxin and de-epoxy-3'-hydroxy HT-2 toxin.

As in other species, T-2 toxin is distributed widely and quickly. In an experiment performed by Chi et al. (1978a) one-day-old broiler chicks were fed a diet containing 2 mg T-2 toxin/kg for 5 weeks and then intubated with a single dose of $^{3}$H-T-2 toxin of 0.5 mg/kg b.w. Maximum tissue concentrations of T-2 toxin and its metabolites were observed at 3 hours for the liver and kidneys and 4 to 6 hours for muscles, adipose tissue and oviducts.

In a similar experiment by Giroir et al (1991) a $^{3}$H-T-2 toxin preparation was administered to 21-day-old chickens and white Pekin ducks. There were few significant differences between the two species in tissue distribution (Table 24). The radioactivity reached maximum levels after 6-12 hours in liver, muscle and kidneys and decreased after.

**Table 24:** T-2 toxin and its metabolites expressed as T-2 toxin equivalents after single oral administration of T-2 toxin (Giroir et al., 1991).

| Species | Route | Dose (mg/kg b.w.) | Tissues | T-2 toxin equivalents (µg/kg) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 6 hours | 12 hours | 24 hours | 2 days |
| Chicken/ Duck | Oral | 0.5 | muscle | 30 | 30 | <10 | <10 |
| | | | liver | 130/90 | 30/40 | 10/<10 | <10 |
| | | | kidneys | 30 | 20 | <10 | <10 |

b.w.: body weight.

In 47 days old broiler chickens, $^{3}$H-T-2 toxin (a single dose of 1.6 mg/kg b.w.) was rapidly metabolised to more polar derivatives. Among the various metabolites, T-2 toxin, HT-2 toxin, neosolaniol and T-2 tetraol were detected. Furthermore, eight unknown derivatives were quantitatively more significant than the other metabolites found (Yoshizawa et al., 1980). T-2 toxin elimination is rapid (more than 50 % in 24 hours) and mainly faecal. Excreta contained 25 % toxin after 6 hours and 60 % after 24 hours. After 24 hours, the large intestine and caecum still contained 25 % of the administered T-2 toxin (Chi et al., 1978a; Giroir et al., 1991). About 80 % of orally administered T-2 toxin was metabolised and eliminated in the excreta within 48 hours (Yoshizawa et al., 1980).

While *in vitro*, chicken hepatic microsomes seemed to be less active than mammalian hepatic microsomes in biotransforming T-2 toxin (Knupp et al., 1987; Kobayashi et al., 1987), the metabolic patterns of T-2 toxin in duck and chicken excreta 18 hours after intraperitoneal (*i.p.*) injection showed an intense metabolic activity and 41 % of 3'-hydroxy-HT-2 toxin, 18 % of HT-2 toxin, 16.5 % of 3'-hydroxy-T-2 toxin, 10 % of 4-deacetylneosalaniol, 5 % of 4-acetoxy-T-2 tetraol and traces of T-2 tetraol, 8-acetoxy-T-2 tetraol, T-2 toxin, T-2 triol and 3-acetoxy-3'-hydroxy-HT-2 toxin were formed (Visconti and Mirocha, 1985). In the liver, non-metabolised T-2 toxin was most abundant, followed by 3'-hydroxy-HT-2 toxin and HT-2 toxin and T-2 triol in lesser quantities. Only traces of 4-deacetyl-neosalaniol, 4-acetoxy-T-2 tetraol, T-2 tetraol and T-2 toxin were detected (Visconti and Mirocha, 1985; Giroir et al., 1991).

### 7.1.4. Companion animals

Only studies dating from the 1980s (Sintov et al., 1986, 1987, 1988), as cited by the JECFA (FAO/WHO, 2001) describing the metabolism in dogs as consisting of Phase I hydrolysis and Phase II glucuronide conjugation could be identified. Consistent data on oral availability are not available. No information about the toxicokinetics in cats has been published. Cats are, however, known to lack glucuronidation capacity and could consequently be expected to be sensitive to the effects of T-2 toxin, since glucuronide conjugation is an important detoxification pathway in other species.

### 7.1.5. Carry over

The carry over of trichothecenes like T-2 toxin or HT-2 toxin from feed to animal derived food is not a major concern as there is no evidence for accumulation of the toxins in specific tissues of animals (e.g. elimination half-life 13.8 minutes in pigs (Beasley et al., 1986). T-2 toxin is extensively metabolised; often accompanied by a reduction of toxicity whereby T-2 toxin is quickly eliminated, mainly via faeces and urine in all investigated species. Therefore, feeding naturally contaminated batches to food producing animals is unlikely to result in significant contributions of T-2 toxin, HT-2 toxin or related metabolites to human exposure from the products of animal origin. Carry over of T-2 and HT-2 toxins from feed to food products of animal origin was not addressed in the previous assessments of the JECFA (FAO/WHO, 2001) or the SCF (SCF, 2001). No recent studies were identified on carry over of T-2 toxin and HT-2 toxin. Older studies are briefly described below.

The carry over of T-2 toxin radioactivity of one cow slaughtered 72 hours after administration of a single dose of $^{3}$H-T-2 toxin, which was calculated to be equivalent to 31.38 mg/kg feed, to muscle, heart, liver and milk was 0.0003, 0.0003, 0.0006 and 0.0004, respectively. The carry over factor was determined by dividing the tissue concentration by the feed concentration (Yoshizawa et al., 1981). Similarly, the carry over factor in swine given a dose equivalent to 1.25 mg $^{3}$H-T-2 toxin/kg feed and slaughtered 18 hours later was 0.002, 0.003 and 0.011 in muscle, heart and liver, respectively, based on the detected radioactivity (Robison et al., 1979 as calculated in Yoshizawa et al., 1981).

In poultry T-2 toxin and other trichothecenes are excreted mainly in their metabolised forms in eggs. Thus in hens, after a single administration of 0.25 mg/kg b.w. of T-2 toxin by gastric intubation, the maximum level of excretion in eggs was reached after 24 hours and only represents 0.175 % of the administered dose. After 2 and 7 days, eggs only contained 0.1 and 0.025 % of the administered dose, respectively. T-2 toxin and/or its metabolites have been reported in the yolk, egg white and shell membranes (Chi et al., 1978a). During chronic administration, the quantities excreted in eggs were higher than after a single administration. In hens, daily oral administration of 0.1 mg/kg b.w. of T-2 toxin for 8 days led to an accumulation of the T-2 toxin and/or its metabolites in the yolk, whereas accumulation levelled off for the egg white and the membranes after 3 days. When exposure ceased, the concentrations of T-2 toxin and its metabolites rapidly decreased in all parts of the egg. On average, the concentration in the edible parts of the egg appeared to be 0.9 µg with feed contaminated with 0.6 mg/kg of T-2 toxin, representing 0.56 % of the T-2 toxin dose administered daily (Chi et al., 1978a).

### 7.1.6. Conclusions

In general, the available information on toxicokinetics is incomplete for each animal species. Comparison of the metabolic profiles and toxicokinetics between species is hampered due to differences in e.g. experimental protocols, available analytical methods, LODs and the lack of available standards for several metabolites. Up to now, five biotransformation pathways of T-2 toxin involving hydrolysis (mainly to HT-2 toxin), hydroxylation, de-epoxidation, glucuronidation and acetylations have been described in different biological systems, resulting in a large number of different metabolites (see Figure 18). Altogether 21 metabolites have been described from studies with pigs by Wu et al. (2010). T-2 toxin and other trichothecenes are extensively de-epoxidated prior to absorption in ruminants. De-epoxide metabolites have also been found in plasma from rats. The main metabolic pathway in all species is via a rapid deacetylation in C-4, resulting in the formation of HT-2 toxin (the only metabolite isolated after incubation with liver, kidney or spleen microsomes from various animal species). This reaction is catalysed by a non-specific carboxyesterase found in blood plasma and in several tissues, primarily in the liver. Depending on the metabolic pathway, HT-2 toxin can be further deacetylated, hydroxylated or conjugated into a large number of metabolites including 3'-hydroxy HT-2 toxin, T-2 triol, 3'-hydroxy T-2 triol or 4-deacetylneosolaniol (which is converted into T-2 tetraol) and their respective glucuronide conjugates. T-2 toxin can also be directly hydroxylated into 3'-hydroxy T-2 toxin in the investigated species. For most of the metabolites of T-2 toxin, no or very limited toxicological information is available. Nevertheless, the de-epoxidation is considered to be an important

detoxification step. The available data show that the carry over of T-2 toxin or HT-2 toxin from feed to food products of animal origin is limited and hence contributes only to a negligible extent to human exposure.

### 7.2. Biochemical modes of action

As the other trichothecenes, T-2 toxin inhibits protein, RNA and DNA synthesis. Data obtained more recently also indicate that T-2 toxin induces apotosis, and in some cell types necrosis, as well as lipid peroxidation affecting cell membrane integrity. These cellular effects may contribute to the toxicity of T-2 toxin (FAO/WHO, 2001; Rocha et al., 2005).

#### 7.2.1. Effects on nucleic acids and protein synthesis

Both synthesis of DNA and RNA were inhibited by T-2 toxin in *ex vivo* cell cultures and *in vitro*. Inhibition of DNA and RNA synthesis by T-2 toxin have been reported at concentrations generally exceeding those that cause an inhibition of protein synthesis (FAO/WHO, 2001).

Various studies reported inhibition of protein synthesis in mammalian cell cultures treated with T-2 toxin *in vitro* (reviewed in FAO/WHO, 2001). *In vivo* inhibition of protein synthesis has also been shown in various organs of rodents that received *i.p*. injection of the T-2 toxin (Thompson and Wannemacher, 1990; FAO/WHO, 2001). *In vitro* studies suggest that T-2 toxin interacts with the peptidyl transferase, which is an integral of the 60S ribosomal subunit, thus inhibiting the transpeptidation of peptide-bond formation. The ultimate result is an inhibition of prolongation and termination of protein synthesis (Liao et al., 1976; Jaradat, 2005).

#### 7.2.2. Apoptosis

*In vitro* T-2 toxin causes apoptosis in various cell types like HL60, Jurkat, U937, Vero cells or human hepatoma cells (SCF, 2001; Bouaziz et al., 2006, 2008; Huang et al., 2007). Apoptosis was also reported *in vivo* lymphoid organs, haematopoeitic tissues, intestinal crypt, brain and skin (SCF, 2001; Grizzle et al., 2004; Sehata et al., 2004a). Apoptosis has also been observed in fetal tissues after *in utero* exposure of rodents (reviewed by Doi et al., 2008)

When T-2 toxin was used at concentrations ranging from 3 to 250 ng/mL, apoptosis was associated with DNA fragmentation and activation of several molecules such as procaspase and caspases-9, -3, -8 and -7 (Minervini et al., 2005; Bouaziz et al., 2006; Chaudhari et al. 2009a) increased expression of c-jun and c-fos, p53 and Bax (Holme et al., 2003; Albarenque and Doi, 2005; Chen et al., 2006) and in some studies the decrease of the anti-apoptotic factor Bcl-xL (Chen et al., 2008). In HL-60, HT-2 toxin (6.25 ng/mL) was also described to induce apoptosis (Holme et al., 2003). In the Jurkat T-cell line, 467 ng/mL of T-2 toxin induces necrosis rather than apoptosis. That was associated with minimal changes in the levels of cytochrome c, procaspase-3 and Bcl-2 (Nasri et al., 2006).

The mechanism of T-2 toxin induced apoptosis is still controversially discussed and two hypotheses have been proposed (Jaradat, 2005). DNA damage could be a secondary effect of protein synthesis inhibition or oxidative stress that can in turn activate mitochondrial apoptotic pathways. Indeed, mitochondria and reactive oxygen species (ROS) seem to have a crucial role in T-2 toxin induced apoptosis in human hepatoma cells (HepG2) and human cervix carcinoma cells (HeLa) *in vitro* (Bouaziz et al., 2008; 2009). As mentioned earlier, various studies report the release of pro-apoptotic factors from mitochondria and increased activity of specific proteases executing this specific apoptotic program. Moreover T-2 toxin increases the expression of p53, a pivotal apoptotic protein, and other proteins such as Bax, Bcl-2, cytochrome-c involved in mitochondrial apoptotic pathway (Doi et al., 2006; Chaudhari et al., 2009a).

Alternatively, apoptotic cell death could be due to the induction of stress-activated protein kinase (SAPK/JNK) and mitogen activated protein kinase (p38/MAPK) either as a secondary effect of protein inhibition or indirectly through lipid peroxidation with subsequent ROS production. It is well documented that T-2 toxin and other trichothecenes induce ribotoxic stress response in different tissues (Rocha et al., 2005). For example in rat keratinocyte, the expression of apoptosis related genes (c-jun and c-fos) and cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) mRNA was markedly elevated before the development of apoptosis (Albarenque and Doi, 2005; Doi et al., 2006). Oral treatment of pregnant rats with T-2 toxin also resulted in upregulated gene expression of c-jun in the rat liver and rat placenta as well as in the fetal liver, further suggesting the involvement of MAPK pathways finally inducing apoptosis. According to Shinozuka et al. (2009), T-2 toxin-induced damage to hepatocytes is mainly through oxidative stress followed by apoptotic cell death. Elevated expression levels of antioxidant genes such as hsp70 and superoxyde dismutase measured in the fetal brain of rats precede the apoptosis in the nervous system (Sehata et al., 2004a). Apoptotic cell death, observed in the human acute monocytic leukemia cell line, THP-1, was preceded by the activation of p38 kinase and is probably mediated by an activation of ATM/H2AX/Chk2 pathways (Rakkestad et al., 2010).

### 7.2.3. Effects on membranes and lipid peroxidation

*In vitro* studies in various cell lines demonstrated lipid peroxidation and the production of ROS. T-2 toxin is an amphophilic molecule, and thus it is thought to be taken up into the cells bilayer membrane and then induce lipid peroxidation by generating free radicals, and thereby damaging cellular membranes. Lipid peroxidation therefore indicates oxidative stress in cells. Furthermore, oxidative stress can be confirmed measuring levels of ROS or antioxidative molecules (such as glutathione (GSH)).

Several studies demonstrate increased ROS levels with subsequent lowering of GSH levels and increased concentrations of malondialdehyde (MDA) *in vitro* and *in vivo* (FAO/WHO, 2001; Vilà et al., 2002; Chaudhari et al., 2009a,b). Ascorbic acid, α-tocopherol and selenium (all substances which protect against free radicals) exerted a protective effect against the T-2 toxin mediated lipid peroxidation and therefore the authors concluded that free radicals are involved (Rizzo et al., 1994; Dvorska et al., 2007). In these experiments T-2 toxin was given either in the feed, 8.1 mg/kg feed, for 21 days in chicken or through a bolus of 3.6 mg/kg b.w. to rats.

Total antioxidant status was reduced and a slight but not significant elevation in plasma and liver MDA content was seen in chickens fed for 17 days a diet containing 10 mg/kg T-2 toxin. However, other studies indicate that T-2 toxin had no effects on glutathione peroxidase activity as well as on plasma and urinary MDA levels in pigs or chicken receiving diet contaminated with 3 and 10 mg T-2 toxin/kg feed (Frankic et al., 2006, 2008).

Taken together there is still some inconsistency among the results shown for the T-2 toxin mediated effects on oxidative stress markers. While several studies showed increased MDA levels, others reported no changes. The extent to which ROS accounts for the observed DNA damage remains also a subject of controversial findings in different cell and animal models. However, biotransformation of the different *in vitro* models used has not always been taken into account.

### 7.2.4. Conclusions

Most of the data on the biochemical mode of action are concerning the T-2 toxin while the biochemical mode of action of the HT-2 toxin has been poorly investigated. T-2 toxin inhibits protein, RNA and DNA synthesis. The more recent results also indicate that T-2 toxin induces apoptosis and lipid peroxidation affecting cell membrane integrity.

## 7.3. Toxicity in experimental animals

### 7.3.1. Acute toxicity

All the studies on acute toxicity were previously considered by the JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001) concluding that T-2 toxin has acute toxicity, with acute oral $LD_{50}$ values in rodents in the range of 5-10 mg/kg b.w. No oral $LD_{50}$ has been established for HT-2 toxin, but its *i.p.* $LD_{50}$ is in the same dose range as that for T-2 toxin in mice (5-10 mg/kg b.w.). In addition to a wide variety of non-specific signs of toxicity, a primary target of toxicity of T-2 toxin was the haematopoetic tissue (SCF, 2001). Toxicity of the gastrointestinal epithelium following acute exposure is also a systemic effect, as it is not only observed following oral, but also parenteral exposure (DeNicola et al., 1978; Pang et al., 1987).

Since the previous evaluations, two new acute toxicity studies have been performed. An acute toxicity study was performed on young Fisher 344 rats (McKean et al., 2006). Doses of 1.0, 2.15, 4.64 or 10 mg/kg b.w. of T-2 toxin were used. The higher doses of T-2 toxin resulted in acute toxic symptoms, such as refusal of food and diarrhoea shortly after the treatment (1-2 hours). Within 24 hours, 100 % mortality (5/5) was observed in animals treated with 4.64 and 10 mg/kg b.w. In animals treated with 2.15 mg/kg b.w. T-2 toxin severe symptoms appeared, such as refusal of food and bloody faeces, however no deaths were observed during the one-week study period. No apparent toxic symptoms were observed in animals treated with the lowest dose and the vehicle control. The $LD_{50}$ value was determined to be 3.71 mg/kg b.w. of T-2 toxin.

In the other study, ICR:CD-1 mice were exposed once to 10 mg/kg b.w. to T-2 toxin. Haematological and blood biochemical examinations and histopathological examination of the liver were done up to 48 hours after treatment. In addition, microarray analysis was done on the gene expression profile of the liver. In T-2 toxin-treated group, aspartate aminotransferarse (AST) and alanine aminotransferase (ALT) levels increased while total cholesterol, total protein, blood, glucose and fibrinogen levels decreased. Histopathologically, T-2 toxin induced hepatocyte modification, characterised by cellular swelling and, at the late stage, by pyknosis with condensed eosinophilic cytoplasm, respectively. Upregulated expression of oxidative stress and apoptosis-related genes and down regulated expression of lipid, glycogen and drug metabolism, and blood coagulation-related genes were observed (Shinozuka et al., 2009, available as an abstract only).

### 7.3.2. Sub-acute and sub-chronic toxicity

Short term toxicity studies showed that T-2 toxin administered orally to rats caused gastric lesions, and dystrophia (at low doses) and necrosis (at high doses) in the liver, kidney and heart as well as immunotoxicity (reviewed in FAO/WHO (2001) and SCF (2001)) (Table 25).

**Table 25:** Studies of the sub-acute toxicity of T-2 toxin.

| Species | Route | Dose (mg/kg b.w. per day) and exposure time | NOAEL or LOAEL (mg/kg b.w. per day) | Effect | Reference |
|---|---|---|---|---|---|
| Rat | Oral (diet) | 0.25-0.75 4 weeks | NOAEL 0.25 | Lesion in the stomach | Ohtsubo and Saito (1977) |
| Monkey | Oral (gavage) | 0.1 4-5 weeks | LOAEL 0.1 | Immunotoxicity | Jagadeesan et al. (1982) |
| Minipig | Oral (gavage) | 0.012-0.06 7 weeks | NOAEL 0.06 | No effect on weight gain, haematology, clinical health and susceptibility to infection | Bernhoft et al. (2000), available as an abstract only |

b.w.: body weight; LOAEL: lowest-observed-adverse-effect-level; NOAEL: no-observed-adverse-effect-level.

Since the previous evaluations, a few other studies have been published mainly dealing with farm animals and only one study in rodents. Chinese hamsters were treated with 10 mL/kg of 7 % dimethylsulfoxide in the control group and 1.0 mg T-2 toxin/kg in the T-2 toxin treated group intragastrically twice a week for a period of 3 weeks. No differences in growth or weight gains appeared during the course of the experiment. The histological examination did not show any changes in the investigated organs in the treated group. A decrease appeared in gamma glutamyltransferase (GMT), alkaline phosphatase and lactate dehydrogenase (LDH) activities, and total and conjugated bilirubin concentrations in the T-2 toxin treated group. A significant increase in the monocyte percent count (9.8 % after T-2 toxin treatment) compared to control (6.65 %) was observed while the differences observed in other leucocyte types were not significant (Rajmon et al., 2001).

### 7.3.3. Chronic toxicity

No chronic toxicity studies on HT-2 toxin are available. Three chronic toxicity studies by the oral route with T-2 toxin are reported in the JECFA evaluation (FAO/WHO, 2001) (Table 26). No new studies in laboratory animals have been published since this evaluation.

**Table 26:** Studies on the chronic toxicity of T-2 toxin. Reported by JECFA (FAO/WHO, 2001).

| Species | Route | Dose/ Exposure time | NOAEL or LOAEL (mg/kg b.w. per day) | Effect | Reference |
|---|---|---|---|---|---|
| Mouse CD1 | Oral (diet) | 0.22-0.45 mg/kg b.w. per day (T-2 toxin 99 % pure), 71 weeks | LOAEL 0.22 | Increase of pulmonary and hepatic adenomas. No haematological troubles | Schiefer et al. (1987) |
| Mouse Kunming Males | Oral (diet) | 0.1 mg/kg b.w. per day (purity of T-2 toxin not reported), 3 times/week, 25 weeks | LOAEL 0.1 | Fore stomach papillomas | Yang and Xia (1988) |
| Mouse DDD females | Oral (diet) | 1.5 and 2.2 mg/kg b.w. per day (purity of T-2 toxin not reported), 52 weeks | NOAEL 0.132 | Fore stomach papillomas. Lesions in oesophagal regions (hyperkeratosis, acanthosis, papillomatosis) | Ohtsubo and Saito (1977) |

b.w.: body weight; LOAEL: lowest-observed-adverse-effect-level; NOAEL: no-observed-adverse-effect-level.

### 7.3.4. Dermal effects

Dermal effects of exposure to T-2 toxin may be of relevance for employees in the milling industry as well as in feed production. T-2 toxin is a potent skin irritant. In rats, a threshold for irritation was reported to be 0.5 µg T-2 toxin/cm² (Fairhurst et al., 1987). The conclusion of the SCF report (SCF, 2001) was that 'T-2 toxin produces oedema, intradermal haemorrhage and necrosis of the skin. Guinea pig is the most sensitive species. The effect on skin has been used as a biological assay for detection of trichothecenes. T-2 toxin can be detected at 0.2 µg with a skin necrosis assay. The minimum effective amount needed to elicit irritation is much less. The mechanism for skin toxicity has not been established.' Further mostly mechanistic work has been carried out since the publication of this report on dermal application of T-2 toxin to rats and mice.

In hairless WBN/ILA-Ht rats given a single applications of T-2 toxin (10 µL of a 0.5 mg/mL solution) on dorsal skin areas, there was a depression of basal cell proliferating activity and subsequent induction of basal cell apoptosis in the epidermis. Additionally T-2 toxin caused infiltration of inflammatory cells including mast cells in the dermis (Albarenque et al., 1999). In a subsequent study in the same experimental system, transforming growth factor-beta1 (TGF-β 1) mRNA of whole skin tissue increased to a significantly higher level 24 hours after treatment, and was suggested to have a close relationship to the induction of epidermal basal cell apoptosis and intradermal infiltration of mast cells and fibroblasts after T-2 toxin application (Albarenque et al., 2000). An increase in the expression of apoptosis-related genes c-fos and to a lesser extent c-jun was also observed (Albarenque et al., 2001a). With regard to cytokines, the level of TNF-α mRNA showed a marked elevation, and to a lesser extent, the levels of IL-1β mRNA increased, in the dorsal skin of WBN/ILA-Ht rats following topical application of T-2 toxin (Albarenque et al., 2001b). Nguansangiam et al. (2003) applied T-2 toxin topically to the footpad of mice (10 µL of a 1.0 mg/mL solution in ethyl acetate) and detected a reduction in Langerhans cell density, and an inflammatory reaction characterised by epidermal desquamation, and necrosis with oedema and inflammatory cell infiltration. The tumour initiation/promotion potential following dermal application of T-2 toxin is described in Section 7.3.10.

In summary, the work carried out on dermal application of T-2 toxin since the JECFA (2001) and the SCF (2001) evaluations has further elucidated mechanistic factors involved in the toxic response, but no dose-response studies have been carried out to further inform the NOAEL or LOAEL.

### 7.3.5. Immunotoxicity

The immune system is one of the main targets of T-2 toxin toxicity. T-2 toxin is reported to be immunotoxic, either by its cytotoxic, apoptotic or immunosuppressive attributes (FAO/WHO, 2001; SCF, 2001; Gutleb et al., 2002; Van Loveren and Piersma, 2004; Minervini et al., 2005; Vlata et al., 2005; Hymery et al., 2006, 2009; Meissonnier et al., 2008b). After acute oral exposure severe damage to actively dividing cells in bone marrow, lymph nodes, spleen, thymus and intestinal mucosa has been observed. Effects of T-2 toxin on both humoral and cellular immune response have been demonstrated in various studies (FAO/WHO, 2001).

#### 7.3.5.1. *In vivo* studies on experimental animals

T-2 toxin exposure induces apoptosis in the thymus and spleen of mice and rats after oral or *i.p.* application. Thymus atrophy in mice has been reported following oral doses of 0.75 mg/kg b.w. (FAO/WHO, 2001). In mice, Nagata et al. (2001) detected apoptotic effects in Peyer patches, mesenteric lymph nodes and thymus 24 hours after a single oral application of 10 mg/kg of T-2 toxin. The degree of lymphocyte apoptosis was prominent in the thymus, moderate in the Peyer's patches and mild in the mesenteric lymph nodes.

#### 7.3.5.2. Effect on inflammatory cells

Few studies have investigated the effects of T-2 toxin on inflammatory cells, but all results concluded that there was an inhibition of the various functions of these cell types (Meissonnier et al., 2008b). *In vitro*, Sorenson et al. (1986) determined the toxic effects of T-2 toxin (4.6 - 46 µg/L) on rat alveolar macrophages. The inhibitory effect on protein synthesis was more remarkable than on RNA synthesis. Phagocytosis of *Staphylococcus aureus* was decreased (> 80 %) for the high exposure concentration (46 µg/L), and macrophage stimulation with lipopolysaccharide (LPS) or lymphokines was reduced for both T-2 toxin concentrations (4.6 and 46 µg/L). Similarly, mouse peritoneal macrophages exposed *in vitro* to T-2 toxin (46 µg/L) displayed a reduced phagocytic activity on *Pseudomonas aeruginosa*. The same observation was reported for peritoneal macrophages from mice injected subcutaneously (*s.c.*) with T-2 toxin (Vidal and Mavet, 1989). A few experiments investigated the toxin effects on cytokine production, protein mediators essential for the processes of immunoregulation. In mice orally dosed on alternate days for 2 weeks with T-2 toxin (0.1, 0.5 or 2.5 mg/kg b.w.), decreased production of TNF, IL-1β and IL-6 was noted on peritoneal macrophages stimulated with LPS (Dugyala and Sharma, 1997). *In vitro*, T-2 toxin reduced release of IL-1β by mouse peritoneal macrophages in a concentration dependent manner. Low doses of T-2 toxin increased the synthesis of interleukin-12 and TNF-α whereas high dose had the opposite effect (Ahmadi and Riazipour, 2008).

#### 7.3.5.3. Effect on cell-mediated immune response

Dendritic cells, the most potent antigen-presenting cells (APCs) of the immune system, have demonstrated sensitivity to trichothecene mycotoxins. T-2 toxin disturbed their maturation process. Two-day incubation with T-2 toxin did not change human leukocyte antigen complex (HLA-DR) or chemokine receptor-7 (CCR7) but decreased cluster of differentiation 86 (CD86) cell surface expression, and reduced the secretion of IL-10 and IL-12 needed for the T-cell response activation (Hymery et al., 2006). Epidermal Langerhans cells are dendritic bone marrow derived cells, and the principal APCs in the skin. They play a central role in delayed-type hypersensitivity (DTH) by presenting antigens to lymphocytes. Experimental topical application of T-2 toxin in mice inhibited the activity of the skin immune system, T-2 toxin reducing the DTH reaction to oxazolone. The antigen transport to draining lymph nodes was not impaired, but the antigen presentation by Langerhans cells was reduced, that may be directly related to the observed decrease in major histocompatibility complex (MHC) class II (Ia) cell-surface antigen expression (Blayloc et al., 1993).

Lymphocyte subsets CD4+ and CD8+ are particularly affected during T-2 toxin induced lymphoid organ depletion. Impairment of lymphoblastogenesis to mitogens has been reported *in vitro* and *in vivo* (reviewed in FAO/WHO, 2001). *In vitro*, proliferation of spleen lymphocytes from mice in the presence of mitogens was enhanced at lower T-2 toxin concentrations (0.23 ng/mL in the presence of concanavalin A (ConA)) and inhibited at higher T-2 toxin concentrations (1.2 ng/mL in the presence of ConA) (FAO/WHO, 2001). More recently, Jaradat et al. (2006) reported that T-2 toxin inhibited mitogen stimulated chicken lymphocyte proliferation *in vitro* at concentrations of 1 ng/mL or higher. Proliferation was completely abolished at 10 ng/mL when the T-2 toxin was added at time zero, while it was decreased by 80 % when the toxin was added to the lymphocytes after 24 hours. Addition of vitamin E provided considerable protection against T-2 toxin inhibition of lymphocyte proliferation.

*In vivo, i.p.* injection of T-2 toxin to mice also gave a bi-phasic response on lymphocyte proliferation, with a stimulation observed at the low doses (0.08 mg/kg b.w per day on days 0, 7 and 14) and inhibition observed at the high doses (1.6 mg/kg b.w. per day on days 0, 7 and 14) (reviewed in FAO/WHO, 2001).

A few experiments investigated the effect of T-2 and HT-2 toxin exposure on lymphocyte cytokine production. On primary CD4+ T-cells from murine spleen stimulated with ConA, T-2 toxin reduced the IL-2 production after a 2-day culture (0.5-2.5 ng/mL) but increased IL-4 and IL-5 production after a 7-day culture. It is of interest to note that the reduction of the IL-2 production was associated with

super-induction of mRNA at the lowest dose (Bondy and Pestka, 2000). Exposure of thymoma cell line EL-4 (model of T-cell) to T-2 toxin did not impair the IL-2 and IL-5 production during the 5 days exposure to phorbol 12-myristate 13-acetate (PMA) (Bondy and Petska, 2000). Splenocytes from mice orally dosed with T-2 toxin on alternate day for 2 weeks showed increased production of IL-2, interferone gamma (IFN- γ) and IL-3 when stimulated *ex vivo* with ConA; IL-2 mRNA expression was particularly increased in comparison with IFN-γ and IL-3 (Kamalavenkatesh et al., 2005). Li et al. (2006a,b) investigated the effect of an *i.p.*-injection of T-2 toxin (1.75 mg/kg b.w.) on cytokine expression in mice intranasally infected with reovirus. T-2 toxin suppressed the induction of IFN- γ by reovirus, but enhanced production of IL-6 and MCP-1 in the bronchoalveolar lavage fluid of the mice (Li et al., 2006a). When mice were infected with reovirus by oral gavage, the exposure to T-2 toxin suppressed the viral induction of IFN-γ and increased the production of IL-6 in the Peyer's patches (Li et al., 2006b).

#### 7.3.5.4. Effect on the humoral immunity

Another immunotoxic effect of T-2 toxin includes the suppression of antibody production. As already mentioned for the effect on lymphocyte proliferation, low amounts of T-2 toxin were found to increase antibody levels, whereas high amounts were found to be immunosuppressive (FAO/WHO, 2001).

In Swiss mice injected daily with T-2 toxin (0.75-1.5 mg/kg b.w.), the antibody production following sheep red blood cell immunisation was decreased (Rosenstein et al., 1979). Recent data confirm the dual effect of T-2 toxin on antibody production. In mice, Li et al. (2006a,b), observed that *i.p.* injection of T-2 toxin followed by an intra-nasal instillation of reovirus, suppressed viral-specific mucosal IgA responses in lung and enteric tract, but potentiated serum IgA and IgG responses in the serum. When the virus was administered by the oral route a transient suppression of virus-specific IgA in faeces was observed as well as specific IgA and IgG2a in serum.

#### 7.3.5.5. Effect on the susceptibility to infections

The above mentioned effect on the immune response led to an increase susceptibility to infectious diseases. Repeated exposure to T-2 toxin increases the susceptibility to a diverse array of pathogens including *Salmonella, Mycobacterium*, *Staphylococcus*, *Listeria, Toxoplasma* and Herpes simplex virus (HSV-1). Effects were seen in rats and mice in a dose range of 0.5 to 5 mg/kg b.w. Enhanced resistance to *Listeria* in the same dose range was observed after short-term preinoculation with T-2 toxin, whereas postinoculation with T-2 toxin resulted in immunosuppression. Such resistance is frequently observed when trichothecenes are administered shortly before the onset of infection (reviewed by Bondy and Petska, 2000).

More recently, Li et al. (2006a,b) investigated in mice the effect of an *i.p.*-injection of T-2 toxin (1.75 mg/kg b.w.) on reovirus infections by the nasal and the oral routes. They demonstrated that 10 days post intra-nasal instillation, virus plaque-forming responses and reovirus L2 gene expression were 10-fold higher in lungs of the T-2 toxin treated mice compared to the control animals. T-2 toxin exposure increased bronchopneumonia and pulmonary cellular infiltration in reovirus-infected mice. When dose response studies were performed, clearance from reovirus from the lung was significantly inhibited at doses of ≥ 0.2 mg/kg b.w. of T-2 toxin. No effects were observed at 0.02 mg/kg b.w. (Li et al., 2006a). The effect of *i.p.* injection of T-2 toxin on an oral gavage of reovirus in mice was also investigated by the same authors (Li et al., 2006b). The T-2 toxin treated mice that had elevated intestinal plaque forming viral titres after 5 days, failed completely to clear the virus from intestine by 10 days, and had significantly increased virus L2 gene RNA levels in faeces. Dose-response analysis revealed that RNA levels were dose-dependently increased with statistically significant effects already observed at the lowest dose tested (0.05 mg/kg b.w.) (Li et al., 2006b).

#### 7.3.5.6. Data on human cells

T-2 toxin inhibited the mitogen-stimulated proliferation of human peripheral lymphocytes in several *in vitro* studies (FAO/WHO, 2001). These findings were confirmed by several studies published more recently. Meky et al. (2001) observed that a 5-day treatment of phytohaemagglutinin-stimulated lymphocytes with T-2 toxin inhibits their proliferation; the 50 % inhibition occurring between 1 and 5 ng/mL. Similarly, Vlata et al. (2005) demonstrated that 10 ng/mL of T-2 toxin had no direct effect on untreated peripheral blood lymphocytes but totally inhibited phytohaemagglutinin-induced T lymphocyte proliferation and caused early apoptosis. Further investigations revealed that T-2 toxin affected all subpopulations studied (CD4+, CD8+, CD45RA+ and CD45RO+). T-2 toxin at sub-toxic concentrations (1 ng/mL) appeared to exhibit costimulatory properties to phytohaemagglutinin-stimulated cells (Vlata et al., 2005).

Using human lymphoid T- and B-cell lines (MOLT-4 and IM-9 cells, respectively), Minervini et al. (2005) also observed immunotoxic effects of T-2 toxin as measured by the cytotoxicity (trypan bleu exclusion), the cell metabolism (MTT test) and the inhibition of cell proliferation (BrdU incorporation). The authors observed that the B cell line was more sensitive to T-2 toxin than the T-cell line and that lower doses were required to alter cell metabolism than proliferation or to observe a cytotoxic effect. After 48 hours of exposure to T-2 toxin, concentrations leading to a 50 % inhibition of cytotoxicity were 6 ng/mL in MOLT-4 cells and 0.2 ng/mL in IM-9 cells. Concentration leading to a 50 % inhibition of cell metabolism was 1 and 0.6 ng/mL in MOLT-4 and IM-9 cells, respectively. Concentrations leading to a 50 % inhibition of cell proliferation were 3 and 0.02 ng/mL in MOLT-4 and IM-9 cells, respectively. Cytotoxicity appeared to be due to early apoptosis in MOLT-4 cells, as indicated by increased Annexin V binding and activation of caspase-3, and to direct cell membrane damage in IM-9 cells (Minervini et al., 2005). In the Jurkat lymphocytic cell line, T-2 toxin was also cytotoxic and the cells became necrotic upon exposure to T-2 toxin (Nasri et al., 2006).

Human lymphocytes stimulated *in vitro* with pokeweed mitogen displayed reduced immunoglobulin (Ig) production (IgA, IgG and IgM) when incubated in the presence of T-2 toxin (Thuvander et al., 1999).

The effect of T-2 and HT-2 toxins were also investigated on human monocytes/macrophages. T-2 toxin was cytotoxic and induced apoptosis in monocytic leukemia U937 cells (Huang et al., 2007) and monocyte/macrophage THP1 cells (Rakkestad et al. 2010). In the human promyelocytic cell line, HL-60, T-2 and HT-2 toxins induced apoptosis in a concentration dependent manner 24 hours after treatment with concentrations starting at 3.1 and 6.25 ng/mL, respectively, (Holme et al., 2003). In macrophages derived from monocytes of healthy blood donors, T-2 toxin was shown to activate caspase-1 and 3, and to strongly enhance LPS-dependent secretion of IL-1β and IL-18 (Kankkunen et al., 2009). Using umbilical cord blood monocytes, Hymery et al. (2009) investigated the effect of T-2 toxin on their differentiation into macrophages or dendritic cells. Monocytes were found to be more sensitive to T-2 toxin than macrophages or dendritic cells with a 50 % inhibitory concentration ($IC_{50}$) of 0.05, 10.1 and 17.5 µg/L, respectively. Monocyte differentiation into macrophages was depressed in the presence of 4.6 µg/L as demonstrated by the expression of CD71, the secretion of TNF-α, the respiratory burst, the endocytosis and the phagocytosis. T-2 toxin also disturbed human monocyte differentiation into dendritic cells as indicated by the expression of CD1a and CD14 (Hymery et al., 2009). Similarly, exposure of dendritic cells to T-2 toxin inhibited their maturation by LPS as indicated by the limited upregulation of HLA-DR and CCR7, the reduced secretion of IL-10 and IL-12 secretions and the inhibition of endocytosis (Hymery et al., 2006).

#### 7.3.5.7. Conclusions

The immune system is one of the main targets of T-2 toxin. This toxin causes cell depletion in lymphoid tissue, inhibits inflammatory cell function and decreases humoral and cell-mediated immune responses

leading to an increased susceptibility to infection. The effect of HT-2 toxin on the immune system is much less documented.

### 7.3.6. Haematotoxicity and myelotoxicity

The JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001) reported several studies describing haematotoxicity and myelotoxicity of T-2 toxin *in vivo* and *in vitro*. A more recent review was published in 2004 by Parent-Massin (2004). Selected studies from the previous evaluations and present studies are summarised in Tables 27 and 28.

**Table 27:** *In vivo* experimental animal studies on haematotoxicity and myelotoxicity of T-2 toxin by oral route.

| Species | Route | Dose/ Exposure time | Effect | Reference |
|---|---|---|---|---|
| Mouse | Oral | 20 mg/kg b.w. per day (purity not reported), 6 weeks | Week 1-3: hypoplasia bone marrow, spleen, anaemia, lymphocytopenia, eosinopenia<br>Week 4-6: bone marrow regeneration | Hayes et al. (1980) |
| Mouse | Oral | 10, 20 mg/kg b.w. per day (crystalline T-2 toxin), 2-4 weeks | Erythroid hypoplasia, severe anaemia | Hayes and Schiefer (1982) |
| Mouse | Oral | 10 mg/kg b.w. per day (crystalline T-2 toxin), 2 weeks | Reversible decrease red blood cells | Hayes and Schiefer (1990) |
| Mouse | Oral | 5 mg/kg b.w. per every 3 days (purity not reported), 2 weeks | Increase in leukocyte counts 2 and 4 weeks | Taylor et al. (1985) |
| Monkey | Gavage | 0.1-5 mg/kg b.w. per day (purity not reported), 2 weeks | Severe leukocytopenia, mild anaemia<br>LOAEL: 0.1 mg/kg b.w. per day | Rukmini et al. (1980) |
| Monkey | Gavage | 100 µg/kg b.w. per day (semi purified) 4-5 weeks | Decrease of 40 % Leucocyte count (reversible) | Jagadeesan et al. (1982) |

b.w.: body weight; LOAEL: lowest-observed-adverse-effect-level.

**Table 28:** *In vitro* studies on haematotoxicity and myelotoxicity of T-2 and HT-2 toxins.

| Species/cells | Toxin | Concentration/ Exposure time | Cytotoxicity dose/ No effect dose (ng/mL) | Effect | Reference |
|---|---|---|---|---|---|
| **Rat** | T-2 | | | | |
| WB Progenitors | toxin | 14 days | CD: 2.5, NED: <0.05 | | Lautraite et al. (1995) |
| **Human** | T-2 | | | | |
| WB Progenitors | toxin | 14 days | CD: 50, NED: <0.05 | | Lautraite et al. |
| Platelet | | 14 days | CD: 5, NED: <0.05 | | (1995); Froquet et al. |
| Progenitor | | 14 days | CD: 5, NED: 1.1 | | (2001); Rio et al. |
| RB progenitors | | | | | (1997) |
| **Human** | T-2 | | | | |
| White Blood cells | toxin | 24 hours | Decrease of 50 %: 5 | | Froquet et al. (2003) |
| Platelet | | | NED: >00 | | |
| Red Blood Cells | | | NED: >500 | | |
| **Rat** | T-2 | | | | |
| Erythrocytes | toxin | 0-11 hours | CD: 250, NED: 130 | Haemolysis | Rizzo et al. (1992) |
| **Guinea pig** | T-2 | 50-200 µg/mL | | Echinocytes | Gyongyossy-Issa et |
| Eryrthrocytes | toxin | 5.2 hours | | | al. (1986) |
| **Rat** | HT-2 | | | | |
| WB Progenitors | toxin | 14 days | CD: 3.75, NED: <0.05 | | Lautraite et al. (1995) |
| **Human** | HT-2 | | | | |
| WB Progenitors | toxin | 14 days | CD: 5, NED: <0.05 | | Lautraite et al. |
| Platelet | | 14 days | CD: 50, NED: 25 | | (1996); |
| Progenitor | | 14 days | CD: 50, NED: <0.05 | | Froquet et al. (2001); |
| RB progenitors | | | | | Rio et al. (1997) |

CD: cytotoxicity dose; NED: no effect dose.

Since the JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001) evaluations, a few new studies have been published. In 2003, Froquet et al. (2003) compared the sensitivity of human circulating blood cells to the sensitivity of human haematopoietic progenitors and some blood parameters. The authors showed that human haematopoietic progenitors are more sensitive to cytotoxic effects of T-2 toxin and concluded that haematological problems associated with T-2 toxin intoxication can be attributed to haematopoiesis inhibition. Ledréan et al. (2005) demonstrated that the haematological effects observed in T-2 toxin mycotoxicosis could then be assigned to haematopoiesis inhibition by apoptosis of $CD^{34+}$ haematopoetic cells.

Taken collectively, these data obtained from *in vitro* and *in vivo* studies show that T-2 and HT-2 toxin are myelotoxic for human and murine progenitors. It is also important to compare *in vitro* and *in vivo* data on haematopoiesis effects of T-2 and HT-2 toxins to *in vivo* and *in vitro* data of T-2 and HT-2 toxin effects on circulating cells. *In vitro* data confirmed that adverse effects of T-2 and HT-2 toxins were likely to be predominantly on progenitor cells rather than on circulating mature blood cells. The characteristic pathologies of T-2 and HT-2 toxin intoxication, i.e., neutropenia, thrombocytopenia and coagulation disorders could be the result of haematopoiesis inhibition by T-2 toxin and HT-2 toxin, after destruction of circulating cells in first phase.

### 7.3.7. Developmental and reproductive toxicity

In 2001 the SCF considered that developmental and reproductive toxicity was not one of the most critical effects from T-2 toxin (SCF, 2001). A NOAEL of 0.45 mg/kg b.w. per day was identified for embryotoxicity or fetotoxicity for CD-1 mice fed for two generations. The similar conclusion of the JECFA in 2001 was that for T-2 toxin 'No embryotoxicity or gross fetal malformations were seen at *i.p.* doses below 0.5 mg/kg b.w. per day' (FAO/WHO, 2001). Continuous administration in the feed of

concentrations equivalent to 0.22 and 0.45 mg/kg b.w. per day did not result in reproductive or gross developmental effects in CD-1 mice, although increased spleen weights were observed in male offspring of exposed dams at both doses (FAO/WHO, 2002). No data were available then on the reproductive toxicity of HT-2 toxin, or have become so subsequently.

One of the studies reviewed in the SCF report (SCF, 2001) was the treatment of pregnant mice with an oral dose of 3 mg/kg b.w. of T-2 toxin on day 11 of gestation (Ishigami et al., 1999). Some apoptosis was seen in embryos (central nervous system, caudal sclerotomic segment, caudal region of the tongue, trachea and facial mesenchyma). This result has been confirmed in a newer study (Ishigami et al., 2001) with pregnant mice given T-2 toxin at various days of gestation. The animals were inoculated orally with 2 mg/kg b.w. of T-2 toxin or the vehicle propylene glycol at gestational day 8.5, 9.5, 10.5, 11.5, 12.5, 13.5, 14.5, 15.5 and 16.5, and the fetuses were examined after 24 hours or at gestational day 17.5. Pyknotic or karyorrhectic cells were observed in the central nervous system, peri-ventricular zone to subventricular zone, in a small number of chondroblasts and chondrocytes, and in the thymus and renal subcapsular parenchyma, the number and region of these cells varying according to the inoculation date. Dead cells were positive by the TUNEL-assay, which uses terminal deoxynucleotidyl transferase dUTP nick end labelling to detect DNA fragmentation by labelling the terminal end of nucleic acids. DNA fragmentation represents a characteristic hallmark of apoptosis. A few fetuses showed skeletal abnormalities such as wavy ribs and short scapula.

The reproductive toxicity of T-2 toxin was also studied by Sehata et al. (2003, 2004a,b, 2005). Pregnant Wistar rats were treated orally with T-2 toxin at a dose of 2 mg/kg b.w. on day 13 of gestation, and were sacrificed at 24 and 48 hours after treatment. They were shown to have single cell necrosis in the thymus, spleen, liver, stomach, intestines, salivary glands and pancreas. Fatty change was observed in the liver. In the fetuses there was an increase in single cell necrosis in the central nervous system at 24 hours after treatment, and after 48 hours an increase in single cell necrosis of haematopoietic cells and of hepatocytes in the liver. The authors suggest that changes observed in the fetuses are caused by the direct effect of T-2 toxin (Sehata et al., 2003).

In subsequent studies Wistar rats were treated similarly and sacrificed 1, 3, 6, 9, 12 or 24 hours later. The number of apoptotic cells was increased in the liver, placenta and fetal liver and peaked at 6, 12 and 9-12 hours after treatment, respectively (Sehata et al., 2004a, 2005). Also apoptotic neuroepithelial cells in the telencephalon increased from 1 hour after treatment (Sehata et al., 2004b). Gene expression studies on samples of liver, placenta and fetal liver showed increases in the expression of apoptosis-related and oxidative stress-related genes (Sehata et al., 2004a, 2005). There was a suppression of expression of lipid metabolism and drug metabolising enzyme-related genes. This indicated that T-2 toxin induces oxidative stress in these tissues, prior to induction of apoptosis. In fetal brain, microarray analysis showed that the expression of oxidative stress-related genes (heat shock protein 70 and haem oxygenase) was strongly induced by T-2 toxin at the peak time of apoptosis induction, 12 hours after treatment (Sehata et al., 2004b). MAPK-related genes (MEKK1 and c-jun) and other apoptosis-related genes (caspase-2 and insulin-like growth factor-binding protein-3) were also induced by T-2 toxin treatment.

In conclusion, the studies carried out on developmental and reproductive toxicity of T-2 toxin since the FAO/WHO (2001) and the SCF (2001) evaluations have been single oral dose investigations to further elucidate the mechanism of toxicity.

### 7.3.8. Neurotoxicity

In 2001 the SCF considered that neurotoxicity was not one of the most critical effects of T-2 toxin (SCF, 2001). T-2 toxin was reported to change the levels of neurotransmitters in rat brain following dietary administration, the LOAEL being 2.0 mg/kg b.w. per day. Behavioural tests for rats given T-2 toxin orally a single dose of 2.0 mg/kg b.w. showed reduced motor activity and performance in a passive avoidance test. The NOAEL for this was 0.4 mg/kg b.w. per day (Sirkka et al., 1992; SCF,

2001). No data were available then on the neurotoxicity of HT-2 toxin or have become so subsequently. The report of the JECFA stated that T-2 toxin showed effects on neurotransmitter levels in rats and chickens, where effects were seen at doses of T-2 in rats as low as 0.1 mg/kg b.w. per day by gavage (FAO/WHO, 2001).

As described in Section 7.3.7. above, Ishigami et al. (1999, 2001) and Sehata et al. (2003, 2004b) carried out studies on pregnant mice and rats exposed orally to T-2 toxin 2-3 mg/kg b.w., which showed apoptotic effects in the fetal brain.

A recently published study evaluated the comparative acute toxicity of percutaneous (dermal) and *s.c.* exposure of T-2 toxin, measuring brain oxidative damage in mice (Chaudary et al., 2010). Following exposure of mice to an $LD_{50}$ dose of T-2 toxin, either by the dermal (5.94 mg/kg b.w.) or *s.c.* (1.54 mg/kg b.w.) route, the animals were sacrificed at 1, 3 or 7 days post-exposure. Significant effects on oxidative damage were produced by T-2 toxin, and some of these persisted even after 7 days post-exposure. Thus both routes of treatment caused an increase in ROS generation, brain GSH depletion, lipid peroxidation and protein carbonyl content in brain. Gene expression studies showed significant changes for antioxidant enzymes (significant increase in superoxide dismutase and catalase with the percutanous route and glutathione reductase and glutathione peroxidase with the *s.c.* route). Although this is not an oral study, involving percutaneous or *s.c.* exposure, it provides some mechanistic information indicating that oxidative damage may be associated with brain toxicity induced by T-2 toxin.

In conclusion, the studies carried out on neurotoxicity of T-2 toxin since the JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001) evaluations have been mechanistic investigations using single doses.

### 7.3.9. Genotoxicity

The assessment of the genotoxicity of T-2 and HT-2 toxins by the SCF in 2001 was that they caused a positive effect in several conventional tests for genotoxicity *in vitro* and in rodents *in vivo*, in particular for clastogenic effects, but that these effects were observed primarily at concentrations also known to inhibit protein and DNA synthesis and produce cytotoxicity (SCF, 2001). The reports of IARC (1993), SCF (2001) and FAO/WHO (2001) summarised the genotoxic effects of T-2 toxin (Tables 29 and 30).

According to these reports, T-2 toxin was shown to be negative in bacterial mutation assays. It produced single strand breaks in mouse lymphocytes and weakly in mouse hepatocytes. Chromosomal damage was found in Chinese hamster V79 fibroblasts and human lymphocytes treated with T-2 toxin. *In vivo* chromosomal aberrations were also apparent in Chinese hamster bone marrow after treatment with 1.7 mg/kg b.w. *i.p.* (weakly positive) and in mice (0.1 mg/kg of feed). However, no micronuclei were found in mice treated with 3 mg/kg b.w. *i.p.* of T-2 toxin. DNA single strand breaks were produced by T-2 toxin in mouse spleen and a weak effect was found in mouse thymus after *i.p.* treatment (3 mg/kg b.w.). The conclusion of the SCF and the JECFA was that it was unclear whether the observed effects are a consequence of interaction of T-2 toxin with genetic material or are secondary to inhibition of protein synthesis (FAO/WHO, 2001; SCF, 2001).

In the earlier studies, DNA damage (single strand breaks) was seen *in vitro* in spleen and thymic lymphocytes of BALB/c mice and weakly in primary hepatocytes (FAO/WHO, 2001). Rakkestad et al. (2010) have now studied DNA damage in the human monocyte cell line THP-1, using the alkaline Comet assay, with and without the enzyme formamidopyrimidine DNA glycosylase (FPG) to detect strand breaks and oxidative DNA damage. Cells were treated with T-2 toxin at a concentration of 4 µM for 3 hours. The T-2 toxin treatment did not result in an increase in DNA damage (increased tail moment). Addition of FPG increased the tail moment but this was not significant.

**Table 29:** *In vitro* studies on the genotoxicity of T-2 toxins as reported by IARC (1993), SCF (2001) and FAO/WHO (2001). (From FAO/WHO, 2001, modified).

| Test system | Test object | Concentration | Results | Reference |
|---|---|---|---|---|
| Reverse mutation | *S. typhimurium*, TA 100, TA 1535, TA 1537, TA 98 | 50 µg/mL | Negative[(a)] | Wehner et al. (1978) |
| | *S. typhimurium*, TA 1535, TA 1537, TA 1538 | 50 µg/mL | Negative[(a)] | Kuczuk et al. (1978) |
| | *S. typhimurium*, TA 100 | 100 µg/plate | Negative[(a)] | Takahashi et al. 1992 |
| SOS DNA repair | *E. coli* PQ37 (spot test) | Not reported | Negative[(a)] | Auffray and Boutibonnes (1986) |
| SOS DNA repair (Chromotest) | *E. coli* PQ37 | 1 µg/mL | Negative[(a)] | Krivobok et al. (1987) |
| Differential study | *B. subtilis rec* strains | 100 µg/plate | Negative | Ueno and Kubota (1976) |
| Mitotic crossing-over | *Saccharomyces cerevisiae ade2* locus | 100 µg/plate | Negative[(a)] | Kuczuk et al. (1978) |
| Petite forward mutation | *Saccharomyces cerevisiae* | 50 µg/mL | Negative | Schappert and Khachatourians (1986) |
| DNA single-strand breaks | BALB/c mouse primary hepatocytes | 0.005 µg/mL | Weakly positive | Lafarge- Frayssinet et al. (1981) |
| | BALB/c mouse spleen lymphocytes | 0.005 µg/mL | Positive | |
| | BALB/c mouse thymic lymphocytes | 0.005 µg/mL | Positive | |
| Sister chromatid exchange | Chinese hamster V79 fibroblasts | 2.3 µg/mL | Weakly positive[(a)] | Thust et al. (1983) |
| | Chinese hamster V79 fibroblasts | 0.1 µg/mL | Weakly positive[(b)] | Zhu et al. (1987) |
| | Human lymphocytes | 0.003 µg/mL | Negative[(a)] | Cooray (1984) |
| Chromosomal aberrations | Chinese hamster V79 fibroblasts | 0.5 µg/mL | Positive[(a)] | Thust et al. (1983) |
| | Chinese hamster V79 fibroblasts | 0.005 µg/mL | Positive | Hsia et al. (1986) |
| | Chinese hamster V79 fibroblasts | 0.05 µg/mL | Weakly positive[(a)] | Zhu et al. (1987) |
| | Chinese hamster V79 fibroblasts | 0.001 µg/mL | Positive | Hsia et al. (1988) |
| | Human lymphocytes | 0.0001 µg/mL | Positive | Hsia et al. (1986) |
| Micronucleus formation | Chinese hamster V79 fibroblasts | 0.05 µg/mL | Positive[(a)] | Zhu et al. (1987) |
| Unscheduled DNA synthesis | Human fibroblasts | 0.005 µg/mL | Positive | Oldham et al. (1980) |
| Inhibition of intercellular communication | Chinese hamster V79 cells | 0.003 µg/mL | Positive | Jone et al. (1987) |

(a): With and without metabolic activation; (b): With metabolic activation.

**Table 30:** *In vivo* studies on the genotoxicity of T-2 toxins as reported by IARC (1993), SCF (2001) and FAO/WHO (2001). (From FAO/WHO, 2001, modified).

| Test system | Test object | Concentration | Results | Reference |
|---|---|---|---|---|
| Polyploidy induction | Allium cepa | 20 µg/mL | Positive | Linnainmaa et al. (1979) |
| Sex-linked recessive lethal mutations | *Drosophila melanogaster* | 63 µg/mL | Weakly positive | Sorsa et al. (1980) |
| | *Drosophila melanogaster* | 100-1000 mg/kg in feed, 2-3 days | Negative | Sorsa et al. (1980) |
| Sex-linked chromosomal loss | Adult *Drosophila melanogaster* | 20 mg/kg in feed, 48 hours | Positive | Sorsa et al. (1980) |
| DNA single-strand breaks | BALB/c mouse liver | 3 mg/kg b.w. (*i.p.*) | Negative | Lafarge-Frayssinet et al. (1981) |
| | BALB/c mouse spleen | 3 mg/kg b.w. (*i.p.*) | Positive | |
| | BALB/c mouse thymus | 3 mg/kg b.w. (*i.p.*) | Weakly positive | |
| Micronucleus induction | Chinese hamster bone marrow | 3 mg/kg b.w. (*i.p.*) | Negative | Norppa et al. (1980) |
| Chromosomal aberrations | Chinese hamster bone marrow | 1.7 mg/kg b.w. (*i.p.*) | Weakly positive | Norppa et al. (1980) |
| | Mice | 0.1 mg/kg of feed | Positive | Bilgrami et al. (1995) |

*i.p.*: intraperitoneal.

In summary the new experimental data since the publication of the SCF and the JECFA evaluations (SCF, 2001, FAO/WHO, 2001) report a negative *in vitro* study on DNA strand breakage using the Comet assay (further data on DNA strand breakage in chickens are reported in Section 7.4.4). No new reports on cytogenetic damage caused by T-2 toxin have been identified since 2001, and no epidemiological evidence is available on genotoxic effects of T-2 toxin.

### 7.3.10. Carcinogenicity

T-2 toxin was assessed for its carcinogenic properties by IARC (IARC, 1993), the JECFA (FAO/WHO, 2001) and the SFC (SCF, 2001). The IARC conclusion was that there were no data available on the carcinogenicity to humans of toxins derived from *Fusarium sporotrichioides*, and that there was limited evidence in experimental animals for the carcinogenicity of T-2 toxin. The latter was based on the study of Schiefer et al. (1987), in which CD-1 mice (groups of 50 male and 50 female weanling mice) were fed a semi-synthetic diet containing 0, 1,5 or 3.0 mg/kg T-2 toxin, the purity of which exceeded 99 %, for 16 months. There was a statistically significant increased incidence of pulmonary and hepatic adenomas in males at the high dose. Studies in trout and in rats treated with T-2 toxin were not considered adequate for assessment by IARC. The overall IARC evaluation was that toxins derived from *Fusarium sporotrichioides* were not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 1993).

Very limited evidence for a weak promotional activity of T-2 toxin has been reported (IARC, 1993). There have been no informative data on *in vivo* activity published since then. Sakai et al. (2007) showed that T-2 toxin was active in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). This assay was developed by Ohmori et al. (2004) to detect tumour promoters and modified by Asada et al. (2005) to detect both tumour initiators and promoters by using different protocols. The characteristics and performance of this assay are described in Sakai et al. (2007). T-2 toxin was demonstrated to be a promoter in this system, being active at concentrations as low as 1 ng/mL in the culture medium. It had no initiation activity.

No epidemiological evidence for human carcinogenicity data on T-2 toxin is available. There are no data available on the carcinogenicity of HT-2 toxin.

The SCF and the IARC previously noted that there is limited evidence for tumourigenicity of T-2 toxin in experimental animals (induction of hepatocellular- and pulmonary adenomas in male mice) (SCF, 2001), and IARC also concluded that there are no data available on the carcinogenicity to humans of toxins derived from *Fusarium sporotrichioides* (IARC, 1993). No further conclusions can be drawn on the carcinogenicity of T-2 toxin and HT-2 toxin on the basis of published results after the FAO/WHO (2001) and the SCF (2001) evaluations.

## 7.4. Adverse effects in livestock, fish and companion animals

### 7.4.1. Ruminants

In general, ruminants are considered to be less sensitive to the effects of trichothecenes such as T-2 toxin, due to the detoxification capacity of the rumen. Therefore young animals, in which the rumen is not fully developed, may be more susceptible to toxic effects of T-2 toxin.

#### 7.4.1.1. Cattle

The previous assessment of T-2 toxin by the JECFA (FAO/WHO, 2001) also included studies on cattle describing reproductive endocrine effects, short term studies e.g. on calves and immunological alterations. However, effects on adult cattle were not fully evaluated. No further studies were published since the JECFA evaluation. Older studies are briefly described below.

In the seventies, field cases of intoxications of cows with mouldy maize were linked to the presence of T-2 toxin in the batches contaminated with *Fusarium tricinctum* (Hsu et al., 1972). Seven out of 35 Holstein cows of a Wisconsin herd died within 5 months and post-mortem examinations revealed extensive haemorrhages on the surface of all internal viscera.

Calves (n = 1/dose) were exposed to T-2 toxin by oral gavage of capsules at doses of 0.08, 0.16, 0.32 or 0.6 mg/kg b.w. per day for 30 days. The highest dose resulted in a hunched stance of the calf, which died on day 20. Evidence of mild enteritis was seen at all doses. Bloody faeces were observed at doses higher than 0.32 mg/kg b.w. per day. Prothrombin times and the activity of serum AST were increased in calves given the two higher doses (Pier et al., 1976).

A gestating cow was intubated with 182 mg of pure T-2 toxin (0.44 mg/kg b.w.) for 15 days after a feed supplemented with 50 mg T-2 toxin/kg was refused. The calf born normally 4 days after the first intubation was dosed with 0.6 mg T-2 toxin/kg b.w. for 7 days and then on alternate days for a total of 16 days. The cow exhibited no clinical effects, abnormal haematology or clinical chemistry. Lesions in the gastrointestinal tract were observed macroscopically and microscopically, however without comparison to untreated animals. The calf developed clinical signs of intoxication (such as hindquarter ataxia, knuckling of the rear feet, listlessness and severe clinical depression) within 20 minutes after the first application. The duration of clinical signs increased from 12 hours to more than 48 hours over the course of the experiment. Nevertheless, no haematological, clinical chemical or histological alterations were detected (Weaver et al., 1980).

The level of several serum proteins and immunoglobulins were altered when calves (n = 6) were given T-2 toxin orally at 0.6 mg/kg b.w. per day for 43 days. Total protein, albumin and immunoglobulin fractions, including the $\alpha$1-, $\beta$1- and $\beta$2-globulin fractions and IgA and IgM and complement protein values were decreased in T-2 toxin treated calves (Mann et al., 1983).

Lymphocytes from calves (n = 2-3) fed a diet containing 0.6 mg/kg of T-2 toxin (source of contamination not reported) for up to 43 days demonstrated a reduced prolierative response to phytohaemagglutinin on days 1, 8 and 29 after beginning of the feeding and decreased response to ConA and pokeweed mitogen on day 29 (Buening et al., 1982).

Neutrophil function and cutaneous reaction to injected phytohaemagglutinin were reduced in 5 calves treated orally with 0.3 mg T-2 toxin/kg b.w. per day for 56 days as compared to pair-fed controls. In another study in which 6 calves were dosed with 0.5 mg T-2 toxin/kg b.w. per day for 28 days the number of B lymphocytes and the response of the B-cell enriched fraction to phytohaemagglutinin were increased (Mann et al., 1984).

The effects on ovarian function of 0.025 mg T-2 toxin/kg b.w. administered orally over a period of 20 days to heifers (n = 4) fed a starch-rich diet to induce acidosis (confirmed by net acid-base excretion during 2/3 of the dosing period) compared to animals fed the same diets but not treated with T-2 toxin (n = 3) were investigated by Huszenica et al. (2000). After $PGF_{2\alpha}$, administration the ovulation occurred later and the plasma progesterone level remained low (< 3 nmol/L) for a longer period in T-2 toxin treated heifers, than their untreated control mates (5.0 ± 0.7 days *vs.* 3.7 ± 0.5 days, $P < 0.05$ and 8.3 ± 0.4 days *vs.* 6.3 ± 0.9 days, $P < 0.01$, respectively).

Effects of T-2 and HT-2 on semen quality have been suspected in bulls (Alm et al., 2002). Bulls at a Finnish artificial insemination station that had been fed with mouldy hay showed a drop in semen quality (low progressive mobility and poor morphology). Analysis of the hay by GC-MS revealed elevated amounts of T-2 toxin (47 µg/kg) and very high amounts of HT-2 toxin (570 µg/kg), although it could not be unequivocally proved that T-2 and HT-2 toxins were the responsible agents for the poor semen quality.

#### 7.4.1.2. Sheep

Lambs (n = 5, 6-8 weeks old) were fed gelatine capsules containing T-2 toxin (99 % purity) dissolved in propylene glycol to result in doses of 0, 0.3 or 0.6 mg T-2 toxin/kg b.w. per day for 21 days. All treated animals developed focal hyperaemia and dermatitis at the mucocutaneous junction of the commissure of the lips, diarrhoea, leukopenia, lymphopenia and lymphoid depletion of the mesenteric lymph nodes and spleen (Friend et al., 1983).

The effects of T-2 toxin (5 or 15 µg/kg b.w., administered orally for 21 days) on ovarian function in ewes was investigated in non acidotic and acidotic ewes (induced by rich concentrate feeding, confirmed by net acid-base excretion) (Huszenica et al., 2000). Ovarian malfunction manifested as lower progesterone peak concentration in the midluteal phase, shortening of the corpus luteum lifespan and prolonged follicular phases. These malfunctions were detected in 3 out of 4 and 3 out of 3 acidotic ewes dosed with 5 and 15 µg T-2 toxin/kg b.w., respectively. Lower progesterone peak concentration was observed in 1 out of 4 ewes fed regular diet and 15 µg T-2 toxin/kg b.w. None of the control and acidotic groups (0 mg T-2 toxin), or ewes fed regular diet with 5 µg T-2 toxin/kg b.w. showed any ovarian malfunction.

#### 7.4.1.3. Conclusions

Exposure to 0.3 mg T-2 toxin/kg b.w. per day or more may result in gastrointestinal lesions, altered serum proteins and haematological alterations in calves or lambs. This could be considered as a LOAEL based on the available data. However, a NOAEL in young ruminants was not identified and investigations using practically relevant concentrations of T-2 toxin are missing. The effects observed in nutritionally challenged heifers and ewes give rise to the assumption that rumen detoxification of T-2 toxin may not always be complete and thus effective to prevent negative effects on ruminants. The limited data on the effects of T-2 toxin on ruminants do not allow a conclusion.

### 7.4.2. Pigs

The effects of T-2 toxin on pigs were considered in the assessments by the JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001), and reviewed by Eriksen and Pettersson (2004). Both, the PMTDI and t-TDI of 0.06 µg/kg b.w. derived by the JECFA and the SCF, respectively, were established on the basis of the LOAEL observed in a short term study on pigs where haematotoxic and immunotoxic effects were observed at dietary concentrations as low as 0.5 mg/kg – equivalent to a dose of 0.029 mg T-2 toxin/kg b.w. per day for 21 days (Rafai et al., 1995a,b). A NOAEL was not identified for pigs. Feeding studies on the effects of T-2-toxins in pigs are summarised in Table 31.

An $LD_{50}$ of 1.2 mg/kg b.w. in pigs (3-50 kg live weight) was established after *i.v.* administration (Weaver et al., 1978a). Acute toxicity was characterised by emesis, posterior paresis, listlessness and lethargy. Within 24 hours, surviving pigs recovered and appeared normal. For dietary exposure, treatment-related decreased feed intake was described in most studies with diets containing 0.5 mg T-2 toxin/kg or more. The reduced feed intake resulted in decreased weight gain at dietary concentrations of 1 mg/kg or more in most cases without affecting feed conversion. Therefore the effects of reduced feed intake and decreased growth are suspected to bias further observations as no pair-fed controls were included in the investigation. However, haematotoxic effects included decreased leukocyte count, decreased haemoglobin concentrations and reduced erythrocyte counts. Immunological effects reported include reduced blastogenic response, immune rosette formation and reduced vaccination/antibody response.

Since the last evaluations, two new studies have been published (Frankic et al., 2008; Meissonnier et al., 2008a). After feeding diets containing 3 mg T-2 toxin/kg (from culture material) to piglets (11.1 kg starting weight, n = 12 in control, n = 9 in experimental groups) for a period of 2 weeks the live weight gain and the feed conversion of exposed piglets was significantly reduced if vitamin E was supplemented to the toxin containing diet but not if only T-2 toxin was administered (Frankic et al., 2008). However, these results have not been confirmed in any other study. In the group administered T-2 toxin, significantly reduced total IgG levels were observed. Increased lymphocyte nuclear DNA damage by T-2 toxin treatment was demonstrated by the Comet assay. Markers of oxidative stress (plasma and 24-hour urinary MDA excretion rate and glutathione peroxidase) were not affected by the treatment (Frankic et al., 2008).

**Table 31:** Feeding studies on the effects of T-2 toxin in pigs.

| Animals | | Toxin | | | Experiment | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | **Initial live weight (kg)** | **Concentration in feed (µg T-2 toxin/kg)** | **Dose (µg T-2/kg b.w. per day)** | **T-2 toxin source** | **Exposure duration (days)** | **N per group** | **Parameter** | **Effect** | **LOEL (µg/kg diet)** | **NOEL (µg/kg diet)** | **Reference** | **Comments** |
| Sow | 171 | 12 000 | 253 | *F. tricinctum* c.e., 97% pure toxin as determined by TLC/GC | 80 - 220 | 3 | Piglets | Only the sow fed for 220 days had only 4 and abnormally small piglets | | | Weaver et al. (1978a) | No controls |
| | | | | | | | Mucosal congestion in intestine and bile | Sows fed for 80 and 122 days | | | | |
| Not reported | 8 | 0, 1000, 2000, 4000, 8000 | 0, 42, 85, 177, 345 | *F. tricinctum* c.e., 97% pure toxin as determined by TLC/GC | 56 | 4 | Feed consumption of the first week | Significantly reduced for the groups fed 1000, 4000 or 8000 mg T-2 toxin rations but not 2000 | 1000 | n.a. | Weaver et al. (1978b) | Not significant (but numerical) effect on feed intake and body weight. No alterations in haematology or clinical chemistry or microscopic lesions |
| Not reported | 10 week old | 12000, 16000, 24000, 32000 | | *F. tricinctum* c.e., 97% pure toxin as determined by TLC/GC | 2 | 2 | Feed refusal | Rations were refused when 16 mg/kg or more were present | 16000 | 12000 | | 2nd experiment, same animals for all treatments (2 day intervals of control and contaminated feed) |
| Not reported | Not reported | | 100 | Pure toxin and crude extracts from *F. tricinctum* cultures on maize | 14/36 | 1/2 | Leucocytes | Decreased in the one pig exposed for 36 days | Dose of 100 for 36 days | Same dose for 14 days | Patterson et al. (1979) | No controls |
| Gilts | 7 | 0, 5000 | | *F. tricinumin* maize culture | 25 | 6 | Feed intake and live weight gain | Reduced but no statistics given | 5000 | n.a. | Rafai et al. (1982) | |
| | | | | | | | Leucocyte counts | Significantly decreased | | n.a. | | |
| | | | | | | | Cortisol | Significantly increased | | n.a. | | |
| | | | | | | | Lymphocyte proliferation | Significantly decreased | | n.a. | | |
| | | | | | | | Antibody development against necrotic enteritis vaccine | Significantly decreased | | n.a. | | |
| | | | | | | | Body weight, thymus and spleen weight | Significantly decreased | | n.a. | | |
| | | | | | | | Adrenal weight | Significantly increased | | n.a. | | |

**Table 31:** Continued.

| Animals | | Toxin | | | Experiment | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | **Initial live weight (kg)** | **Concentration in feed (µg T-2 toxin/kg)** | **Dose (µgT-2 toxin/kg b.w. per day)** | **Toxin source** | **Exposure duration (days)** | **N per group** | **Parameter** | **Effect** | **LOEL (µg/kg diet)** | **NOEL (µg/kg diet)** | **Reference** | **Comments** |
| Female | 11 | 0, 5000 | | Produced in the lab - no further description | 21 | 6 | Coliform bacteria in the GIT (stomach - caecum) | Increased | 5000 | n.a. | Tenk et al. (1982) | |
| | | | | | | | Cortisol | Significantly increased | 5000 | n.a. | | |
| Not reported | Not reported | 0, 13520 | | *F. sporotrichioides* extract | Not reported | Not reported | Feed intake and live weight gain | Partially refused feed and did not gain weight | 13520 | n.a. | Palyusik et al. (1990) | +5420 µg HT-2 toxin/kg, no exact measurements given |
| | | | | | | | Uterus | Atrophy | 13520 | n.a. | | |
| Barrows | 15.9 | 0, 10000 | | 99% pure toxin as determined by NMR/MS | 28 | 6 | Live weight gain | Significantly decreased | 10000 | n.a. | Harvey et al. (1990a,b) | No feed intake measured as all piglets of one group were in the same box |
| | | | | | | | Serum biochemistry | Significantly increased triglycerides was the only effect detected at 21 day that was still evident at 28 days | 10000 | n.a. | | |
| | | | | | | | Haematology | Red blood cell measures significantly affected (PCV, haemoglobin, MCV, MCH, MCHC) | 10000 | n.a. | | |
| | | | | | | | Organs | Heart weight significantly increased | 10000 | n.a. | | |
| | | | | | | | Skin lesions | Obvious at snout and prepuce after 21 days, but without signs of pain | 10000 | n.a. | | |

**Table 31:** Continued.

| Animals | | Toxin | | | Experiment | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | **Initial live weight (kg)** | **Concentration in feed (µg T-2 toxin/kg)** | **Dose (µg T-2 toxin/kg b.w. per day)** | **Toxin source** | **Exposure duration (days)** | **N per group** | **Parameter** | **Effect** | **LOEL (µg/kg diet)** | **NOEL (µg/kg diet)** | **Reference** | **Comments** |
| Sow | Not reported | 0, 8000 | 24 mg/day sowA and 6 mg/day sow B | *F. tricinctum* c.m. (on rice) | approx. 32 days | 1 | Piglet health | Piglets of sow A became ill within 72 hours after birth (at the time of farrowing; before colostrum intake all piglets were healthy and similar to control) | | | Ványi et al. (1991) | N=1, no results on the sows |
| Mixed (SPF) | 38 | 0, 400, 800, 1600, 3200 | | Purified from c.m. of *F. sporotrichioides* 99.8% of substances identified as T-2 toxin | 35 | 6 | Feed intake | Significantly influenced as indicated by ANOVA, no group differences evaluated, but 1600-group had highest feed intake (might be biased by analysis of covariance), 3200-lowest | n.a. | n.a. | Friend et al. (1992) | Significantly different starting live weight in different groups. Necropsy and clinical chemistry and haematology data showed no T-2 toxin effect |
| Barrows | 18 | 0, 8000 | | >99% pure toxin as determined by NMR/MS) | 30 | 9/3 | Feed intake and live weight gain | Significantly reduced | 8000 | n.a. | Harvey et al. (1994) | T-2 toxin had no effect on blastogenesis. No synergistic, but additive effects with ochratoxin A |
| | | | | | | | Alkaline phosphatase activity | Significantly reduced | 8000 | n.a. | | |
| | | | | | | | Haemoglobin concentration | Significantly reduced | 8000 | n.a. | | |

**Table 31:** Continued.

| Animals | | Toxin | | | Experiment | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | **Initial live weight (kg)** | **Concentration in feed (µg T-2 toxin/kg)** | **Dose (µg T-2 toxin/kg b.w. per day)** | **Toxin source** | **Exposure duration (days)** | **N per group** | **Parameter** | **Effect** | **LOEL (µg/kg diet)** | **NOEL (µg/kg diet)** | **Reference** | **Comments** |
| Not reported[(a)] | 9 | 0, 500, 1000, 2000, 3000, 4000, 5000, 10000, 15000 | 0, 29, 62, 105, 129 | Purified from c.m. of *F. tricinum*, 90% of substances identified as T-2 toxin | 21 | 10 | Macroscopic lesions | Snout/oral cavity | 4000 | 3000 | Rafai et al. (1995a) | 2 experiments |
| | | | | | | | Live weight gain | Significantly decreased | 2000 | 1000 | | |
| | | | | | | | Feed intake | Significantly decreased | 500 | n.a. | | |
| | | | | | | | Glucose in blood | Significantly decreased | 1000 | 500 | | |
| | | | | | | | Inorganic phosphorus and magnesium | Significantly increased | 1000 | 5000 | | |
| Not reported[(a)] | 9 | 0, 500, 1000, 2000, 3000 | 0, 29, 62, 105, 129 | Purified from c.m. of *F. tricinum*, 90% of substances identified as T-2 toxin | 21 | 10 | Anti-horse globulin (A-HG) titre | Significantly decreased at all measured time points (day 7, 14, 21) | 500 | n.a. | Rafai et al. (1995b) | Same experiment as Rafai et al. (1995a) |
| | | | | | | | Lymphocyte stimulation with A-HG | Significantly decreased after 21 days | 2000 | 1000 | | |
| | | | | | | | Lymphocyte stimulation with PHA | Significantly decreased after 21 days | 2000 | 1000 | | |
| | | | | | | | Lymphocyte stimulation with ConA | Significantly decreased after 21 days | 500 | n.a. | | |
| | | | | | | | Leucocyte counts | Significantly decreased | 500 | n.a. | | |
| | | | | | | | t-lymphocytes | Significantly decreased | 500 | n.a. | | |
| | | | | | | | Histological changes in thymus, spleen and lymph nodes | | 500 | n.a. | | |

**Table 31:** Continued.

| Animals | | Toxin | | | Experiment | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | **Initial live weight (kg)** | **Concentration in feed (µg T-2 toxin/kg)** | **Dose (µg T-2 toxin/kg b.w. per day)** | **Toxin source** | **Exposure duration (days)** | **N per group** | **Parameter** | **Effect** | **LOEL (µg/kg diet)** | **NOEL (µg/kg diet)** | **Reference** | **Comments** |
| Male castrated | 11.4 | 0, 540, 1324, 2102 | | Pure toxin (>98%) | 28 | 5 | Anti-ovalbumin antibody production | Significantly reduced on day 21 but not on day 28 | 1324 | 540 | Meissonnier et al. (2008a) | 213 µg deoxynivalenol/kg feed, 38 µg zearalenone/kg feed. No feed intake recorded |
| | | | | | | | Live weight gain | Significantly reduced | 2102 | 1324 | | |
| | | | | | | | IgA in serum | Significantly increased at day 7 but not at later time points | 2102 | 1324 | | |
| | | | | | | | P450 1A protein expression | Significantly reduced | 2102 | 1324 | | |
| Male castrated | 11.7 | 0, 3000 | | Commercial fungal c.m. | 14 | 9 - 12 | Live weight gain | Only significantly reduced when vitamin E (100 mg/kg feed) was additionally supplemented | 3000 | n.a. | Frankic et al. (2008) | Fed restrictively |
| | | | | | | | Feed conversion | Only significantly reduced when vitamin E (100 mg/kg feed) was additionally supplemented | 3000 | n.a. | | |
| | | | | | | | DNA-damage in lymphocytes | Significantly increased by 27% | 3000 | n.a. | | |
| | | | | | | | Total serum IgG | Significantly increased | 3000 | n.a. | | |

N: number of animals; LOEL: lowest-observed-effect level; NOEL: no-observed-effect level; TLC: thin layer chromatography; GC: gas chromatography; NMR: nuclear magnetic resonance; MS: mass spectrometry; GIT: gastrointestinal tract; PCV: packed cell volume; MCV: average red blood cell size; MCH: haemoglobin amount per red blood cell; MCHC: mean corpuscular haemoglobin concentration; A-HG: anti-horse globulin; PHA: phytohaemagglutinin; ConA: concanavalin A; Ig: immunoglobulin; P450 1A: Cytochrome P450 1A; c.e.: culture extract; c.m. culture material; n.a.: not applicable; approx.: approximately.
(a): Possibly mixed, not clearly reported in the study.

Feeding diets containing 2.102 mg T-2 toxin/kg (> 98 % purity) for 28 days to piglets (11.4 ± 0.3 kg live weight at the start, n = 5) resulted in a significant decrease in live weight gain (87 % of control) while at lower dietary concentrations the effect was not significant (90 and 92 % at 0.54 and 1.324 mg T-2 toxin/kg, respectively) (Meissonnier et al., 2008a). Pigs fed 1.324 or 2.102 mg T-2 toxin/kg exhibited reduced anti-ovalbumin antibody production on day 21 without significant alteration to specific lymphocyte proliferation. The CONTAM Panel noted that there was no increase in the severity of the anti-ovalbumin antibody production effects after day 21. Immunoglobulin concentrations in plasma of the pigs were not altered over the experiment with the exception of a significantly increased level of IgA on day 7 in the group fed the highest contaminated diet. The livers of pigs exposed to T-2 toxin presented normal cytochrome P450 content, UGT 1A and P450 2B, 2C or 3A protein expression, and glutathione- and UDP glucuronosyl-transferase activities. However, CYP1A related activities (ethoxyresorufin O-demethylation and benzo[*a*]pyrene hydroxylation) were reduced for all pigs given T-2 toxin, with CYP1A protein expression decreased in pigs fed the highest dose. In addition, T-2 toxin exposure reduced certain N-demethylase activities. However, feed intakes were not reported by Meissonnier et al. (2008a).

In conclusion, the published investigations demonstrate that pigs are among the most susceptible animals towards the effects of T-2 toxin, the most sensitive endpoints being immunological or haematological effects that occur from doses of 29 µg T-2 toxin/kg b.w. per day (equivalent to 500 µg T-2 toxin/kg feed). Based on the available data 29 µg T-2 toxin/kg b.w. per day could be considered as a LOAEL. So far, no NOAEL is identified for pigs.

### 7.4.3. Poultry

Effects of acute and chronic toxicity of T-2 toxin on poultry were previously reported by the JECFA (FAO/WHO, 2001) and were recently reviewed by Dohnal et al. (2008), AFSSA (2009), van der Fels-Klerx and Stratakou (2010) and Li et al. (2011). Since the JECFA evaluation, several studies on the effects of chronic T-2 toxin intoxication in poultry after oral administration have become available. In addition, a few studies on the chronic effects of the combination of T-2 and HT-2 toxins are available. Chronic toxicity studies on HT-2 toxin were not identified.

The $LD_{50}$ values for T-2 toxin and HT-2 toxin in poultry as reported in the previous evaluation are summarised in Table 32. More recent studies on acute toxicity in poultry were not identified.

**Table 32:** Studies of the acute toxicity of T-2 toxin and HT-2 toxin by oral administration in poultry.

| Species | Sex | Toxin | $LD_{50}$ (mg/kg b.w.) | Reference |
|---|---|---|---|---|
| 7-day-old broiler chickens | Male | T-2 toxin | 4 | Hoerr et al. (1981) |
| Laying hens | Female | T-2 toxin | 6.3 | Chi et al. (1977b) |
| Day-old cockerels | Male | T-2 toxin | 1.84 | Lansden et al. (1978) |
| 1-day-old broiler chickens | Not reported | T-2 toxin | 5 | Chi et al. (1977b, 1978b) |
| 1-day-old broiler chickens | Not specified | HT-2 toxin | 7.2 | Chi et al. (1978b) |

b.w.: body weight.

Symptoms of T-2 toxin acute intoxication are dominated by nervous and digestive disorders (Grevet, 2004). In the minutes following oral administration, hyperpnoea appears, accompanied by lethargy, drooping head and wings and a loss of balance. Signs of nervous abnormalities disappear quickly and digestive troubles start. Digestive disorders are characterised by repeated deglutition, diarrhoea and complete refusal to eat or drink. Death occurs 3.5 to 13.5 hours after the T-2 toxin has been administered. Lesions are dominated by a haemorrhagic syndrome localised in the digestive system and in the muscles (Grevet, 2004).

The most relevant chronic toxicity studies on the different types of effects based on dose and duration of exposure in several poultry species are summarised in Table 33. Most of the recent studies published since 2001 are briefly described below.

Dietary concentrations of up to 1 mg/kg feed of T-2 toxin (as well as the combined treatment of T-2 toxin with diacetoxyscirpenol up to 1 mg/kg feed) given for 5 weeks to one day old broiler chickens and turkeys (20 and 12 per dose group, respectively) did not affect body weight gains or feed conversion ratios. However, mouth and intestine lesions were observed at 0.5 and 1.0 mg T-2 toxin/kg feed, respectively for both species (Sklan et al., 2001, 2003). A diet containing 2 mg/kg of T-2 toxin for 28 days induced in broiler chickens (n = 6 per group and 5 replicates) a decrease in body weight and an increase in feed/body weight gain ratio (Diaz et al., 2005). Another feeding study (doses of 0, 0.5, 1.5, 4.5 or 13.5 mg T-2 toxin/kg during 17 days) on the same species (broiler chickens) was conducted by Rezar et al. (2007). Reductions in feed consumption and in body weight gain were observed at the dose of 4.5 mg T-2 toxin/kg feed (Rezar et al., 2007). Lesions in liver, lymphoid organs, proventriculus and intestine were observed in broiler chickens after a diet of 4 mg T-2 toxin/kg feed (Rajeev et al., 2003). These pathological effects were also noticed by Krisnamoorthy et al. (2007) from day 12 of a 28 day feeding study at a low dose of 0.5 mg T-2 toxin/mg feed in broiler chickens (n = 12).

Two studies were conducted by Grizzle et al. (2005) for adult bobwhite quails to determine the effect of chronic *vs.* intermittent exposure to T-2 toxin on reproductive performance. In a first study, 180 hens received by gavage 0 % ($LD_0$), 20 % ($LD_{20}$), 40 % ($LD_{40}$) or 60 % ($LD_{60}$) of the acute 100 % lethal dose of T-2 toxin (0, 12.4, 14.0 or 15.5 mg T-2 toxin/kg b.w., respectively). One quarter of the dosage was administered each week for 3 weeks. Date of puberty was delayed 5 days as a result of the highest dose of T-2 toxin. There were no differences in hen-day egg production or fertile hatchability of eggs as a result of intermittent exposure to T-2 toxin. Fertility and total hatchability of eggs collected from hens (15.5 mg T-2 toxin/kg b.w. treatment) during week 2 following puberty were less than from control hens. In the second study, 139 hens were fed commercial breeder diets fortified with T-2 toxin at 0, 12, 16 and 20 mg T-2 toxin/kg feed for a 4 week period. Puberty was delayed 11 days among hens fed 20 mg/kg feed as compared to hens not fed T-2 toxin. Similarly, feed consumption was lower among birds consuming any levels of T-2 toxin as compared to controls. No differences in total hen-day egg production were found. However, percent fertility and total hatchability of eggs were lower among hens receiving 20 mg T-2 toxin/kg feed as compared to control hens during the first 7 days following puberty. Results from these studies indicate that reproductive failure in wild bobwhite quail may be a consequence of T-2 toxin exposure (Grizzle et al., 2005).

At a single dose level, 1 mg T-2 toxin/kg in diet to broiler chickens (3 birds per group and per time point) caused statistically significant induction of apoptosis in thymus at 6, 12, 24 and 36 hours post-treatment. A slight increase of apoptotic cells was also observed in spleen, but this effect was not statistically significant (Venkatesh et al., 2005). Macroscopic examination of broiler chickens fed 4 mg/kg of T-2 toxin for 28 days also revealed atrophy of lymphoid organs (bursa of Fabricus, thymus and spleen) mainly due to lymphocytolysis (Nataraja et al., 2003). Kamalavenkatesh et al. (2005) also observed lymphocytolysis and lymphoid depletion in lymphoid organs, decrease in thymic CD4+ and CD8+ lymphocytes in broiler chickens (n = 10) exposed to 1 mg/kg T-2 toxin in diet for 28 days from the day of hatch.

Adverse effects on immunoglobulin formation after parenteral immunisation (Newcastle disease virus) were not observed for concentrations up to 1 mg/kg of T-2 toxin in diet for chickens and turkeys (conditions described above) by Sklan et al. (2001, 2003). However, a diet at a T-2 toxin concentration of 1 mg/kg showed decreased haemagglutination inhibition titres to Newcastle disease virus in chickens (conditions described above) (Kamalavenkatesh et al., 2005). Similarly, immunmodulatory effects were observed in an experiment with 23 day old broiler cockerels (n = 20 per dose) exposed to 2.35 or 4.18 mg/kg T-2 toxin in feed for 14 days (Weber et al., 2006). At the low dose immunostimulatory effects were observed and at the high dose immunosuppressive effects occurred (endpoint: inhibition titres against Newcastle disease virus). Further investigations of the same group showed that oral

application of vitamin E increased antibody formation against Newcastle disease in animals, an effect which could not be suppressed by exposure to 2.35 mg/kg T-2 toxin in diet (Weber et al., 2008).

Two more recent studies report on oral administration of both T-2 and HT-2 toxins. In the experiment of Pál et al. (2009), 90 days old broiler cockerels (n = 30) were fed for 21 days with a naturally contaminated diet at 0.31 and 0.26 mg of T-2 and HT-2 toxins, respectively per kg feed (equivalent to doses of 0.033-0.05 and 0.03-0.04 mg T-2 toxin and HT-2 toxin, respectively per kg b.w. per day). Body weight gain and feed consumption were not affected. An increased content of reduced GSH in blood plasma and heart and a decreased content in liver and pancreas in comparison to the control were observed. However, only the observed effect in the heart was statistically significant. Moreover, the presence of antioxidants (vitamin E and selenium) in feed may mask clinical signs of intoxication (Pál et al., 2009). The effects of combining T-2 and HT-2 toxins at different doses in the starter (0-21 days: 1.04 mg T-2 toxin and 0.49 mg HT-2 toxin/kg feed), and finisher diets (22-39 days: 0.12 mg T-2 toxin and 0.02 mg HT-2 toxin/kg feed) were investigated in 40 day old broiler cockerels (n = 20) by Weber et al. (2010). The partially purified toxins obtained from inoculated maize were sprayed onto the complete feed. Pathological signs such as lesions in the oral cavity and on the tongue (inflammation in the small intestine) were found for half of the birds at the end of treatment. Body weight was significantly lower as a result of feeding T-2 and HT-2 toxin contaminated diet at the end of the starter diet (Weber et al., 2010).

Recently DNA damage has been observed in chickens (n = 10 per group) exposed to T-2 toxin in feed (fortified to 10 mg/kg), with or without addition of dietary nucleotides (2 g nucleotides/kg feed) (Frankic et al., 2006). The objective was to study the protective effect of dietary nucleotides in the case of DNA damage induced in leukocytes by T-2 toxin. After 17 days of treatment the Comet assay was used to measure DNA damage in spleen leukocytes. T-2 toxin was found to significantly induce DNA damage, and this effect was reduced when the feed was supplemented with nucleotides. A study by Rezar et al., (2007) also showed a significant increase in fragmented DNA in a study with male broiler chickens (n = 10 per group) that received feed containing 13.5 mg/kg T-2 toxin for 17 days, using the Comet assay of spleen leukocytes. T-2 toxin at 4.5 mg/kg feed or lower did not show a significant effect.

Sokolovic et al. (2007) used chicken nucleated blood cells as a cellular model for genotoxicity testing using the alkaline Comet assay. Chickens (n = 5 per group) were administered a single dose of 0.5 mg T-2 toxin/kg b.w. into the crop. Blood was collected immediately prior to the treatment and 24 hours after the application of the treatment dose. T-2 toxin caused a significant increase in mean tail lengths and mean tail moments indicating the formation of DNA damage.

**Table 33:** Effects of chronic intoxication by oral administration of T-2 toxin or the combination of T-2 and HT-2 toxins in poultry.

| | Species: Concentration[(a)] and duration | Symptoms | Reference |
|---|---|---|---|
| **Zootechnical Performance** | 1 day old broiler chickens (n=40): 1-16 mg T-2 toxin/kg, 3 weeks | Decrease in body weight gain and feed consumption to 4 mg T-2 toxin/kg diet | Wyatt et al. (1973) |
| | 8 week old broiler chickens (n=12/dose of T-2 toxin): 0.2-4 mg T-2 toxin/kg, 9 weeks | Decrease in body weight gain and feed consumption to 4 mg T-2 toxin/kg diet | Chi et al. (1977b) |
| | 1 day old broiler chickens (n=36): 4 mg T-2 toxin/kg, 3 weeks | Decrease in body weight gain and feed consumption | Kubena et al. (1989a) |
| | 1 day old broiler chickens (n=60): 4 mg and 8 mg T-2 toxin/kg, 3 weeks | Decrease in body weight gain and feed consumption | Kubena et al. (1989b); Kubena et al. (1990) |
| | 33 week old laying hens (n=10); 2 mg T-2 toxin/kg; 24 days; | No effect on body weight gain but decrease in feed consumption | Diaz et al. (1994) |
| | 1 day old chickens (n=20): 0.1-1 mg T-2 toxin/kg, 5 weeks | No effect on body weight gain | Sklan et al. (2001) |
| | 1 day old turkey poults (n=12): 0.2-1 mg T-2 toxin/kg, 5 weeks | No effect on body weight gain at 1 mg T-2 toxin/kg | Sklan et al. (2003) |
| | 1 day old broiler chickens (n=6 per 5 replicates): 2 mg T-2 toxin, 28 days | Decrease in body weight gain | Diaz et al. (2005) |
| | Male broiler chicks (n=10): 0.5-13.5 mg T-2 toxin/kg, 17 days | Decrease in body weight gain and feed consumption to 4.5 mg T-2 toxin/kg diet | Rezar et al. (2007) |
| | 40 day old broiler cockerels (n=20); Starter diet: 1.04 mg T-2 toxin + 0.49 mg HT-2 toxin/kg, 0-21 days, finisher diet: 0.12 mg T-2 toxin + 0.02 mg HT-2 toxin/kg, 22-39 days | Decrease in body weight gain to 1.04 mg T-2 toxin + 0.49 mg HT-2 toxin/kg, at 21 days, | Weber et al. (2010) |
| | 90 days old broiler cockerels (n=30): 0.31 and 0.26 mg of T-2 and HT-2 toxins, respectively per kg feed 0-21 days | No change in body weight gain and feed consumption to 0.03-0.05 mg T-2 toxin and 0.03-0.04 mg HT-2 toxin/kg b.w. per day | Pál et al. (2009) |
| | Laying hens (n=10): 20 mg T-2 toxin/kg, 3 weeks | Decrease in body weight gain and feed consumption to 0.86-0.91 mg T-2 toxin/kg b.w. per day | Wyatt et al. (1975) |
| | 27 week old laying hens (n=8/dose of T-2 toxin): 0.5-8 mg T-2 toxin/kg, 8 weeks | Decrease in feed consumption to 8 mg T-2 toxin/kg | Chi et al. (1977a) |
| | Adult bobwhite quails (n=180): 12, 16 and 20 mg T-2 toxin/kg, 4 weeks | Decrease in body weight gain to 12 mg T-2 toxin/kg | Grizzle et al. (2005) |
| | 1 day old white Pekin fattening ducks (n=10 per dose of T-2 toxin): 0.2-4 mg T-2 toxin/kg, 7 weeks | Decrease in body weight gain and feed consumption to 0.2 mg/kg | Rafai et al. (2000) |

**Table 33:** Continued.

| | Species: Concentration[(a)] and duration | Symptoms | Reference |
|---|---|---|---|
| **Reproduction** | Laying hens (n=10): 20 mg T-2 toxin/kg, 3 weeks | Reduction in egg production and thinner egg shell to 0.86-0.91 mg T-2 toxin/kg b.w. per day | Wyatt et al. (1975) |
| | 27 week old laying hens (n=4/dose of toxin): 0.5-8 mg T-2 toxin/kg, 8 weeks | Decrease in egg laying to 8 mg T-2 toxin/kg, increase in number of unfertilised eggs and drop in hatchability to 2 mg T-2 toxin/kg | Chi et al. (1977a); |
| | 33 week old laying hens (n=10): 2 mg T-2 toxin/kg, 24 days | Reduction in egg production | Diaz et al. (1994) |
| | Laying geese (n=110): 0.2-3 mg T-2 toxin/kg b.w.[(b)], 18 days | Drop in egg laying and hatchability | Ványi et al. (1994) |
| | Adult bobwhite quails (n=180): 12, 16 and 20 mg T-2 toxin/kg, 4 weeks | Delay of puberty, decrease of egg fertility and hatchability to 20 mg/kg | Grizzle et al. (2005) |
| **Skin and mucous membranes** | 1 day old broiler chickens (n=40): 1-16 mg T-2 toxin/kg, 3 weeks | Necrosis of mucous membranes in the mouth cavity from the third week to 1 mg T-2 toxin/kg diet | Wyatt et al. (1973) |
| | 1 day broiler chickens (n=36): 0.2-4 mg T-2 toxin/kg, 9 weeks | Oral lesions to 4 mg T-2 toxin/kg diet | Chi et al. (1977a) |
| | Chicken (n=not reported): 2-10 mg T-2 toxin/kg, 4 weeks | Slight necrosis in a few animals to 10 mg T-2 toxin/kg | Richard et al. (1978) |
| | 1 day old broiler chickens (n=36): 4 mg T-2 toxin/kg, 3 weeks | Oral lesions | Kubena et al. (1989a) |
| | 1 day old broiler chickens (n=60): 4 mg and 8 mg T-2 toxin/kg, 3 weeks | Oral lesions | Kubena et al. (1989b,1990) |
| | 1 day old white Pekin fattening ducks (n=10 per dose of T-2 toxin): 0.2-4 mg T-2 toxin/kg, 7 weeks | Necrosis of the skin and the mucous membranes of the tongue, palate, near the mouth and the pharynx up to 3 mg T-2 toxin/kg | Rafai et al. (2000) |
| | 1 day old chickens (n=20): 0.1-1 mg T-2 toxin/kg, 5 weeks | Oral lesions at 0.5 mg T-2 toxin/kg and mild lesions in the intestine (decrease in the surface of villi) at 1 mg T-2 toxin/kg | Sklan et al. (2001) |
| | 1 day old turkeys poults (n=12): 0.2-1 mg T-2 toxin/kg, 5 weeks | Oral lesions at 0.5 mg T-2 toxin/kg and mild lesions in the intestine at 1 mg T-2 toxin/kg | Sklan et al. (2003) |
| | 1 day old broiler chickens (n=50): 4 mg T-2 toxin/kg, 35 days | Lesions in the intestine | Rajeev et al. (2003) |
| | Chickens (n=12): 0.5 mg T-2 toxin/kg, 28 days | Epthelial necrosis, intestinal glandular fibrosis to 0.5 mg T-2 toxin/kg | Krishnamoorthy et al. (2007) |
| | 40 day old broiler cockerels (n=20); Starter diet: 1.04 mg T-2 toxin + 0.49 mg HT-2 toxin/kg, 0-21 days, finisher diet: 0.12 mg T-2 toxin + 0.02 mg HT-2 toxin/kg, 22-39 days | Lesions in the oral cavity and on the tongue | Weber et al. (2010) |

**Table 33:** Continued.

| | Species: Concentration[(a)] and duration | Symptoms | Reference |
|---|---|---|---|
| **Immune system** | Turkeys (n=not reported): 10 mg T-2 toxin/kg, 4 weeks | Decrease in the size of the Bursa of Fabricius, accelerated thymus involution | Richard et al. (1978) |
| | Chicken (n=40): 1-16 mg T-2 toxin/kg, 3weeks | Decrease in the weight of the spleen and the bursa of Fabricius to 8 mg/kg | Wyatt et al. (1973) |
| | 1 day old broiler chickens (n=36): 4 mg T-2 toxin/kg, 3 weeks | Increase in the weight of the bursa of Fabricius | Kubena et al. (1989a) |
| | 1 day old white Pekin fattening ducks (n=10 per dose of T-2 toxin): 0.2-4 mg T-2 toxin/kg, 7 weeks | Decrease in lymphocyte response to mitogenic and blastogenic agents, lower lymphocyte levels in lymphoid organs to 3-4 mg T-2 toxin/kg. Lymphocyte depletion in the spleen and bursa of Fabricius to 3-4 mg/kg | Rafai et al. (2000) |
| | 1 day old broiler chickens (n=50): 4 mg T-2 toxin/kg, 28 days | Atrophy in lymphoid organs (bursa Fabricius, thymus and spleen) | Nataraja et al. (2003) |
| | 1 day old broiler chickens (n=50): 4 mg T-2 toxin/kg, 35 days | Lesions in lymphoid organs (bursa Fabricius and thymus) | Rajeev et al. (2003) |
| | 1 day old broiler chickens (n=6 per 5 replicates): 2 mg T-2 toxin, 28 days | No change in the weight of the bursa Fabricius and spleen | Diaz et al. (2005) |
| | 28-day old broiler chicks (n=3/group and time point): 1 mg T-2 toxin/kg (single dose); 6, 12, 24, 36 hours | Induction of apoptosis in thymus (peak induction to 24 hours) | Venkatesh et al. (2005) |
| | Broiler chickens (n=10): 1 mg T-2 toxin/kg, 28 days | Lymphoid depletion, decrease in haemagglutination inhibition titres against Newcastle disease virus | Kamalvenkatesh et al. (2005) |
| | 23 day old broiler cockerels (n=20): 4.18 mg T-2 toxin/kg, 14 days | Immunosuppressive effects (haemagglutination inhibition titres against Newcastle disease virus) | Weber et al. (2006) |
| | Chickens (n=10 per group): 10 mg T-2 toxin/kg, 17 days | Increase of DNA fragmentation in spleen leukocyte | Frankic et al. (2006) |
| | Broiler chickens (n=10): 13.5 mg T-2 toxin/kg, 17 days | Increase of DNA fragmentation in spleen leukocytes | Rezar et al. (2007) |

(a): Unless otherwise stated, toxin concentrations are expressed in mg/kg feed; (b): Dose expressed in mg/kg live body weight (b.w).

In conclusion, based on the available data, during chronic intoxication mostly cutaneous lesions (oral cavity and intestine membrane) and/or changes in zootechnical and reproduction performances (e.g. growth, egg production and hatchability) are apparent. As first effects, lesions occur in the oral cavity at concentrations from 0.5 mg T-2 toxin/kg in feed in one day old broiler chickens and one day old turkey poults. In one day old fattening ducks concentrations as low as 0.2 mg/kg feed caused a significant reduction in body weight gain. Immunmodulatory effects are obvious at concentrations above 1 mg T-2 toxin/kg in feed. Growth depression was observed in a dose from 2 mg T-2 toxin/kg in diet fed to chickens. Effects on reproduction i.e. infertility of eggs and/or reduction of egg production were seen at concentrations starting from 2 mg T-2 toxin/kg feed for laying hens. DNA damage has been observed in spleen leukocytes above 4.5 mg T-2 toxin/kg feed. Direct administration of T-2 toxin into the crop showed positive Comet assay results in peripheral blood cells at a dose of 0.5 mg/kg b.w. So far, no NOAEL has been identified for poultry. However, based on the available data 40 µg/kg b.w. per day and 48 µg/kg b.w. per day (equivalent to 0.5 mg T-2 toxin/kg of feed) could be considered as LOAEL for broiler chickens and fattening turkeys, respectively.[30] For fattening ducks a LOAEL of 40 µg/kg b.w. kg per day (equivalent to 0.2 mg T-2 toxin/kg feed)[30] and for laying hens a LOAEL of 120 µg/kg b.w. per day (equivalent to 2 mg T-2 toxin/kg feed)[31] were identified.

### 7.4.4. Rabbits

Few studies of the effects of T-2 toxin on rabbits were reported in the previous assessments of the JECFA (FAO/WHO, 2001) or the SCF (SCF, 2001). Additional studies on oral administration are reported below.

A single oral dose of T-2 toxin at 2.0 mg/kg b.w. was given to 5 New Zealand white rabbits by gavage. Oral lesions, diarrhoea and anorexia in the animals were observed but no significant alteration in haematological and biochemical parameters were noticed. However, one of the 5 rabbits was found dead 36 hours after the treatment (Gentry and Cooper, 1981). In an experiment performed by Glávits et al. (1989), groups of 14 rabbits were treated with single oral doses of 1, 2, 4, 6, 8, 10 or 15 mg/kg b.w. of T-2 toxin. With the doses of 4 mg/kg b.w. or higher the animals died within 24-48 hours. Acute catarrhal gastroenteritis, necrosis of lymphoid cells of the gastrointestinal mucosa, centrolobular dystrophy of the liver, necrosis of cells of the mononuclear phagocyte system in the liver, tubulonephrosis, focal dystrophy of the adrenal cortex, lymphocyte depletion involving both T- and B-cell-dependent zones of the lymphoid organs (spleen, lymph and ampulla ilei), and depletion and necrosis of the myelopoietic cell colonies of the bone marrow were observed. Similar but milder changes were noticed in surviving rabbits exsanguinated 48 hours after treatment. In addition to the direct damage done to the digestive tract mucosa and liver, T-2 toxin severely damaged the cells participating in humoral and cell-mediated immunity and in the local defence of the intestinal mucosa, and markedly impaired phagocytosis and granulocytopoiesis. In another sub acute experiment, 15 rabbits were given oral doses of 2 mg/kg b.w. of T-2 toxin daily for 10 days. One or two rabbits were killed by bleeding every day. In rabbits killed after day 7 sub acute catarrhal gastritis, emaciation and hypertrophy of the adrenal cortex were observed (Glávits et al., 1989).

Feeds containing sub lethal T-2 toxin concentrations of 12.5 and 25 mg/kg were fed to 12 4-month-old New Zealand White female rabbits for 10 days i.e. 0.19 and 0.28 mg T-2 toxin/kg b.w. per day, respectively (Fekete et al., 1989). The animals ate 60-70 % less toxin-containing food. The rabbits showed emaciation, sub acute catarrhal gastritis, necrosis of the lymphoid cells of the intestinal mucosa, depletion and necrosis in the lymphoid follicles of the ampulla ilei, spleen and lymph nodes. Necrosis of the cells of mononuclear phagocyte system and myeloid haemacytogenesis was characteristic. The T-2 toxin concentrations of faeces, cecotroph and urine were proportional to intake (Fekete et al., 1989).

[30] LOAELs calculated by using the live weight and feed intake as reported by authors in the respective studies.
[31] LOAEL calculated by using the live weight and feed intake as presented in Appendix C, Table C2.

Groups of 10 New Zealand white rabbits were fed with naturally contaminated wheat at 0.19 mg/kg feed for 32 days, equivalent to 0.008 mg T-2 toxin/kg b.w. per day (Fekete and Huszenicza, 1993). A control group was fed uncontaminated wheat. The treated animals were then given gonadotropin-releasing hormone to induce false gestation plus T-2 toxin treatment for a further 18 days more (total treatment duration 50 days). Progesterone levels were monitored during this period of time. Two animals died during the 32-day experiment period and one animal died during the subsequent treatment period. No control animal died. No morphological differences were noticed between the three treated and the three control animals killed after 32 days. Serum creatinine and ALT activities were higher in treated than in control animals and the serum cholinesterase concentration decreased. Three out of the five animals given both gonadotropin-releasing hormone and T-2 toxin treatment showed abnormal progression of progesterone concentrations (Fekete and Huszenicza, 1993).

Effects of 4-7-week feeding of naturally contaminated wheat grains containing 0.284 mg T-2 toxin/kg feed were investigated in sexually mature, virgin female rabbits. Three out of 10 animals died before the end of the experiment (acute, fibrinous-purulent peritonitis and pneumonia). Hepatic damage was indicated by a significant increase in serum ALT activity and AST (slight), gamma-glutamyl transferase, malate dehydrogenase activities, and decreased cholinesterase activity compared to control animals. Impaired kidney function was indicated by a significantly higher creatinine level, as compared to the control. T-2 toxin impaired ovarian functions, reflected by unaltered progesterone concentration, macro- and microscopical pictures after GnRH-stimulation (Szilágyi et al., 1994). However, the findings of these two studies are based on the use of naturally contaminated feed for which co-contamination with other mycotoxins may occur. Moreover, the findings of Szilágyi et al. (1994) on reproductive endocrine effects were considered by the CONTAM Panel to be inconclusive.

Guerre et al. (2000) investigated whether exposure at low doses could alter metabolism of xenobiotics by the liver. Three doses of 0.10, 0.25, or 0.50 mg T-2 toxin/kg b.w. dissolved in olive oil were administered orally to New Zealand white rabbits daily for 5 days. At 0.50 mg/kg b.w. per day, three out of five animals died, whereas only a slight decrease in body weight gain and moderate signs of toxicity occurred in rabbits receiving 0.25 mg/kg b.w. per day, and the body weight increased without signs of toxicity at 0.10 mg/kg b.w. per day. Regarding the metabolism of xenobiotics by the liver, the observed results that no significant effects on drug metabolising enzymes were seen at a T-2 toxin dose of 0.1 mg/kg b.w. per day suggested that a short exposure time to T-2 toxin would not be associated with any significant changes.

In conclusion, the available toxicological data from chronic exposure studies in rabbits show that doses ranging from 0.5-2.0 mg of T-2 toxin/kg b.w. per day result in a decrease of body weight gain and other signs of toxicity such as gastritis and intestinal necrosis. Only moderate signs including haematological and hormonal effects and no signs of toxicity have been observed at doses of 0.2-0.5 mg of T-2 toxin/kg b.w. per day. A NOAEL of 0.1 mg T-2 toxin/kg b.w. per day was identified.

### 7.4.5. Farmed fish

Few studies of the effects of T-2 toxin in fish have been reported. Effects of T-2 toxin on fish species were not evaluated in the previous assessments of the JECFA (FAO/WHO, 2001) or the SCF (SCF, 2001).

Rainbow trout (n = 1000, reduced to 400 after 9 months) were given 0.2 or 0.4 mg crystalline T-2 toxin/kg feed. The livers of five fish from each treatment group were examined every month until the experiment was finished after 12 months. No signs of neoplasia were found (Marasas et al., 1969).

Feeding crystalline T-2 toxin in the diet to juvenile rainbow trout (3 jars with 50 fish in each/dose, fish weight from 1.0 g) for 16 weeks resulted in reductions in feed intake, growth rates, feed efficiency and haematocrit at concentrations of 2.5 mg/kg to 15 mg/kg feed. Feed concentrations of 10 and 15 mg T-

2 toxin/kg feed increased the mortality to 11.3 % or 12.7 %, respectively, compared to 1.3 % in the control group (Poston et al., 1982).

Decreased survival was reported for channel catfish (*Ictalurus punctatus*) given 2.5 mg (survival rate 78.8 %) or 5.0 (survival rate 80.0 %) mg pure crystalline T-2 toxin/kg feed mixed into a semi purified basal diet (n = 80/group). The survival was not affected in groups given 0.625 or 1.25 mg T-2 toxin/kg feed (survival rates 98.8-100 %) (Manning et al., 2003). Feed intakes and weight gains were reduced in the fish given 1.25 mg/kg of T-2 toxin in feed or more in the diet compared to control. The feed conversion ratio, compared to a pair-fed control group, was only reduced in the highest dose group. The haematocrit values were significantly reduced in fish given 1.25, 2.5 or 5.0 mg T-2 toxin/kg feed compared to both ad lib and pair fed control groups. Histopathological alterations in stomach and head and trunk kidneys were observed in fish from the highest dose group and haematopoietic effects affecting erythrocytic and leukocytic precursors in an unspecific manner in the head kidney was reported in the high dose group (n = 4).

Channel catfish were given 1.0 or 2.0 mg pure crystalline T-2 toxin per kg of feed. Eight aquaria, with 20 fish in each, was used for each treatment. After six weeks of feeding, the fish were challenged *in situ* by immersion with a suspension of *Edwardsiella ictaluri* (approximately 2.25 x $10^6$ colony forming units/mL) and then exposed to the T-2 toxin for another 21-day period. The survival rate was not affected during the initial 6 weeks. The mortality of the fish post-challenge increased from 68.8 % in the control group to 84.11 and 99.3 % in the groups given 1.0 or 2.0 mg T-2 toxin/kg feed (Manning et al., 2005). Apoptosis of leukocytes was found the head kidney from tilapia (*Oreochromis nilocticus*) given three *i.p.* injections on alternate days of 0.3 mg T-2 toxin/kg b.w. (Gogal et al., 2000).

In conclusion, only few feeding studies with T-2 toxin in fish feed are available, while no feeding studies with HT-2 toxin on fish have been reported. At present, no effect has been reported from fish given feed containing 0.63 mg T-2 toxin/kg feed or less. Thus based on the available data 13 µg/kg b.w. per day could be considered as NOAEL[32]. The reported effects occurring at the doses from about 1.0 mg T-2 toxin/kg feed were reduced feed intake, growth and haematocrit values as well as an increased mortality compared to an untreated control group.

### 7.4.6. Companion animals (pets and horses)

Effects of T-2 toxin on cats were previously reported by the JECFA (FAO/WHO, 2001) and the SCF (SCF, 2001). Effects on horses were not included in the previous assessments. Only a few old studies are available on cats and horses. No toxicity data were identified for dogs.

#### 7.4.6.1. Cats

T-2 toxin was administered to four groups of 4 to 6 cats orally in gelatin capsules on alternate days at doses of 0.06, 0.08 or 0.1 mg/kg b.w. per day, until death. The T-2 toxin had been isolated from culture material, but the purity was not defined (reports are from the late 1970s). The animals survived for 6-40 days. The mean death time varied according to the dose ranging from 13.5 to 34.5 days according to the highest to the lowest dose, respectively. Emesis, anorexia, bloody diarrhoea, and ataxia were observed. The cats lost weight and became emaciated. The gross lesions observed included multiple petechiae to ecchymotic haemorrhages of the intestinal tract, lymph nodes, and heart. The lumen of the gut contained copious amounts of dark-red material. The microscopic lesions included haemorrhages in the gut, lymph nodes, heart, and meninges, necrosis of the gastrointestinal epithelium, and decreased cellularity of the bone marrow, lymph nodes and spleen. The mean survival

[32] NOAEL calculated by using the live weight and feed intake as presented in Appendix C, Table C2.

time was inversely related to the dose of T-2 toxin (Lutsky et al., 1978). Lutsky and Mor (1981) conducted a study on cats (n = 10) to reproduce the signs of human ATA. Similar symptoms (medullary aplasia, pancytopenia, haemostatic abnormalities) were observed after oral administration of 0.08 mg T-2 toxin/kg b.w. every second day. All 10 cats in the study died within 32 days (mean survival time 21 days). The particular sensitivity of cats to T-2 toxin might be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation. Because of the severe effects observed in cats at low dose levels these data cannot be used to identify a NOAEL or LOAEL.

#### 7.4.6.2. Horses

An intoxication case involving two horses that had consumed feed containing barley contaminated with T-2 toxin (25 mg/kg) in British Columbia was reported by Greenway and Puls (1976). These animals showed apathy, hypersialorrhea and hyperthermia. Another case involved 30 horses having consumed a mixture of seed maize, maize cobs and wheat bran containing 204 mg T-2 toxin/kg. Twelve animals died after a maximum of 4 weeks after having shown serious locomotive disorders. In particular, dramatic changes in blood chemistry (leukocytosis, anaemia) and fatty degeneration of the liver were observed (Gabal et al., 1986).

The effect of long term administration of T-2 toxin was studied in 6 Trotter mares by Juhasz et al. (1997). Oral administration of 7 mg T-2 toxin per day for 30 to 42 days only showed perilabial dermatitis that quickly disappeared at the end of the trial in three mares. No deleterious effects were observed on ovarian activity or reproductive function (Juhasz et al., 1997).

#### 7.4.6.3. Conclusions

Cats seem to be amongst the most sensitive animal species and the clinical symptoms observed closely resemble clinical findings in humans. The particular sensitivity of cats to T-2 toxin might be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation. Due to the limited data and the serious effects observed for cats at low dose levels (i.e. mortality), the available data could not be used to identify a NOAEL or LOAEL. For dogs, no toxicity data were identified.

The sensitivity of horses to T-2 toxin cannot be evaluated since only one experimental study and very few outbreaks involving a small number of animals are available in the literature. Due to the lack of available data, a NOAEL or LOAEL for horses cannot be identified.

### 7.5. Combined effects with other mycotoxins

Co-exposure to more than one mycotoxin in animals and humans through feed and food is likely to occur. Previously the SCF reported the combined effects *in vitro* of several trichothecenes including T-2 and HT-2 toxins (SCF, 2002). The *in vivo* effects of T-2 toxin have mainly been investigated in combination with aflatoxins, ochratoxin A and other *Fusarium* toxins (fumonisins and trichothecenes). Most of these studies were performed on farm animals fed with naturally contaminated diets (SCF, 2002; Grenier and Oswald, 2011).

Friend et al. (1992) and Kubena et al. (1989a) focused on the interaction between deoxynivalenol and T-2 toxin. In swine, a less than additive effect between the two toxins was observed on body weight gain and feed intake, reflecting mainly the effect of deoxynivalenol (Friend et al., 1992). In broiler chickens exposed to both toxins, most of the observed effects (oral lesions, total protein, albumin and LDH) were due to T-2 toxin (Kubena et al., 1989a) and the combined effect of deoxynivalenol and T-2 toxin resulted in a less than additive effect. However, an additive effect was obtained on the body weight gain and on the serum cholesterol concentration.

In laying hens, an additive effect of T-2 toxin and diacetoxyscirpenol was observed on feed intake and oral lesions (Diaz et al., 1994). In this experiment, a lower egg production was noticed in hens receiving either T-2 toxin or diacetoxyscirpenol contaminated diet but this recovered gradually during the experiment. In hens fed the co-contaminated diet, the egg production decreased throughout the experiment.

Interaction between fumonisins (mainly fumonisin B1) and T-2 toxin was reported in turkeys and in chickens (Kubena et al., 1995, 1997). Both experiments reported an additive effect on the body weight gain and an increased relative weight of gizzard that was related to the irritant property of T-2 toxin. The difference between these two studies concerned the effect of the combined treatment on the activity of hepatic enzymes. In one experiment, T-2 toxin potentiates the effect of fumonisin (Kubena et al., 1995) on AST and LDH, whereas in the other experiment, the effect on these enzymes was lower than the one observed with fumonisin alone (Kubena et al. 1997).

Studies on the combined effect of HT-2 toxin with other mycotoxins *in vivo* were not identified.

In conclusion, most of the *in vivo* data dealing with the combined effects of T-2 toxin with other mycotoxins revealed a dose additivity or an antagonism. However, at present the database describing possible effects of combined exposure to T-2 toxins and other mycotoxins or the combined exposure to T-2 toxin and other trichothecenes is very weak and not sufficient for establishing either the nature of combined effects or the relative potencies of trichothecenes.

## 7.6. Human data

### 7.6.1. Observations in humans

Many reports have reviewed the human toxicosis incidences possibly linked to intake of *Fusarium* fungi and trichothecene contaminated food historically (FAO/WHO, 2001; SCF, 2001; IARC, 1993; Sudakin, 2003; van der Fels-Klerx and Stratakou, 2010). A brief summary of these findings is given below. Up to now, there is no conclusive evidence that any human disease is exclusively attributed to T-2 and HT-2 toxin dietary exposure.

In the cases of small-scale laboratory contact, reversible dermal effects including irritation, loss of sensitivity and skin desquamation were reported to be caused by fungal cultures containing T-2 toxins (200 mg/L) but effects from other mycotoxins potentially present in the cultures could not be ruled out (FAO/WHO, 2001; IARC, 1993).

ATA outbreaks during war time (1931-1947) in the former Union of Soviet Socialist Republics (USSR) were widely reported. ATA is a human disease associated with ingestion of mouldy grains infested by T-2 toxin producing strains *F. poae* and *F. sporotrichioides*. Studies carried out years after the toxicosis suggested that *F. sporotrichioides* isolated from the food collected during the epidemic produced 4.1 g of T-2 toxin/kg of infected millet (Joffe, 1986). The lethal disease involved four stages. First, hyperaemia of the oral mucosa, weakness, fever, nausea and vomiting may occur. Acute oesophagitis, gastritis and gastroenteritis, even circulatory failure and convulsions happened in severe cases. At the following stage a significantly reduced number of white blood cells was observed, which included leukopenia, granulopenia and progressive lymphocytosis. The third stage was accompanied by severe haemorrhagic diathesis, necrotic pharyngitis laryngitis and further lowered leukocyte count, platelet diminution and anemia resulting in anoxia and up to 50 % fatal cases occurred due to the total closure of the patient's larynx. Finally, the recovery stage may be complicated with secondary infection.

Human toxicosis related to ingestion of *Fusarium* infected grain (Scabby grain diseases) was previously reported in several countries, including Japan and Korea during the period of 1946-1963

(FAO/WHO, 2001; SCF, 2001), China (Luo, 1988; Wang et al., 1993), and India (Bhat et al., 1987, 1989) and these were well summarised in the evaluations of the JECFA and the SCF (FAO/WHO, 2001; SCF, 2001). Briefly, nausea, vomiting, diarrhoea and abdominal pain were common symptoms associated with the toxicosis. In the outbreaks in Japan and Korea, recovery usually occurred within a few days time and no lethal cases were reported. *F. graminearum* was isolated from suspected cereals suggesting a possibility of deoxynivalenol contamination but not T-2 and HT-2 toxins. The number of cases involved was over 100 in both the two China and the two India outbreaks, although none was lethal. Up to 4 mg/kg of T-2 toxin were detected in wheat flour samples from India (Bhat et al., 1987, 1989) and 0.2-0.4 mg/kg of T-2 toxin was detected in rice from China using an ELISA method (Wang et al., 1993). However, in the Wang et al. (1993) study, the fungal species were not of T-2 and HT-2 toxin producing nature, and a possible contribution from other trichothecene toxins could not be ruled out.

Building-related indoor exposure to fungal species such as *Stachybotrys* has given rise to health concerns in the US as reviewed by Kuhn and Ghannoum (2003). The review described representative studies including the mould-related infant idiopathic pulmonary haemorrhage, and the building-related dermatologic and respiratory syndrome in adults. The studies focused on *Stachybotrys* species fungi as the potential pathogens, amongst which *S. chartarum* is the species most closely associated with type D trichothecene and T-2 toxin, and indoor air contamination.

The occupational contact to T-2 toxin and T-2 toxin potential usage as a chemical warfare agent are also noted. However, these studies are recognised as less relevant to food exposure. It is therefore agreed not to include such exposure in the content of the present opinion.

In summary, there are reports on human cases of intoxications, but these could not be conclusively linked to dietary exposure levels. No new studies than those reported above were identified in the literature in the context of dietary exposure to T-2 and HT-2 toxins and human diseases.

### 7.6.2. Biomarkers

It appears that there is no validated human exposure or specific effect biomarker for T-2 and HT-2 toxins. Little information on human toxicokinetics is available to date. In animals, T-2 toxin is rapidly absorbed and metabolised without specific organ accumulation and the half-life is usually less than 30 minutes in pigs, rats and cattle (Beasley et al., 1986; Eriksen and Pettersson, 2004; Larsen et al., 2004). Experiment on T-2 toxin metabolites in blood and urine of the Cynomolgus monkey (Naseem et al., 1995) indicated that metabolites such as T-2 tetraol in blood and urine can be detected days following *i.v.* injection. Based on this result and the stability study of T-2 toxin metabolites in biological fluids (Pace and Matson, 1988), T-2 tetraol in urine could potentially be used as a marker for diagnostic purposes. However, these studies did not take account of any variable factors during intestinal absorption and digestion. Glucuronide conjugates of T-2 toxin, HT-2 toxin and other metabolites were formed rapidly and extensively (63 %) in the plasma in pigs (Corley et al, 1985), so the conjugates and other potential metabolites need to be considered in the biomarker development. However, a similar study in rats raised uncertainty about these biomarkers because of the lack of a dose-relationship between T-2 toxin and the key metabolites in urine, including 3'-hydroxy-HT-2 toxin and T-2 tetraol (Pfeiffer et al., 1988).

GC-MS methods have been developed to detect T-2 toxin, HT-2 toxin and other metabolites in spiked human blood (Begley et al., 1986) and urine samples (Black et al., 1986); however these studies could not be related to the level of exposure or internal dose. Recent rapid development in techniques of purification and detection, including IA column or other SPE clean-up followed by HPLC with FLD after derivatization (Visconti et al., 2005), or by HPLC/UHPLC-MS/MS, have significantly improved the analytical quality and sensitivity. Such developments are promising for the establishment of biomarkers.

In conclusion, it is recognised that biomarkers for T-2 and HT-2 toxins are not a well-developed area due to the low exposure levels as well as rapid and complex metabolism. These factors are major obstacles in human epidemiology studies.

### 7.7. Dose response modelling

Since the SCF evaluation (SCF, 2001) there has been no new evidence that other toxic effects including dermal toxicity, developmental and reproductive toxicity and neurotoxicity occur at doses lower than those causing immunotoxicity and haematotoxicity in pigs. Based on the available data, the JECFA (FAO/WHO, 2001) identified that there was substantial evidence for the immunotoxicity and haematotoxicity of T-2 toxin in several species and based their PMTDI assessment on a LOAEL of 0.029 mg/kg b.w. per day in a short term pig study (Rafai et al, 1995a,b). This was considered to be close to the NOAEL. The SCF also considered the study in pigs by Rafai et al. (1995b) in its evaluation (SCF, 2001) and established a t-TDI in-line with the PMTDI established for T-2 and HT-2 toxins by the JECFA.

Since the study of Rafai et al. (1995a,b) on pigs, only one new dose-response study suitable for risk assessment has become available. Although cats have been shown to be a very sensitive species, their particular sensitivity to T-2 toxin is likely to be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation. Because of the difference in metabolic pathway with that of humans, data for cats are not suitable for human risk assessment.

In the study of Rafai et al. (1995a,b) nine to ten pigs per feeding group of unreported gender (mean weight of piglets at the beginning of the study was about 9 kg) were fed diets containing 0, 0.5, 1, 2 or 3 mg T-2 toxin per kg diet (average daily feed intakes 0, 0.38, 0.81, 1.24, and 1.43 mg, respectively) over a period of 21 days resulting in doses of 0, 29, 62, 105 or 129 µg T-2 toxin/kg b.w. per day, respectively (see Table 31). The feed intake of the animals was already significantly decreased by 13 % at the lowest level of exposure. This may result in a bias of all further observations by the reduced nutrient intake which is also reflected in the decreased weight gain (significant decreases were observed at doses of 1 mg T-2 toxin/kg diet and above) as no pair fed controls were investigated. However, decreased lymphocyte stimulation by ConA, decreased leucocyte counts and T-cells as well as reduced anti-horse globulin titre formation and histological changes in thymus, spleen and lymph nodes observed in the group receiving 29 µg T-2 toxin/kg b.w. per day were reported.

More recently a feeding trial investigating similar concentrations of T-2 toxin (purity greater than 98 %) in the diet at concentrations of 0, 0.54, 1.32 and 2.10 mg/kg feed for piglets of similar starting body weight (11.4 kg, five male piglets per feeding group) was reported by Meissonnier et al. (2008a) (see Table 31). Anti-ovalbumin antibody production was one of the sensitive parameters and was significantly reduced on day 21 in groups receiving 1.32 or 2.10 mg T-2 toxin/kg diet. The overall weight gain was significantly reduced for piglets receiving the highest concentration in diet. In contrast to the study of Rafai (1995a,b), Meissonnier et al. (2008a) used a smaller number of animals. Furthermore, the feed intakes of the pigs were not measured and therefore information on the T-2 toxin exposure was not available from the study. For this reason the study of Meissonnier et al. (2008a) was only modelled to ascertain if the results of this study was supportive of that of Rafai et al. (1995a,b). The results obtained on day 21 in the Meissonnier et al. (2008a) study were chosen for the benchmark dose (BMD) analysis, because this time point allows the development of the specific immune response and it was the longest time period investigated in the study of Rafai et al. (1995a,b). Although the most sensitive parameter determined in the study of Meissonnier et al. (2008a) was liver microsomal drug metabolising enzyme activity of the piglets fed the lowest concentration in the diet, (namely significantly reduced O-dealkylation of ethoxy-resorufin but not methoxy-resorufin and significantly reduced hydroxylation of benzo-[*a*]-pyrene but not aniline), this was not considered suitable for risk assessment.

From the studies of Rafai et al. (1995a,b) and Meissonnier et al. (2008a) the CONTAM Panel chose for dose-response modelling the specific antibody response, anti-horse globulin response and anti-ovalbumin response, respectively, where a dose dependent response was observed. The two studies support each other as both identified impairment in the antigen response at low T-2 toxin exposure levels as the critical immunotoxicological effect. In addition, although the two studies were performed independently from each other with a 10 years time difference, they were based on comparable designs and aims, and provided data on immunological effects of T-2 toxin which can be compared. These data were used for a BMD analysis.

As a specific antibody response to a foreign protein represents a function of the immune system, a significant reduction in an antibody response implies a significant reduction in the functionality of the immune system. When applying the BMD approach the CONTAM Panel noted that the specific antibody responses to foreign antigens can be modeled as continuous data where the benchmark response (BMR) should be defined as a percent change in the average magnitude of the response when compared to that predicted at background i.e. a relative deviation from background. Ideally the BMR should reflect an effect size that is negligible or non-adverse (EFSA, 2009b), but at the same time not too small to avoid extrapolation outside the range of observation. The default value for continuous data recommended by EFSA is a BMR of 5 %. In the absence of statistical or toxicological considerations supporting deviation from the default value, the CONTAM Panel chose the BMR of 5 % when applying the BMD approach for the available dose-response data on anti-horse globulin titre and anti-ovalbumin.

The CONTAM Panel performed a BMD analysis (for details see Appendix E) and calculated a 95 % lower confidence limit for the benchmark dose response of 5 % ($BMDL_{05}$) of 10 µg T-2 toxin/kg b.w. per day which was identified for a decrease in anti-horse globulin titre values. The CONTAM Panel used this value as a reference point for the risk characterisation for T-2 and HT-2 toxins.

### 7.8. Derivation of TDI

In view of the rapid metabolism of T-2 toxin to HT-2 toxin, and the fact that the toxicity of T-2 toxin might at least partly be attributed to HT-2 toxin, a group TDI was established for the sum of T-2 and HT-2 toxins. Based on the $BMDL_{05}$ of 10 µg T-2 toxin/kg b.w. per day, the CONTAM Panel established a group TDI of 100 ng/kg b.w. for the sum of T-2 and HT-2 toxins using the default uncertainty factor of 100. The CONTAM Panel decided that no extra uncertainty factor was required because there is no need for extrapolation from LOAEL to NOAEL due to the use of a $BMDL_{05}$. The CONTAM Panel also noted that there was no increase in response after day 21, which was the day chosen for the BMD analysis, and considered that this also indicates that an extra uncertainty factor is not required. In addition the use of $BMDL_{05}$ partially accounts for some uncertainties associated with the study of Rafai et al. (1995a,b), such as the small sample size, inter-animal variability and the non-monotonous dose-response relationship. Furthermore, the recent data available from the study of Meissonnier et al. (2008a) reporting a comparable dose-response for a similar specific antibody response reduces uncertainty, although these data did not provide a suitable basis for dose-response analysis due to the lack of data on feed intakes. Thus as new relevant evidence has become available since the previous t-TDI was established by the SCF in 2001, and as the present assessment was based on a $BMDL_{05}$, the CONTAM Panel concluded that a full TDI of 100 ng/kg b.w. can now be established.

## 8. Risk characterisation

With regard to human risk characterisation, the Scientific Opinion included an updated dietary exposure assessment of T-2 toxin and HT-2 toxin which used recent analytical results on the occurrence of T-2 and HT-2 toxin in food and the consumption patterns of specific groups of the population. For animal risk characterisation, the daily exposure levels of T-2 toxin and HT-2 toxin for

the different animal species were estimated. Only T-2 and HT-2 toxins were considered in this Scientific Opinion although combined exposures with other trichothecenes and mycotoxins may occur.

### 8.1. Human health risk characterisation

The dietary exposures were expressed as the sum of T-2 toxin and HT-2 toxin, which was considered appropriate by the CONTAM Panel in view of the fact that the T-2 toxin is rapidly metabolised to HT-2 toxin.

For calculating the chronic dietary exposure to the sum of T-2 and HT-2 toxins, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.1.1). The mean dietary exposure (average consumption in the total population) and the high dietary exposure ($95^{th}$ percentile food consumption in the total population) to the sum of T-2 and HT-2 toxins were calculated separately for each dietary survey using consumption data recorded at the individual level. Individual food consumption data were combined with the mean occurrence values in order to provide mean and high percentile exposure estimates ($95^{th}$ percentile). Exposure estimates were calculated for both LB and UB scenarios.

Using LB and UB concentrations, the chronic dietary exposure to the sum of T-2 and HT-2 toxins in adult populations across 19 European countries,has been estimated to range from 3.4 to 18 ng/kg b.w. per day for average consumers (range represents the minimum LB to maximum UB from the different countries), and 7.2 to 39 ng/kg b.w. per day for $95^{th}$ percentile consumers. Toddlers (age $\geq$ 12 months to < 36 months) had the highest exposure to the sum of T-2 and HT-2 toxins, with a range from 12 to 43 ng/kg b.w. per day for average consumers, and 23 to 91 ng/kg b.w. per day for $95^{th}$ percentile consumers. In the elderly and very elderly population, the chronic dietary exposure to the sum of T-2 and HT-2 was slightly lower compared to other adults.

Estimates of chronic dietary exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data are below the group TDI of 100 ng/kg b.w. for populations of all age groups, and therefore not a health concern.

There are limited data on dietary habits of vegetarians with data available for only five European countries, with very few subjects in four of them. These limited data do not indicate significant differences in dietary exposure between the vegetarians and the general population.

### 8.2. Animal health risk characterisation

Because of the limited knowledge on the effects of T-2 and HT-2 toxins on farm and companion animals, and the absence of a comprehensive database on feed consumption by livestock in the EU, it has not been possible to properly assess the risks of these toxins for animal health. However, the exposure values at the LB and UB concentrations for the sum of T-2 and HT-2 toxins in diets have been estimated for a number of farm livestock and companion animal categories, based on expected feed intakes and example diets, and these have been compared with identified NOAELs/LOAELs (Table 34), or with the calculated $BMDL_{05}$ for pigs.

A $BMDL_{05}$ of 10 µg/kg b.w. per day was calculated for pigs (Appendix E). Instead of the LOAELs identified for pigs and poultry (Table 34), the CONTAM Panel used this $BMDL_{05}$ as a reference point for risk characterisation for both pigs and poultry. The latter was considered acceptable, as there was no indication from identified LOAELs that poultry are more sensitive than pigs. In the absence of NOAELs or LOAELs for horses and dogs, the CONTAM Panel also decided to use the same reference point as that derived for pigs to give an indication on the possible risk, since the toxicokinetics of T-2 and HT-2 toxins in horses and dogs are not substantially different to that of pigs. However, due to the

differences in oral bioavailability and metabolism in ruminants and fish, the $BMDL_{05}$ for pigs was not used for the risk characterisation for these species. The identified NOAELs or LOAELs were used for risk characterisation for ruminants, fish and rabbits. For cats the health risk from the exposure to the sum of T-2 and HT-2 toxins could not be assessed as no NOAEL or LOAEL has been identified, and as there is a lack of sufficient data on the feline-specific biotransformation and toxicodynamics. However, cats seem to be amongst the most sensitive animal species to T-2 toxin and HT-2 toxin intoxication.

For cattle, the highest estimated exposure of the sum of T-2 and HT-2 toxins by dairy cows, based on the available mean UB concentrations was 1082 µg/day or 1.7 µg/b.w. per day. While no NOAEL/LOAELs have been identified for mature healthy cattle, a LOAEL for calves of 300 µg/kg b.w. per day has been identified and no studies using lower doses were available. Since the predicted exposure (based on the available mean UB concentrations) is < 1 % of the LOAEL (for ruminating calves), it is reasonable to assume that exposure of adult dairy cows and cattle to the sum of T-2 and HT-2 toxins is unlikely to be a health concern.

Under normal farming conditions, only pregnant and lactating sheep and goats are fed diets that are likely to contain significant proportions of cereal grains and cereal by-products, and these usually represent < 50 % of the total diet. Although no LOAELs for mature healthy sheep or goats have been identified, exposure to the sum of T-2 and HT-2 toxins is likely to be low because their diets consist predominantly of non-cereal feeds. Therefore, the CONTAM Panel concluded that the risk of adverse health effects of feed containing T-2 and HT-2 toxins would also be low for these animals. In this context, it should be noted that in ruminants, T-2 and HT-2 toxins are largely detoxified due to de-epoxidation by rumen microorganisms. As a result, the susceptibility of ruminants to these toxins is influenced by rumen conditions. In acidotic heifers, doses of 25 µg/kg b.w. per day and in acidotic ewes 5 µg/kg b.w. per day T-2 toxin were reported as LOAELs, indicating the increased susceptibility associated with rumen dysfunction. Pre-ruminant calves, lambs and kids may be more susceptible to T-2 and HT-2 toxins if cereals represent a major part of their transitional diet from liquid to solid feed.

**Table 34:** Identified NOAELs and LOAELs with the estimated exposure at LB and UB levels for different farm livestock species and companion animals.

| Animal species for which a NOAEL/LOAEL were identified | NOAEL µg/kg b.w. per day | LOAEL µg/kg b.w. per day | Animal species for which exposure has been estimated | LB µg/kg b.w. per day | UB µg/kg b.w. per day |
|---|---|---|---|---|---|
| **Ruminants** | | | **Ruminants** | | |
| Dairy cows | - | - | Dairy cows | 0.16 | 1.7 |
| Calves | - | 300 | | - | - |
| | | | Beef cattle | 0.06 | 0.11 |
| Sheep | - | - | Lactating sheep | 0.26 | 0.51 |
| Goats | - | - | Milking goats | 2.7 | 3.3 |
| | | | Fattening goats | 0.91 | 1.2 |
| **Pigs** | | | **Pigs** | | |
| Piglets | - | 29 | Piglets | 0.27 | 1.3 |
| | | | Growing pigs | 0.28 | 0.87 |
| **Poultry** | | | **Poultry** | | |
| Laying hens | | 120[(a)] | Laying hens | 0.49 | 1.6 |
| Broiler chickens | - | 40[(b)] | Broilers | 0.95 | 1.8 |
| Fattening turkeys | - | 48[(b)] | Fattening turkeys | 0.27 | 0.95 |
| Fattening ducks | - | 40[(b)] | Fattening ducks | 0.35 | 1.2 |
| **Rabbits** | 100 | - | **Rabbits** | 0.98 | 1.7 |
| **Farmed fish** | 13[(c)] | - | **Farmed fish** | 0.090 | 0.19 |
| **Companion animals** | | | **Companion animals** | | |
| Cats | - | - | Cats | 0.20 | 0.34 |
| Dogs | - | - | Dogs | 0.23 | 0.38 |
| Horses | - | - | Horses[(d)] | 1.1 | 1.2 |

NOAEL: No-observed-adverse-effect-level; LOAEL: Lowest-observed-adverse-effect-level b.w.: body weight, LB: lower-bound; UB: upper bound; -: not identified.
(a): Expressed as mg T-2 toxin/kg feed in Section 7.4.3. and converted to µg/kg b.w. per day by using the live weight and feed intake (expressed as dry matter) as presented in Appendix C,T able C2; (b) Expressed as mg T-2 toxin/kg feed in Section 7.4.3. In addition, converted to µg/kg b.w. per day by using the live weight and feed intake as reported by authors in the respective studies; (c) Expressed as mg T-2 toxin/kg feed in Section 7.4.5. In addition, converted to µg/kg b.w. per day by using the live weight and feed intake (expressed as dry matter) as presented in Appendix C,T able C2; (d): Moderate activity.

For piglets, the $BMDL_{05}$ compares with an estimated exposure of 1.3 µg/kg b.w. per day for piglets and 0.87 µg/kg b.w. per day for growing pigs based on the available mean UB concentrations for the sum of T-2 and HT-2 toxins. The exposure to the sum of T-2 and HT-2 toxins is 13 % of the $BMDL_{05}$ for piglets and 9 % of the $BMDL_{05}$ for growing pigs indicating that the risk of adverse health effects of feed containing T-2 and HT-2 toxins is low. For poultry the UB exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data is up to 18 % of the $BMDL_{05}$ depending on the species (Table 34). As for pigs, these data suggest that the risk of adverse health effects of feed containing T-2 and HT-2 toxins in poultry is low.

For rabbits and farmed fish, the estimated UB exposure to the sum of T-2 and HT-2 toxins based on the reported occurrence data in feed is well below the identified NOAELs and therefore considered unlikely to be a health concern for these species (Table 34).

For dogs the estimated UB exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data is 4 % of the $BMDL_{05}$ and for horses 12 % of the $BMDL_{05}$ indicating that the risk of adverse health effects of feed containing T-2 and HT-2 toxins is low.

In summary, for ruminants, rabbits and farmed fish the estimated exposures to the sum of T-2 and HT-2 toxins based on the available occurrence data are considered unlikely to be a health concern. For

pigs, poultry, dogs and horses, comparison of the estimates of exposure based on the reported levels of the sum of T-2 and HT-2 toxins in feeds to the $BMDL_{05}$ for pigs indicate that the risk of adverse health effects as a result of consuming feed containing T-2 and HT-2 toxins is low. For cats, the lack of LOAELs/NOAELs precludes the estimation of health risk associated with T-2 and HT-2 toxins in feed.

## 9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to T-2 and HT-2 toxins has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on "Characterizing and Communicating Uncertainty in Exposure Assessment" has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters). In addition other uncertainties were considered.

### 9.1. Assessment objectives

The objectives of the assessment were defined in the terms of reference. The CONTAM Panel assessed the new data available since the latest assessment by the SCF of T-2 toxin and HT-2 toxin in food, which enabled a consideration of the possibility of refining the previously established t-TDI. The CONTAM Panel also produced an updated human dietary exposure assessment, and estimated exposures for different animal species where toxicity became evident. Levels of carry-over from feed to food of animal origin were also estimated, and these were considered only a minor contributor to human exposure. The types of feed that were considered the major sources of contamination by T-2 toxin and HT-2 toxin were identified. There was no uncertainty in addressing these objectives.

### 9.2. Exposure scenario and model

For food, the vast majority of occurrence data were on grains and grain-based foods. The groups 'Grain milling products' and 'Breakfast cereals' dominated the product coverage. The use of the UB approach for high percentage of occurrence data < LODs/LOQs is conservative, i.e. it represents an overestimation of exposure.

Limited data on vegetarians indicate some uncertainty in their exposure assessment. There was a lack of dietary surveys reporting consumption data for children younger than 1 year, which led to an uncertainty in this area.

The occurrence data on T-2 and HT-2 toxins in compound feed were limited and were not used to estimate the exposures for farm and companion animals. In addition, the occurrence data were not representative for all feed materials in which T-2 and HT-2 toxins could be present. This introduced uncertainties in the animal exposure estimates. High variability of feedingstuffs and feeding systems used for livestock in Europe add to the overall uncertainty of animal exposure estimates.

### 9.3. Other uncertainties

The lack of certified calibrants and certified reference materials for various matrices are limitations, and add thereby to the overall uncertainty.

There is a body of evidence that the domestic pig is amongst the most sensitive species to the immunotoxic and haematotoxic effects of T-2 and HT-2 toxins and hence has been used in previous assessments to establish group TDIs. One uncertainty of the study by Rafai et al. (1995a,b) is the incorporation of T-2 toxin into the diets by the inclusion of a partially purified extract from liquid

cultures of *F. tricintum*. The T-2 toxin content of this, measured by gas and liquid chromatography, was greater than 90 %. Therefore, up to 10 % of the substance administered in this study is not identified. Assuming the unknown 10 % of substance to be nontoxic would underestimate the toxicity of T-2 toxin in this investigation on pigs. On the other hand, assuming the remaining 10 % of the substance to be highly toxic, risk assessment using figures of the mentioned study would overestimate the toxicity of T-2 toxin. However, the CONTAM Panel considered that the proportion of 10 % of the unknown substance is unlikely to markedly contribute to the overall toxicity.

Lack of individual feed intake data for experimental and farm animals, pair feeding of control animals, possible impurities of administered T-2 toxin and limited toxicity data for some of the farm and companion animals contribute to the overall uncertainty.

Co-exposure of T-2 and HT-2 toxins with other toxins (e.g. trichothecenes) may occur but the toxic effect of such combinations is not well known, and this therefore contributes to the uncertainty.

### 9.4. Summary of uncertainties

In Table 35, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

**Table 35:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the human and animal dietary exposure to the sum of T-2 and HT-2 toxins.

| Sources of uncertainty | Direction[(a)] |
|---|---|
| Uncertainty of the analytical measurements | +/- |
| Occurrence data on feed not representative for all feed materials in which T-2 and HT-2 toxins could be present | +/- |
| Effect of food and feed processing | +/- |
| High variability of feedstuffs used and feeding systems for livestock | +/- |
| Use of UB occurrence data in the exposure estimations | + |
| Use of LB occurrence data in the exposure estimations | - |
| Limited exposure data on infants | +/- |
| Limited data on exposures for vegetarians | +/- |
| No toxicokinetic data on T-2 and HT-2 toxins in humans and in most animal species | +/- |
| Lack of information on the contribution of the toxicity of HT-2 toxin and other metabolites to overall toxicity | +/- |
| Combined effects with other mycotoxins or other toxic substances in food and feed | +/- |

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel concluded that given the uncertainties, the risk assessment of human and animal exposure to the sum of T-2 and HT-2 toxins is more likely to over- than under-estimate the risk.

# CONCLUSIONS AND RECOMMENDATIONS

## CONCLUSIONS

### *General*

- T-2 toxin and HT-2 toxin are mycotoxins and are members of the large group of fungal sesquiterpenes, commonly denoted as trichothecenes. They are produced by various *Fusarium* species. Generally, the *Fusarium* species grow and invade crops, and may produce these toxins under moist cool conditions.

### *Methods of analysis*

- Accurate quantification of T-2 and HT-2 toxins is mostly carried out by liquid chromatography coupled with (multi-stage) mass spectrometry often within a multianalyte approach. This methodology can be applied for the analysis of cereals, food and feed as well as samples of human and animal origin.

- For rapid screening, several immunochemical methods have become available but they may suffer from undesired cross reactivity with other trichothecenes and increased measurement uncertainty.

- None of the applied methods have been formally validated in interlaboratory validation studies and there are no certified reference materials available for T-2 and HT-2 toxins.

### *Occurrence and effect of processing*

- In general, HT-2 toxin concentration represents two-thirds of the sum of T-2 toxin and HT-2 toxin concentration.

- A total of 17,683 analytical results for T-2 toxin, 16,536 for HT-2 toxin and 20,519 for the sum of T-2 and HT-2 toxins collected between 2005 and 2010 from 22 European countries were used in the evaluation.

- Overall, 65 % of results were below the limit of detection (LOD) or limit of quantification (LOQ) with variation for the individual toxins across food, feed and unprocessed grains between 53 % to 77 %.

- The highest mean concentrations for the sum of T-2 and HT-2 toxins in food, feed and unprocessed grains were observed in grains and grain milling products, notably in oats and oat products.

- Higher concentrations were observed in unprocessed grains compared to grain products for human consumption. This suggests that processing applied to grains results in lower T-2 toxin and HT-2 toxin concentrations in grain milling products for human consumption.

- During grain milling, T-2 and HT-2 toxins are not destroyed but unevenly redistributed between the milling fractions.

- Because T-2 and HT-2 toxins are mostly attached to the outer hull of the grain, cleaning, sorting, sieving and, de-hulling of grains lead to marked increases in T-2 and HT-2 toxins in cereal by-products, e.g. bran.

- During baking and cooking, T-2 and HT-2 toxins seem to be relatively stable.

- Malting leads to substantially lower levels of T-2 and HT-2 toxins in malt compared to the original barley, although the ratio varies considerably.

- Manufacturing of compound feedstuffs does not affect T-2 and HT-2 toxins levels.

***Human exposure***

- The chronic dietary exposure in the adult population across 14 European countries, using lower bound (LB) and upper bound (UB) concentrations, ranged from 3.4 to 18 ng/kg body weight (b.w.) per day for average consumers, and 7.2 to 39 ng/kg b.w. per day for 95$^{th}$ percentile consumers. In elderly and very elderly populations, the chronic dietary exposure to the sum of T-2 and HT-2 toxins was slightly lower compared to other adults.

- The highest chronic exposure was estimated in toddlers (age $\geq$ 12 months to < 36 months) ranging from 12 to 43 ng/kg b.w. per day for average consumers, and 23 to 91 ng/kg b.w. per day for 95$^{th}$ percentile consumers.

- Grains and grain-based foods made the largest contribution to the sum of T-2 and HT-2 toxin exposure. Important contributors were bread, fine bakery wares, grain milling products and breakfast cereals. In infants, the highest contributors were in the food group 'Foods for infants and small children', mainly cereal-based foods.

- The limited data on vegetarians do not indicate a significant difference in the dietary exposure to the sum of T-2 and HT-2 toxins between the vegetarians and the general population.

***Animal exposure***

- Animal exposure to the sum of T-2 and HT-2 toxins is primarily from consuming cereal grains and cereal by-products; levels in forages and oilseed meals are generally low.

- For dairy cows, the calculated LB and UB exposure to the sum of T-2 and HT-2 toxins increased with milk yield, with the LB and UB of 0.75 and 1.7 µg/kg b.w. per day, respectively, for milk yield of 50 kg/day (for high producing cows fed high proportion of compound feed). For beef cattle fed a cereal-based ration, the LB and UB exposures were 0.39 and 0.76 µg/kg b.w. per day, respectively.

- For small ruminants, the estimated LB and UB exposures were 0.3 and 0.59 µg/kg b.w. per day, respectively, for sheep, and ranged from 0.91 to 3.3 µg/kg b.w. per day, respectively, for fattening and milking goats.

- For adult pigs, the estimated LB and UB exposures were 0.28 and 0.87 µg/kg b.w. per day, respectively. The LB exposure was at the same level for piglets whereas the UB exposure was about 1.5 times higher.

- The LB and UB exposure estimates for laying hens were 0.49 and 1.6 µg/kg b.w. per day, respectively, and higher for broilers 0.95 and 1.8 µg/kg b.w. per day, respectively. The estimated LB and UB exposures for fattening turkeys were 0.27 and 0.95 µg/kg b.w. per day, respectively, and somewhat higher for fattening ducks.

- For rabbits, the estimated LB and UB exposures were 0.98 and 1.7 µg/kg b.w. per day, respectively.

- The LB and UB exposure estimates of 0.090 and 0.19 µg/kg b.w. per day, respectively, were calculated for farmed fish.

- Estimated LB and UB exposure for dogs and cats were similar (0.20-0.38 µg/kg b.w. per day) although slightly higher for dogs. For horses, the LB and UB exposures were 1.1 and 1.2 µg/kg b.w. per day, respectively.

***Hazard identification and characterisation***

***Toxicokinetics***

- The available information on the toxicokinetics of T-2 and HT-2 toxins is incomplete.

- T-2 toxin is rapidly metabolised by at least five biotransformation pathways including hydrolysis, hydroxylation, de-epoxidation, glucuronidation and acetylations, resulting in a large number of different metabolites. HT-2 toxin is a major metabolite of T-2 toxin.

- T-2 toxin and metabolites are rapidly distributed to several tissues and rapidly excreted without any accumulation.

- The de-epoxide metabolites are considered to be considerably less toxic. For the other metabolites, very little, or no toxicity data are available.

- In ruminants, there may be a significant de-epoxidation by rumen microorganisms prior to absorption.

- The carry over of T-2 and HT-2 toxins from feed to food products of animal origin is limited and hence contributes only to a negligible extent to human exposure.

***Toxicity of T-2 and HT-2 toxins***

- T-2 and HT-2 toxins are known to impair protein and DNA synthesis, and to induce haematotoxicity and myelotoxicity associated with impairment of haematopoiesis in bone marrow. The lowest-observed-adverse-effect-level (LOAEL) is equal to 100 µg/kg b.w. per day of T-2 toxin in two studies in monkeys and 29 µg/kg b.w. per day in pigs.

- There is no new evidence that the other toxic effects, including dermal toxicity, developmental and reproductive toxicity and neurotoxicity, occur at doses lower than those causing immunotoxicity and haematotoxicity in pigs.

- The assessment of the genotoxicity of T-2 and HT-2 toxins indicated a positive effect in several conventional tests for genotoxicity *in vitro* and in rodents *in vivo*, in particular for clastogenic effects, but these effects were observed primarily at concentrations also known to inhibit protein and DNA synthesis and produce cytotoxicity.

- No new reports on cytogenetic damage caused by T-2 toxin have been identified since the evaluation of the Scientific Committee for Food (SCF).

- There is limited evidence reported in the previous evaluation of the SCF for tumourigenicity of T-2 toxin in experimental animals (induction of hepatocellular- and pulmonary adenomas in male mice). No further conclusions can be drawn on the carcinogenicity of T-2 toxin and HT-2 toxin on the basis of published results since the SCF evaluation.

- The International Agency for Research on Cancer concluded in 1993 that there were no data available on the carcinogenicity to humans of toxins derived from *Fusarium sporotrichioides* and no new data since then have been found.

- No new epidemiological data were identified in the literature in the context of dietary exposure to T-2 and HT-2 toxins and human diseases.

- Cats are amongst the most sensitive animal species. This particular sensitivity of cats to T-2 toxin is likely to be associated with their inability to excrete T-2 toxin and its metabolites via glucuronide conjugation and therefore data for cats are not suitable for human risk assessment.

- Currently available toxicity studies in pigs confirm that the pig is also amongst the most sensitive animal species. Only limited new dose-response data on toxicity in pigs have been found.

- The CONTAM Panel concluded that a reduction in specific antibody response in pigs is the critical effect for human risk assessment.

- The 95 % lower confidence limit for the benchmark dose response of 5 % ($BMDL_{05}$) of 10 µg T-2 toxin/kg b.w. per day, was calculated for anti-horse globulin titre values, and used as a reference point for the risk characterisation for T-2 and HT-2 toxins.

- In view of the rapid metabolism of T-2 toxin to HT-2 toxin, and the fact that the toxicity of T-2 toxin might at least partly be attributed to HT-2 toxin, a group TDI was established for the sum of T-2 and HT-2 toxins.

- An uncertainty factor of 100 was applied to the $BMDL_{05}$, to establish a group TDI of 100 ng/kg b.w. for the sum of T-2 and HT-2 toxins. As new relevant evidence has become available since the previous t-TDI was established by the SCF in 2001, and as the present assessment was based on a $BMDL_{05}$, the CONTAM Panel concluded that a full TDI of 100 ng/kg b.w. can now be established.

***Adverse effects in livestock, fish and companion animals***

- In young ruminants, exposure to 300 µg T-2 toxin/kg b.w. per day or more may result in gastrointestinal lesions, altered serum proteins and haematological alterations. This could be considered as a LOAEL. A no-observed-adverse-effect-level (NOAEL) for this animal category was not identified and investigations using practically relevant concentrations of T-2 toxin are missing.

- In ruminants, the effects observed in nutritionally challenged heifers and ewes give rise to the assumption that rumen detoxification of T-2 toxin may not always be complete.

- For pigs, the published investigations demonstrate that they are among the most susceptible animals towards the effects of T-2 toxin, the most sensitive endpoints being immunological or haematological effects, which occur from doses of 29 µg/kg b.w. per day. This could be considered as a LOAEL. So far, no NOAEL has been identified for pigs.

- In poultry, first effects (e.g. mucosal damage in oral cavity) occur at a dose of 40 µg T-2 toxin/kg b.w. per day and 48 µg T-2 toxin/kg b.w. per day for broiler chickens and fattening turkeys, respectively. In fattening ducks a dose of 40 µg/kg b.w. per day of T-2 toxin caused a significant reduction in body weight gain. Infertility of eggs and/or reduction of egg production were seen at doses of 120 µg/kg b.w. per day of T-2 toxin for laying hens. These doses could be considered as LOAELs for poultry. So far, no NOAELs have been identified.

- For rabbits, doses ranging from 500-2000 µg of T-2 toxin/kg b.w. per day result in decreased body weight gain and mucosal damage. Only moderate signs including haematological and hormonal effects have been observed for doses ranging from 200-500 µg of T-2 toxin/kg b.w. per day. A NOAEL of 100 µg T-2 toxin/kg b.w. per day was identified.

- Reduced feed intake, growth and haematocrit values together with an increased mortality have been reported for fish. The lowest NOAEL of 13 µg T-2 toxin/kg b.w. per day was identified for catfish.

- Cats are amongst the most sensitive animal species. Due to the limited data and the severe effects i.e. mortality observed for cats at the low dose levels (60-100 µg T-2 toxin/kg b.w. per day), the available data cannot be used to identify a NOAEL or LOAEL.

- For dogs, no toxicity data are available.

- The available data do not allow identification of a NOAEL or LOAEL for horses.

- The available data describing possible effects of combined exposure to T-2 and HT-2 toxins with other mycotoxins are too limited to draw any conclusions.

***Human health risk characterisation***

- Estimates of chronic dietary exposure for populations of all age groups to the sum of T-2 and HT-2 toxins based on the available occurrence data are below the group TDI of 100 ng/kg b.w., and therefore there is no health concern.

- There are limited data on dietary habits of vegetarians with data available for only five European countries, with very few subjects in four of them. These limited data do not indicate significant differences in dietary exposure between the vegetarians and the general population.

***Animal health risk characterisation***

- Based on estimates of feed intake and the available occurrence data on feedingstuffs, the exposures to the sum of T-2 and HT-2 toxins for ruminants are substantially lower than the LOAELs identified, and are therefore considered unlikely to be a health concern.

- For pig and poultry, the CONTAM Panel used the $BMDL_{05}$ for pigs as a reference point. The estimates of exposure based on the reported levels of the sum of T-2 and HT-2 toxins in feed indicate that the risk of adverse health effects as a result of consuming feed containing T-2 and HT-2 toxins is low for these species.

- The limited data available for rabbits and fish suggest that the estimated exposures to the sum of T-2 and HT-2 toxins in feed at the currently reported concentrations is well below the identified NOAELs, and therefore considered unlikely to be a health concern.

- For dogs and cats, it was not possible to identify NOAELs or LOAELs. For cats, the health risk from exposure to the sum of T-2 and HT-2 toxins could not be assessed. However, the CONTAM Panel concluded that for dogs the $BMDL_{05}$ for pigs could be applied as a reference point. The estimated exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data indicate that the risk of adverse health effects of feed containing T-2 and HT-2 toxins is low for dogs.

- For horses, it was also not possible to identify a NOAELs or a LOAEL. However, the CONTAM Panel concluded that the $BMDL_{05}$ for pigs could be applied as a reference point.

The estimated exposure to the sum of T-2 and HT-2 toxins based on the available occurrence data indicate that the risk of adverse health effects of feed containing T-2 and HT-2 toxins is low for horses.

## RECOMMENDATIONS

- (Certified) reference materials (both matrix reference materials as well as calibrants) should be made available for the determination of T-2 and HT-2 toxins in food and feed.
- Further research on the effects of food processing (i.e. cooking) including the characterisation of the degradation products of T-2 and HT-2 toxins is needed.
- Harmonised reporting for compound feed occurrence data on T-2 and HT-2 toxins across the European countries is needed.

## DOCUMENTATION PROVIDED TO EFSA

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# APPENDICES

## A. CONCENTRATIONS OF THE SUM OF T-2 AND HT-2 TOXINS, T-2 TOXIN AND HT-2 TOXIN IN FOOD SUB-GROUPS

Concentrations of the sum of T-2 and HT-2 toxins, T-2 toxin and HT-2 toxin (µg/kg) across food sub-groups as used in the exposure assessment are presented in Tables A1-A3.

**Table A1:** Concentration of the sum of T-2 and HT-2 toxins (µg/kg) across food groups as used in the exposure assessment.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| **Grains for human consumption** | | | | | | | | |
| Wheat grain | 156 | 29 % | LB | 14 | 20 | 20 | 20 | 26 |
| | | | UB | 15 | 20 | 20 | 20 | 26 |
| Barley grain | 36 | 25 % | LB | 10 | 4.2 | 9.3 | 75 | 89 |
| | | | UB | 13 | 7.0 | 12 | 75 | 89 |
| Rye grain | 39 | 69 % | LB | 4.2 | 0.0 | 1.1 | 61 | 61 |
| | | | UB | 9.5 | 6.3 | 10 | 61 | 61 |
| Oats, grain | 71 | 27 % | LB | 31 | 26 | 45 | 66 | 124 |
| | | | UB | 34 | 32 | 47 | 66 | 124 |
| Rice | 49 | 71 % | LB | 0.56 | 0.0 | 0.1 | 3.3 | 13 |
| | | | UB | 2.9 | 0.9 | 6.0 | 6.0 | 15[(c)] |
| Other grains | 17 | 76 % | LB | 0.36 | -[(b)] | -[(b)] | -[(b)] | 3.1 |
| | | | UB | 5.0 | -[(b)] | -[(b)] | -[(b)] | 10[(c)] |
| **Grain milling products** | | | | | | | | |
| Grain milling products (undefined) | 58 | 62 % | LB | 2.3 | 0.0 | 1.0 | 10 | 50 |
| | | | UB | 4.6 | 2.0 | 6.3 | 11 | 50 |
| Wheat milling products | 1310 | 73 % | LB | 1.7 | 0.0 | 0.5 | 7.3 | 75 |
| | | | UB | 7.6 | 5.0 | 10 | 20 | 75 |
| Rye milling products | 205 | 58 % | LB | 1.1 | 0.0 | 1.1 | 3.0 | 38 |
| | | | UB | 4.3 | 3.6 | 6.3 | 10 | 38 |
| Buckwheat milling products | 14 | 43 % | LB | 5.2 | -[(b)] | -[(b)] | -[(b)] | 52 |
| | | | UB | 6.1 | -[(b)] | -[(b)] | -[(b)] | 52 |
| Maize milling products | 265 | 35 % | LB | 5.8 | 1.3 | 4.7 | 20 | 204 |
| | | | UB | 8.2 | 5.2 | 9.0 | 21 | 204 |
| Oat milling products | 229 | 56 % | LB | 10 | 5.9 | 14 | 32 | 118 |
| | | | UB | 11 | 7.0 | 16 | 32 | 118 |
| Spelt milling products | 197 | 59 % | LB | 0.69 | 0.0 | 0.7 | 2.8 | 12 |
| | | | UB | 3.3 | 2.5 | 6.3 | 8.1 | 16[(c)] |

**Table A1:** Continued.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| **Bread and rolls** | | | | | | | | |
| Bread and rolls (undefined) | 78 | 36 % | LB | 1.3 | 1.0 | 1.8 | 3.3 | 8.8 |
| | | | UB | 2.6 | 1.6 | 2.8 | 8.8 | 20[(c)] |
| Wheat bread and rolls | 107 | 50 % | LB | 0.7 | 0.5 | 1.0 | 2.2 | 5.5 |
| | | | UB | 4.0 | 1.3 | 10 | 10 | 10[(c)] |
| Rye bread and rolls | 66 | 30 % | LB | 0.85 | 0.8 | 1.1 | 2.6 | 4.5 |
| | | | UB | 2.0 | 1.1 | 2.4 | 6 | 10[(c)] |
| Mixed wheat and rye bread and rolls | 114 | 40 % | LB | 0.81 | 0.6 | 1.4 | 2.7 | 5.0 |
| | | | UB | 2.4 | 1.4 | 2.2 | 10 | 10[(c)] |
| Multigrain bread and rolls | 22 | 18 % | LB | 2.6 | 1.9 | 3.2 | 6.4 | 6.9 |
| | | | UB | 3.6 | 2.6 | 6.0 | 6.9 | 10[(c)] |
| Unleavened bread, crisp bread and rusk | 131 | 36 % | LB | 1.3 | 0.7 | 1.5 | 4.3 | 27 |
| | | | UB | 2.8 | 1.6 | 4.0 | 6.0 | 27 |
| Other bread | 99 | 55 % | LB | 0.85 | 0.0 | 1.3 | 2.9 | 13 |
| | | | UB | 6.1 | 1.8 | 10 | 20 | 20[(c)] |
| **Pasta** | 513 | 26 % | LB | 1.7 | 1.1 | 2.1 | 5.0 | 17 |
| | | | UB | 2.5 | 1.5 | 2.8 | 10 | 17 |
| **Breakfast cereals** | | | | | | | | |
| Breakfast cereals (undefined) | 77 | 34 % | LB | 6.3 | 1.4 | 3.9 | 36 | 87 |
| | | | UB | 8.8 | 3.7 | 6.3 | 36 | 87 |
| Cereal flakes | 1187 | 53 % | LB | 13 | 8.0 | 18 | 42 | 197 |
| | | | UB | 14 | 9.0 | 18 | 42 | 197 |
| Oat flakes | 1089 | 53 % | LB | 14 | 9.1 | 19 | 43 | 197 |
| | | | UB | 15 | 10 | 19 | 43 | 197 |
| Spelt flakes | 32 | 72 % | LB | 0.29 | 0.0 | 0.07 | 1.9 | 2.5 |
| | | | UB | 1.6 | 1.0 | 1.5 | 6.0 | 6.0[(c)] |
| Cereal flakes (undefined) | 66 | 50 % | LB | 2.7 | 0.4 | 3.1 | 9.5 | 32 |
| | | | UB | 5.6 | 5.0 | 7.0 | 14 | 32 |
| Corn flakes | 162 | 51 % | LB | 2.1 | 0.10 | 1.1 | 5.1 | 123 |
| | | | UB | 5.1 | 2.8 | 6.3 | 13 | 123 |
| Muesli | 249 | 12 % | LB | 5.6 | 4.2 | 7.7 | 17 | 49 |
| | | | UB | 6.2 | 5.0 | 8.4 | 17 | 49 |
| Grits | 58 | 74 % | LB | 2.4 | 0.0 | 0.60 | 13 | 60 |
| | | | UB | 3.6 | 0.90 | 1.9 | 13 | 60 |
| Other breakfast cereals | 75 | 31 % | LB | 1.6 | 0.70 | 1.4 | 7.5 | 20 |
| | | | UB | 2.6 | 1.2 | 4.2 | 7.5 | 20 |
| **Fine bakery wares** | | | | | | | | |
| Fine bakery wares (undefined) | 68 | 51 % | LB | 0.94 | 0.60 | 1.7 | 3.6 | 4.3 |
| | | | UB | 3.0 | 4.0 | 4.0 | 6.5 | 6.5[(c)] |
| Pastries and cakes | 72 | 64 % | LB | 0.66 | 0.0 | 0.7 | 2.0 | 20 |
| | | | UB | 1.8 | 0.80 | 1.3 | 6.5 | 20 |
| Biscuits (cookies) | 391 | 31 % | LB | 2.2 | 1.1 | 2.6 | 8.4 | 66 |
| | | | UB | 4.2 | 2.6 | 5.1 | 14 | 66 |

**Table A1:** Continued.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| **Snack food** | 36 | 44 % | LB | 4.9 | 0.70 | 4.5 | 20 | 20 |
| | | | UB | 5.6 | 2.0 | 5.2 | 20 | 20 |
| **Vegetables and vegetable products** | | | | | | | | |
| Soya | 50 | 26 % | LB | 1.1 | 0.9 | 1.8 | 2.9 | 4.4 |
| | | | UB | 1.2 | 0.9 | 1.8 | 2.9 | 4.4 |
| Oilseeds | 48 | 13 % | LB | 1.6 | 1.1 | 1.5 | 4.4 | 10 |
| | | | UB | 1.6 | 1.1 | 1.5 | 4.4 | 10 |
| Other vegetables | 49 | 47 % | LB | 1.1 | 0.30 | 1.0 | 6.2 | 10 |
| | | | UB | 1.2 | 0.50 | 1.0 | 6.2 | 10 |
| Fungi (dried)[(d)] | 20 | 5 % | LB | 9.5 | 3.4 | 12 | 40 | 45 |
| | | | UB | 9.5 | 3.4 | 12 | 40 | 45 |
| **Composite food (cereal-based)** | | | | | | | | |
| Cereal-based dishes | 55 | 58 % | LB | 0.65 | 0.0 | 1.3 | 2.7 | 4.4 |
| | | | UB | 3.7 | 1.3 | 10 | 10 | 10[(c)] |
| **Alcoholic beverages** | | | | | | | | |
| Beer and beer-like beverage | 59 | 5 % | LB | 0.82 | 0.70 | 1.0 | 2.1 | 2.4 |
| | | | UB | 0.82 | 0.70 | 1 | 2.1 | 2.4 |
| Wine | 97 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 0.18 | -[(b)] | -[(b)] | -[(b)] | 0.2[(c)] |
| **Food for infants and small children** | | | | | | | | |
| Food for infants and small children (undefined) | 33 | 45 % | LB | 0.92 | 0.50 | 1.1 | 3.2 | 7.8 |
| | | | UB | 2.1 | 0.80 | 2.2 | 10 | 10[(c)] |
| Cereal-based food for infants and young children | 390 | 29 % | LB | 2.7 | 1.0 | 3.3 | 11 | 31 |
| | | | UB | 3.5 | 1.6 | 4.3 | 13 | 33[(c)] |
| **Products for special nutritional use** | | | | | | | | |
| Fine bakery products and breakfast cereals for diabetics | 51 | 37 % | LB | 1.7 | 0.80 | 2.9 | 5.1 | 12 |
| | | | UB | 2.9 | 1.4 | 4.4 | 10 | 17[(c)] |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit; (d): The concentration introduced in the calculation of the dietary exposure was corrected by multiplying the mean value obtained on dehydrated products by 0.1 (90 % water content in fresh fungi).

**Table A2:** T-2 toxin concentration (µg/kg) across food groups as used in the exposure assessment.

| Food group | N[a] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Grains for human consumption** | | | | | | | | |
| Wheat | 149 | 29 % | LB | 7.0 | 10 | 10 | 10 | 16 |
| | | | UB | 7.4 | 10 | 10 | 10 | 16 |
| Barley | 36 | 25 % | LB | 3.4 | 0.60 | 2.4 | 18 | 34 |
| | | | UB | 3.8 | 1.0 | 2.9 | 18 | 34 |
| Rye | 39 | 79 % | LB | 0.79 | 0.0 | 0.0 | 11 | 11 |
| | | | UB | 3.0 | 2.9 | 5.0 | 11 | 11 |
| Oats | 71 | 27 % | LB | 7.5 | 7.0 | 10 | 21 | 41 |
| | | | UB | 8.7 | 8.0 | 10 | 21 | 41 |
| Rice | 37 | 84 % | LB | 0.35 | 0.0 | 0.0 | 0.10 | 13 |
| | | | UB | 1.8 | 0.10 | 3.0 | 10 | 13 |
| Other grains | 13 | 85 % | LB | 0.25 | -[b] | -[b] | -[b] | 3.1 |
| | | | UB | 2.5 | -[b] | -[b] | -[b] | 5.0[c] |
| **Grain milling products** | | | | | | | | |
| Grain milling products (undefined) | 55 | 76 % | LB | 0.56 | 0.0 | 0.10 | 5.0 | 10 |
| | | | UB | 1.8 | 1.0 | 2.9 | 5.0 | 10 |
| Wheat milling products | 862 | 86 % | LB | 0.58 | 0.0 | 0.0 | 0.53 | 45 |
| | | | UB | 3.2 | 2.5 | 5.0 | 10 | 45 |
| Rye milling products | 169 | 75 % | LB | 0.28 | 0.0 | 0.06 | 1.7 | 10 |
| | | | UB | 1.7 | 1.0 | 2.9 | 5.0 | 10 |
| Buckwheat milling products | 7 | 86 % | LB | 0.60 | -[b] | -[b] | -[b] | 4.2 |
| | | | UB | 1.1 | -[b] | -[b] | -[b] | 4.2 |
| Maize milling products | 232 | 43 % | LB | 3.2 | 0.43 | 2.8 | 12 | 81 |
| | | | UB | 4.4 | 2.9 | 5.0 | 12 | 81 |
| Oat milling products | 204 | 63 % | LB | 2.4 | 0.0 | 3.6 | 11 | 34 |
| | | | UB | 3.2 | 0.50 | 4.8 | 11 | 34 |
| Spelt milling products | 148 | 78 % | LB | 0.08 | 0.0 | 0.0 | 0.30 | 2.4 |
| | | | UB | 1.3 | 0.25 | 2.5 | 5.0 | 10[c] |
| **Bread and rolls** | | | | | | | | |
| Bread and rolls (undefined) | 34 | 76 % | LB | 0.30 | 0.0 | 0.10 | 4.4 | 4.4 |
| | | | UB | 1.4 | 0.50 | 1.0 | 5.0 | 10[c] |
| Wheat bread and rolls | 74 | 91 % | LB | 0.07 | 0.0 | 0.0 | 0.23 | 3.5 |
| | | | UB | 3.6 | 3.0 | 5.0 | 5.0 | 5.0[c] |
| Rye bread and rolls | 36 | 47 % | LB | 0.19 | 0.10 | 0.20 | 0.9 | 1.5 |
| | | | UB | 1.0 | 0.20 | 1.2 | 5.0 | 5.0[c] |
| Mixed wheat and rye bread | 48 | 71 % | LB | 0.06 | 0.0 | 0.07 | 0.50 | 0.6 |
| | | | UB | 1.7 | 0.10 | 3.0 | 5.0 | 5.0[c] |
| Multigrain bread and rolls | 8 | 50 % | LB | -[b] | -[b] | -[b] | -[b] | 4.5 |
| | | | UB | -[b] | -[b] | -[b] | -[b] | 5.0[c] |
| Unleavened bread, crisp bread and rusk | 66 | 68 % | LB | 0.21 | 0.0 | 0.32 | 1.0 | 2.0 |
| | | | UB | 1.5 | 2.0 | 2.0 | 3.0 | 4.5[c] |

**Table A2:** Continued.

| Food group | N[(a)] | ND | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| Other bread | 52 | 67 % | LB | 0.18 | 0.0 | 0.2 | 1.2 | 2.3 |
| | | | UB | 2.6 | 2.7 | 5.0 | 5.0 | 5.0[(c)] |
| **Pasta** | 129 | 55 % | LB | 0.43 | 0.0 | 0.4 | 1.4 | 12 |
| | | | UB | 1.8 | 0.6 | 3.0 | 5.0 | 12 |
| **Breakfast cereals** | | | | | | | | |
| Breakfast cereals (undefined) | 55 | 47 % | LB | 1.9 | 0.30 | 0.9 | 22 | 22 |
| | | | UB | 3.6 | 1.0 | 5.0 | 22 | 22 |
| Cereal flakes | 1106 | 60 % | LB | 3.2 | 0.0 | 5.5 | 14 | 64 |
| | | | UB | 3.9 | 2.0 | 5.7 | 14 | 64 |
| Oat flakes | 1003 | 58 % | LB | 3.5 | 0.0 | 5.7 | 15 | 64 |
| | | | UB | 4.0 | 2.0 | 5.9 | 15 | 64 |
| Spelt flakes | 56 | 95 % | LB | 0.003 | 0.0 | 0.0 | 0.05 | 0.07 |
| | | | UB | 2.6 | 3.0 | 3.0 | 6.0 | 6.0[(c)] |
| Cereal flakes (undefined) | 47 | 70 % | LB | 1.0 | 0.0 | 0.3 | 8.8 | 9.5 |
| | | | UB | 2.4 | 3.0 | 3.0 | 8.8 | 9.5 |
| Corn flakes | 132 | 64 % | LB | 1.0 | 0.0 | 0.2 | 2.8 | 56 |
| | | | UB | 2.6 | 0.50 | 3.0 | 10 | 56 |
| Muesli | 104 | 27 % | LB | 1.7 | 0.9 | 2.7 | 5.2 | 15 |
| | | | UB | 2.1 | 1.3 | 3.0 | 5.2 | 15 |
| Grits | 47 | 89 % | LB | 0.80 | 0.0 | 0.0 | 6.3 | 16 |
| | | | UB | 1.6 | 0.4 | 1.9 | 6.3 | 16 |
| Other breakfast cereals | 31 | 61 % | LB | 0.58 | 0.0 | 0.4 | 2.5 | 10 |
| | | | UB | 1.2 | 0.25 | 2.4 | 3.0 | 10 |
| **Fine bakery wares** | | | | | | | | |
| Fine bakery wares (undefined) | 41 | 83 % | LB | 0.20 | 0.0 | 0.0 | 1.5 | 2.0 |
| | | | UB | 1.9 | 2.0 | 2.0 | 2.0 | 5.0[(c)] |
| Pastries and cakes | 15 | 93 % | LB | 0.67 | -[(b)] | -[(b)] | -[(b)] | 10 |
| | | | UB | 2.5 | -[(b)] | -[(b)] | -[(b)] | 10 |
| Biscuits (cookies) | 241 | 61 % | LB | 0.88 | 0.0 | 0.8 | 3.3 | 24 |
| | | | UB | 3.0 | 2.0 | 5.0 | 10 | 24 |
| **Snack food** | 18 | 50 % | LB | 4.3 | -[(b)] | -[(b)] | -[(b)] | 10 |
| | | | UB | 4.9 | -[(b)] | -[(b)] | -[(b)] | 10 |
| **Vegetables and vegetable products** | | | | | | | | |
| Soya | 4 | 50 % | LB | 0.13 | -[(b)] | -[(b)] | -[(b)] | 0.31 |
| | | | UB | 0.18 | -[(b)] | -[(b)] | -[(b)] | 0.31 |
| Other vegetables | 3 | 0 % | LB | 0.13 | -[(b)] | -[(b)] | -[(b)] | 0.28 |
| | | | UB | 0.13 | -[(b)] | -[(b)] | -[(b)] | 0.28 |
| **Composite food (cereal-based)** | | | | | | | | |
| Cereal-based dishes | 20 | 85 % | LB | 0.13 | 0.0 | 0.0 | 1.3 | 1.7 |
| | | | UB | 4.3 | 5.00 | 5.0 | 5.0 | 5.0[(c)] |
| **Food for infants and small children** | | | | | | | | |
| Food for infants and small children (undefined) | 9 | 56 % | LB | 0.19 | -[(b)] | -[(b)] | -[(b)] | 0.50 |
| | | | UB | 2.1 | -[(b)] | -[(b)] | -[(b)] | 5.0[(c)] |

**Table A2:** Continued.

| Food group | N[(a)] | ND | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| Cereal-based food for infants | 141 | 45 % | LB | 0.88 | 0.10 | 0.94 | 5.2 | 7.7 |
| | | | UB | 1.7 | 0.70 | 2.1 | 7.7 | 10[(c)] |
| **Products for special nutritional use** | | | | | | | | |
| Fine bakery products and breakfast cereals for diabetics | 13 | 62 % | LB | 0.32 | -[(b)] | -[(b)] | -[(b)] | 2.4 |
| | | | UB | 2.0 | -[(b)] | -[(b)] | -[(b)] | 5.0[(c)] |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit.

**Table A3:** HT-2 toxin concentration (µg/kg) across food groups as used in the exposure assessment.

| Food group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| **Grains for human consumption** | | | | | | | | |
| Wheat | 148 | 26 % | LB | 7.3 | 10 | 10 | 10 | 16 |
| | | | UB | 8.2 | 10 | 10 | 10 | 16 |
| Barley | 33 | 52 % | LB | 7.3 | 3.0 | 6.8 | 55 | 57 |
| | | | UB | 9.1 | 6.0 | 8.7 | 55 | 57 |
| Rye | 35 | 86 % | LB | 3.6 | 0.0 | 0.0 | 50 | 50 |
| | | | UB | 7.5 | 5 | 6 | 50 | 50 |
| Oats | 70 | 17 % | LB | 24 | 25 | 36 | 51 | 100 |
| | | | UB | 25 | 25 | 36 | 51 | 100 |
| Rice | 37 | 100 % | LB | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | | | UB | 1.6 | 0.5 | 3 | 3 | 3[(c)] |
| Other grains | 13 | 92 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.5 |
| | | | UB | 3.7 | -[(b)] | -[(b)] | -[(b)] | 6.0[(c)] |
| **Grain milling products** | | | | | | | | |
| Grain milling products (undefined) | 54 | 76 % | LB | 0.9 | 0.0 | 0.4 | 5.0 | 17 |
| | | | UB | 4.5 | 4.2 | 6.0 | 10 | 17 |
| Wheat milling products | 745 | 87 % | LB | 1.0 | 0.0 | 0.0 | 4.0 | 39 |
| | | | UB | 4.4 | 4.0 | 6.0 | 10 | 39 |
| Rye milling products | 157 | 85 % | LB | 0.6 | 0.0 | 0.0 | 2.4 | 33 |
| | | | UB | 3.3 | 3.0 | 5.0 | 6.0 | 33 |
| Buckwheat milling products | 7 | 71 % | LB | 1.1 | -[(b)] | -[(b)] | -[(b)] | 7.0 |
| | | | UB | 2.5 | -[(b)] | -[(b)] | -[(b)] | 7.0 |
| Maize milling products | 216 | 57 % | LB | 3.4 | 0.0 | 2.3 | 11 | 147 |
| | | | UB | 5.5 | 3.0 | 6.0 | 11 | 147 |
| Oat milling products | 204 | 43 % | LB | 7.7 | 4.1 | 12 | 25 | 84 |
| | | | UB | 8.1 | 4.8 | 12 | 25 | 84 |

**Table A3:** Continued.

| Food group | N[(a)] | ND | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| Spelt milling products | 132 | 87 % | LB | 0.4 | 0.0 | 0.0 | 2.1 | 12 |
| | | | UB | 3.4 | 3.0 | 6.0 | 6.0 | 12 |
| **Bread and rolls** | | | | | | | | |
| Bread and rolls (undefined) | 43 | 72 % | LB | 0.50 | 0.0 | 1.1 | 2.5 | 3.0 |
| | | | UB | 4.1 | 2.5 | 6.0 | 10 | 10[(c)] |
| Wheat bread and rolls | 44 | 82 % | LB | 0.20 | 0.0 | 0.0 | 1.8 | 2.6 |
| | | | UB | 4.2 | 5.0 | 5.0 | 5.0 | 5.0[(c)] |
| Rye bread and rolls | 36 | 67 % | LB | 0.40 | 0.0 | 0.5 | 3.0 | 3.3 |
| | | | UB | 1.6 | 0.70 | 3.0 | 5.0 | 5.0[(c)] |
| Mixed wheat and rye bread and rolls | 48 | 83 % | LB | 0.10 | 0 | 0 | 0.90 | 2.4 |
| | | | UB | 1.9 | 0.40 | 3.0 | 5.0 | 5.0[(c)] |
| Multigrain bread and rolls | 9 | 56 % | LB | 0.90 | -[(b)] | -[(b)] | -[(b)] | 2.5 |
| | | | UB | 3.2 | -[(b)] | -[(b)] | -[(b)] | 10[(c)] |
| Unleavened bread, crisp bread and rusk | 66 | 86 % | LB | 0.5 | 0.0 | 0.0 | 3.0 | 9.3 |
| | | | UB | 2.1 | 2.0 | 3.0 | 4.5 | 9.3 |
| Other bread | 52 | 71 % | LB | 0.7 | 0.0 | 0.6 | 3.8 | 11 |
| | | | UB | 3.6 | 5.0 | 5.0 | 8.0 | 11 |
| **Pasta** | 129 | 61 % | LB | 1.2 | 0.0 | 1.5 | 5.9 | 15 |
| | | | UB | 2.9 | 2.8 | 5.0 | 6.9 | 15 |
| **Breakfast cereals** | | | | | | | | |
| Breakfast cereals (undefined) | 63 | 57 % | LB | 5.0 | 0.0 | 3.2 | 29 | 66 |
| | | | UB | 7.9 | 3.2 | 10 | 29 | 66 |
| Cereal flakes | 1074 | 35 % | LB | 9.7 | 7.0 | 14 | 33 | 159 |
| | | | UB | 10 | 7.0 | 14 | 33 | 159 |
| Oat flakes | 1003 | 31 % | LB | 10 | 7.6 | 15 | 34 | 159 |
| | | | UB | 11 | 7.6 | 15 | 34 | 159 |
| Spelt flakes | 24 | 96 % | LB | 0.10 | 0.0 | 0.0 | 0.0 | 2.5 |
| | | | UB | 1.1 | 0.5 | 1.5 | 3.0 | 3.0[(c)] |
| Cereal flakes (undefined) | 47 | 81 % | LB | 1.6 | 0.0 | 0.0 | 13 | 26 |
| | | | UB | 4.2 | 4.0 | 4.0 | 13 | 26 |
| Corn flakes | 127 | 83 % | LB | 1.6 | 0.0 | 0.0 | 3.0 | 75 |
| | | | UB | 4.1 | 3.0 | 5.0 | 10 | 75 |
| Muesli | 108 | 43 % | LB | 6.0 | 2.0 | 6.3 | 27 | 88 |
| | | | UB | 6.8 | 3.9 | 6.3 | 27 | 88 |
| Grits | 47 | 89 % | LB | 1.8 | 0.0 | 0.0 | 7.3 | 43 |
| | | | UB | 2.6 | 0.5 | 2.3 | 7.3 | 43 |
| Other breakfast cereals | 31 | 77 % | LB | 0.8 | 0.0 | 0.0 | 5.1 | 10 |
| | | | UB | 2.6 | 3.0 | 4.0 | 6.0 | 10 |
| **Fine bakery wares** | | | | | | | | |
| Fine bakery wares (undefined) | 41 | 90 % | LB | 1.8 | 0.0 | 0.0 | 3.2 | 58 |
| | | | UB | 3.7 | 2.0 | 2.0 | 4.5 | 58 |

**Table A3:** Continued.

| Food group | N[(a)] | ND | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximu |
| Pastries and cakes | 15 | 93 % | LB | 0.9 | 0.0 | 0.0 | 10 | 10 |
| | | | UB | 3.2 | 2.0 | 4.5 | 10 | 10 |
| Biscuits (cookies) | 200 | 63 % | LB | 1.5 | 0.0 | 2.0 | 6.2 | 42 |
| | | | UB | 3.8 | 2.6 | 5.3 | 10 | 42 |
| Snack food | 23 | 65 % | LB | 3.1 | 0.0 | 10 | 10 | 10 |
| | | | UB | 5.8 | 10 | 10 | 10 | 10 |
| **Vegetables and vegetable products** | | | | | | | | |
| Soya | 4 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 0.30 | -[(b)] | -[(b)] | -[(b)] | 0.30[(c)] |
| Other vegetables | 3 | 67 % | LB | 0.3 | -[(b)] | -[(b)] | -[(b)] | 0.90 |
| | | | UB | 0.50 | -[(b)] | -[(b)] | -[(b)] | 0.90 |
| **Composite food (cereal-based)** | | | | | | | | |
| Cereal-based dishes | 19 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.3 | -[(b)] | -[(b)] | -[(b)] | 5.0[(c)] |
| **Food for infants and small children** | | | | | | | | |
| Food for infants and small children (undefined) | 9 | 56 % | LB | 0.30 | -[(b)] | -[(b)] | -[(b)] | 1.0 |
| | | | UB | 2.2 | -[(b)] | -[(b)] | -[(b)] | 5.0[(c)] |
| Cereal-based food for infants and young children | 140 | 64 % | LB | 2.7 | 0.00 | 2.9 | 17 | 25 |
| | | | UB | 3.9 | 2.0 | 5.7 | 17 | 25 |
| **Products for special nutritional use** | | | | | | | | |
| Fine bakery products and breakfast cereals for diabetics | 13 | 69 % | LB | 1.1 | -[(b)] | -[(b)] | -[(b)] | 12 |
| | | | UB | 3.7 | -[(b)] | -[(b)] | -[(b)] | 12 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit.

## B. CONCENTRATIONS OF THE SUM OF T-2 AND HT-2 TOXINS, T-2 TOXIN AND HT-2 TOXIN IN FEED

Concentrations of the sum of T-2 and HT-2 toxins, T-2 toxin and HT-2 toxin (µg/kg) across feed groups as used in the animal exposure assessment are presented in Tables B1-B3.

**Table B1:** Concentrations (µg/kg) of the sum of T-2 and HT-2 toxins across feed groups.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Cereal grains, their products and by-products** | | | | | | | | |
| Undefined cereal grains, their products and by-products | 7 | 86 % | LB | 34 | -[(b)] | -[(b)] | -[(b)] | 235 |
| | | | UB | 71 | -[(b)] | -[(b)] | -[(b)] | 235 |
| Oats | 177 | 13 % | LB | 152 | 70 | 192 | 460 | 3061 |
| | | | UB | 170 | 90 | 200 | 460 | 3061 |
| Oat middlings | 220 | 1.8 % | LB | 300 | 153 | 423 | 118 | 1711 |
| | | | UB | 300 | 153 | 423 | 118 | 1711 |
| Barley | 228 | 69 % | LB | 21 | 0.0 | 18 | 75 | 667 |
| | | | UB | 52 | 44 | 50 | 95 | 667 |
| Sorghum | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 29 | -[(b)] | -[(b)] | -[(b)] | 40[(c)] |
| Wheat | 215 | 92 % | LB | 1.9 | 0.0 | 0.0 | 16 | 71 |
| | | | UB | 27 | 40 | 44 | 44 | 73[(c)] |
| Wheat middlings | 60 | 38 % | LB | 46 | 17 | 32 | 331 | 628 |
| | | | UB | 52 | 20 | 32 | 331 | 628 |
| Wheat bran | 9 | 67 % | LB | 9.4 | -[(b)] | -[(b)] | -[(b)] | 41 |
| | | | UB | 24 | -[(b)] | -[(b)] | -[(b)] | 44[(c)] |
| Wheat gluten | 71 | 4.2 % | LB | 177 | 161 | 231 | 354 | 429 |
| | | | UB | 177 | 161 | 231 | 354 | 429 |
| Triticale | 46 | 89 % | LB | 5.7 | 0.0 | 0.0 | 20 | 141 |
| | | | UB | 20 | 15 | 16 | 44 | 141 |
| Maize | 219 | 69 % | LB | 38 | 0.0 | 16 | 249 | 862 |
| | | | UB | 45 | 8.0 | 28 | 249 | 862 |
| Maize middlings | 33 | 52 % | LB | 54 | 0.0 | 43 | 437 | 493 |
| | | | UB | 60 | 8.0 | 47 | 437 | 493 |
| Maize gluten feed | 18 | 28 % | LB | 37 | 17 | 46 | 157 | 157 |
| | | | UB | 40 | 21 | 46 | 157 | 157 |
| **Oil seeds, oil fruits, their products and by-products** | | | | | | | | |
| Soya (bean), toasted | 14 | 93 % | LB | 1.2 | -[(b)] | -[(b)] | -[(b)] | 17 |
| | | | UB | 8.9 | -[(b)] | -[(b)] | -[(b)] | 21[(c)] |
| Sunflower seed | 16 | 94 % | LB | 5.3 | -[(b)] | -[(b)] | -[(b)] | 85 |
| | | | UB | 13 | -[(b)] | -[(b)] | -[(b)] | 89[(c)] |

**Table B1:** Continued.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Forages and roughage (including maize silage)** | | | | | | | | |
| Lucerne meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 8.0 | -[(b)] | -[(b)] | -[(b)] | 8.0[(c)] |
| Grass meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 19 | -[(b)] | -[(b)] | -[(b)] | 40[(c)] |
| Maize silage[(d)] | 124 | 90 % | LB | 6.0 | 0.0 | 0.0 | 36 | 239 |
| | | | UB | 42 | 44 | 44 | 56 | 259 |
| **Compound feedingstuffs** | | | | | | | | |
| Compound feedingstuffs (undefined) | 890 | 72 % | LB | 12 | 0.0 | 11 | 52 | 567 |
| | | | UB | 19 | 8.0 | 16 | 52 | 567 |
| Compound feedingstuffs for calves | 10 | 90 % | LB | 3.3 | -[(b)] | -[(b)] | -[(b)] | 33 |
| | | | UB | 41 | -[(b)] | -[(b)] | -[(b)] | 53[(c)] |
| Compound feedingstuffs for cattle | 27 | 96 % | LB | 1.5 | 0.0 | 0.0 | 0.0 | 40 |
| | | | UB | 38 | 40 | 44 | 44 | 53[(c)] |
| Compound feedingstuffs for piglets | 36 | 94 % | LB | 1.2 | 0.0 | 0.0 | 15 | 28 |
| | | | UB | 35 | 40 | 44 | 44 | 48[(c)] |
| Compound feedingstuffs for pigs | 29 | 93 % | LB | 1.1 | 0.0 | 0.0 | 14 | 17 |
| | | | UB | 40 | 44 | 44 | 44 | 44[(c)] |
| Compound feedingstuffs for sows | 17 | 76 % | LB | 8.3 | -[(b)] | -[(b)] | -[(b)] | 55 |
| | | | UB | 42 | -[(b)] | -[(b)] | -[(b)] | 75[(c)] |
| Compound feedingstuffs for poultry | 14 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 34 | -[(b)] | -[(b)] | -[(b)] | 44[(c)] |
| Other feed | 156 | 80 % | LB | 9.2 | 0.0 | 0.0 | 59 | 245 |
| | | | UB | 38 | 40 | 44 | 73 | 245 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50th percentile; P75: 75th percentile; P95: 95th percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit; (d): concentration reported as µg/kg 88 % dry matter.

**Table B2:** Concentrations (µg/kg) of T-2 toxins across feed groups.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Cereal grains, their products and by-products** | | | | | | | | |
| Undefined cereal grains, their products and by-products | 34 | 71 % | LB | 40 | 0.0 | 38 | 351 | 559 |
| | | | UB | 51 | 20 | 38 | 351 | 559 |
| Oats | 164 | 62 % | LB | 24 | 0.0 | 37 | 107 | 268 |
| | | | UB | 40 | 30 | 37 | 107 | 268 |
| Oat middlings | 220 | 3.2 % | LB | 114 | 51 | 145 | 470 | 825 |
| | | | UB | 114 | 51 | 145 | 470 | 825 |
| Barley | 242 | 91 % | LB | 4.2 | 0.0 | 0.0 | 25 | 221 |
| | | | UB | 23 | 20 | 30 | 30 | 221 |
| Sorghum | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 15 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Wheat | 237 | 100 % | LB | 0.1 | -[(b)] | -[(b)] | -[(b)] | 14 |
| | | | UB | 14 | -[(b)] | -[(b)] | -[(b)] | 35[(c)] |
| Wheat middlings | 8 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.0 | -[(b)] | -[(b)] | -[(b)] | 4.0[(c)] |
| Wheat bran | 9 | 89 % | LB | 2.4 | -[(b)] | -[(b)] | -[(b)] | 22 |
| | | | UB | 10 | -[(b)] | -[(b)] | -[(b)] | 22 |
| Wheat gluten | 72 | 5.6 % | LB | 85 | 72 | 115 | 184 | 199 |
| | | | UB | 86 | 72 | 115 | 184 | 199 |
| Triticale | 41 | 95 % | LB | 1.4 | 0.0 | 0.0 | 0.0 | 38 |
| | | | UB | 6.9 | 5.0 | 5.0 | 19 | 38 |
| Maize | 231 | 79 % | LB | 13 | 0.0 | 0.0 | 72 | 415 |
| | | | UB | 18 | 4.0 | 13 | 75 | 415 |
| Maize middlings | 33 | 82 % | LB | 15 | 0.0 | 0.0 | 120 | 171 |
| | | | UB | 18 | 4.0 | 4.0 | 120 | 171 |
| Maize gluten feed | 18 | 61 % | LB | 13 | -[(b)] | -[(b)] | -[(b)] | 109 |
| | | | UB | 15 | -[(b)] | -[(b)] | -[(b)] | 109 |
| **Oil seeds, oil fruits, their products and by-products** | | | | | | | | |
| Soya (bean), toasted | 15 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.4 | -[(b)] | -[(b)] | -[(b)] | 10[(c)] |
| Sunflower seed | 20 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 5.3 | -[(b)] | -[(b)] | -[(b)] | 10[(c)] |

**Table B2:** Continued.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P5 | P75 | P95 | Maximum |
| **Forages and roughage (including maize silage)** | | | | | | | | |
| Lucerne meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.0 | -[(b)] | -[(b)] | -[(b)] | 4.0[(c)] |
| Grass meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 9.3 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Maize silage[(d)] | 129 | 99 % | LB | 1.8 | 0.0 | 0.0 | 0.0 | 206 |
| | | | UB | 20 | 20 | 20 | 20 | 206 |
| **Compound feedingstuffs** | | | | | | | | |
| Compound feedingstuffs (undefined) | 895 | 85 % | LB | 3.3 | 0.0 | 0.0 | 20 | 321 |
| | | | UB | 7.0 | 4.0 | 4.0 | 20 | 321 |
| Compound feedingstuffs for calves | 10 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 19 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Compound feedingstuffs for cattle | 42 | 64 % | LB | 9.3 | 0.0 | 25 | 25 | 38 |
| | | | UB | 21 | 20 | 25 | 25.0 | 38 |
| Compound feedingstuffs for piglets | 36 | 100 % | LB | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | | | UB | 17 | 20 | 20 | 20 | 20[(c)] |
| Compound feedingstuffs for pigs | 33 | 88 % | LB | 3.0 | 0.0 | 0.0 | 25 | 25 |
| | | | UB | 20 | 20 | 20 | 25 | 25 |
| Compound feedingstuffs for sows | 17 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 18 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Compound feedingstuffs for poultry | 18 | 78 % | LB | 5.6 | -[(b)] | -[(b)] | -[(b)] | 25 |
| | | | UB | 18 | -[(b)] | -[(b)] | -[(b)] | 25 |
| Other feed | 180 | 90 % | LB | 9.1 | 0.0 | 0.0 | 69 | 352 |
| | | | UB | 24 | 20 | 20 | 69 | 352 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit; (d): concentration reported as µg/kg 88 % dry matter.

**Table B3:** Concentrations (µg/kg) of HT-2 toxin across feed groups.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Cereal grains, their products and by-products** | | | | | | | | |
| Undefined cereal grains, their products and by-products | 7 | 86 % | LB | 17 | -[(b)] | -[(b)] | -[(b)] | 116 |
| | | | UB | 37 | -[(b)] | -[(b)] | -[(b)] | 116 |
| Oats | 164 | 13 % | LB | 107 | 65 | 156 | 280 | 905 |
| | | | UB | 109 | 65 | 156 | 280 | 905 |
| Oat middlings | 220 | 1.8 % | LB | 186 | 103 | 272 | 710 | 1068 |
| | | | UB | 186 | 103 | 272 | 710 | 1068 |
| Barley | 228 | 69 % | LB | 17 | 0.0 | 17 | 56 | 484 |
| | | | UB | 29 | 20 | 24 | 56 | 484 |
| Sorghum | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 15 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Wheat | 215 | 92 % | LB | 1.8 | 0.0 | 0.0 | 16 | 57 |
| | | | UB | 15 | 20 | 24 | 24 | 57 |
| Wheat middlings | 8 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.0 | -[(b)] | -[(b)] | -[(b)] | 4.0[(c)] |
| Wheat bran | 9 | 67 % | LB | 7.0 | -[(b)] | -[(b)] | -[(b)] | 25 |
| | | | UB | 14 | -[(b)] | -[(b)] | -[(b)] | 25 |
| Wheat gluten | 71 | 5.6 % | LB | 91 | 84 | 122 | 198 | 241 |
| | | | UB | 91 | 84 | 122 | 198 | 241 |
| Triticale | 41 | 93 % | LB | 4.5 | 0.0 | 0.0 | 12 | 103 |
| | | | UB | 13 | 10 | 10 | 30 | 103 |
| Maize | 219 | 71 % | LB | 25 | 0.0 | 13 | 164 | 447 |
| | | | UB | 29 | 4.0 | 15 | 164 | 447 |
| Maize middlings | 33 | 52 % | LB | 39 | 0.0 | 27 | 317 | 322 |
| | | | UB | 41 | 4.0 | 27 | 317 | 322 |
| Maize gluten feed | 18 | 28 % | LB | 24 | -[(b)] | -[(b)] | -[(b)] | 80 |
| | | | UB | 26 | -[(b)] | -[(b)] | -[(b)] | 80 |
| **Oil seeds, oil fruits, their products and by-products** | | | | | | | | |
| Soya (bean), toasted | 14 | 93 % | LB | 1.2 | -[(b)] | -[(b)] | -[(b)] | 17 |
| | | | UB | 4.9 | -[(b)] | -[(b)] | -[(b)] | 17 |
| Sunflower seed | 16 | 94 % | LB | 5.3 | -[(b)] | -[(b)] | -[(b)] | 85 |
| | | | UB | 9.1 | -[(b)] | -[(b)] | -[(b)] | 85 |

**Table B3:** Continued.

| Feed group | N[(a)] | LC | LB/UB | Concentration (µg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean | P50 | P75 | P95 | Maximum |
| **Forages and roughage (including maize silage)** | | | | | | | | |
| Lucerne meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 4.0 | -[(b)] | -[(b)] | -[(b)] | 4.0[(c)] |
| Grass meal | 3 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 9.3 | -[(b)] | -[(b)] | -[(b)] | 20[(c)] |
| Maize silage[(d)] | 124 | 90 % | LB | 6.0 | 0.0 | 0.0 | 36 | 239 |
| | | | UB | 24 | 24 | 24 | 36 | 239 |
| **Compound feedingstuffs** | | | | | | | | |
| Compound feedingstuffs (undefined) | 891 | 75 % | LB | 8.7 | 0.0 | 11 | 39 | 545 |
| | | | UB | 12 | 4.0 | 11 | 39 | 545 |
| Compound feedingstuffs for calves | 10 | 90 % | LB | 3.3 | -[(b)] | -[(b)] | -[(b)] | 33 |
| | | | UB | 22 | -[(b)] | -[(b)] | -[(b)] | 33 |
| Compound feedingstuffs for cattle | 27 | 96 % | LB | 1.5 | 0.0 | 0.0 | 0.0 | 40 |
| | | | UB | 20 | 20 | 24 | 24 | 40 |
| Compound feedingstuffs for piglets | 36 | 94 % | LB | 1.2 | 0.0 | 0.0 | 15 | 28 |
| | | | UB | 18 | 20 | 24 | 24 | 28 |
| Compound feedingstuffs for pigs | 29 | 93 % | LB | 1.1 | 0.0 | 0.0 | 14 | 17 |
| | | | UB | 21 | 24 | 24 | 24 | 24[(c)] |
| Compound feedingstuffs for sows | 17 | 76 % | LB | 8.3 | -[(b)] | -[(b)] | -[(b)] | 55 |
| | | | UB | 24 | -[(b)] | -[(b)] | -[(b)] | 55 |
| Compound feedingstuffs for poultry | 14 | 100 % | LB | 0.0 | -[(b)] | -[(b)] | -[(b)] | 0.0 |
| | | | UB | 17 | -[(b)] | -[(b)] | -[(b)] | 24[(c)] |
| Other feed | 156 | 81 % | LB | 7.3 | 0.0 | 0.0 | 52 | 99 |
| | | | UB | 21 | 20 | 24 | 52 | 99 |

N: number of samples; LC: left censored data (values below the limit of detection or limit of quantification); LB: lower-bound; UB: upper-bound; P50: 50$^{th}$ percentile; P75: 75$^{th}$ percentile; P95: 95$^{th}$ percentile.
(a): If N < 60 then the calculated P95 should be considered as an indicative value only due to the limited number of data (EFSA, 2011b); (b): not calculated where all data were left-censored or the number of data was very limited; (c): value represent the left-censoring limit; (d): concentration reported as µg/kg 88 % dry matter.

## C. INTAKES AND COMPOSITION OF DIETS USED IN ESTIMATING ANIMAL EXPOSURE TO THE SUM OF T-2 AND HT-2 TOXINS

This Appendix gives feed intakes for different livestock, fish and companion animals used in this Scientific Opinion. The composition of diets for each of the major farm livestock species are based on published guidelines on nutrition and feeding (AFRC, 1993; Carabano and Piquer, 1998; NRC 2007a,b; Leeson and Summers, 2008; EFSA, 2009a; OECD, 2009; McDonald et al., 2011) and data on EU manufacture of compound feeds (FEFAC, 2009). They are therefore estimates of the Panel on Contaminants in the Food Chain (CONTAM Panel), but are in agreement with common practice. In addition, the calculated lower bound (UB) and upper bound (UB) mean concentrations for the sum of T-2 and HT-2 toxins in the estimated diets for the farm livestock species and companion animals are given in this Appendix.

### C1. Feed intake

### C1.1. Cattle, sheep and goats

The diets of cattle, sheep and goats consist of predominantly of forages, supplemented where necessary with cereal grains, vegetable proteins etc as necessary (see Section 5.2.). Since levels of the sum of T-2 and HT-2 toxins in forages are low or non-existent (see Section 4.1.3 and Appendix B, Table B1), it has been assumed that they make no contribution to exposure. Therefore, exposure has been estimated for the non-forage feeds only,[33] and in Table C1 their proportion in the diet is also given. Live weights, feed intakes and growth rates/productivity are from AFRC (1993) and NRC (2007a). These live weights, feed intakes and growth rates/productivity for cattle, sheep and goats, and diet composition, are used in this Scientific Opinion (Table C1).

**Table C1:** Live weights, growth rate/productivity, dry matter intake for cattle, sheep and goats, and the proportions of the diet as non-forage.

| | Live weight (kg) | Growth rate or productivity | Dry matter intake (kg/day) | % of diet as non-forage feed | Reference |
|---|---|---|---|---|---|
| Dairy cows, lactating | 650 | 30-50 kg milk/day | 20.7 | 40 | AFRC (1993) |
| Fattening cattle: beef[(a)] | 400 | 1 kg/day | 9.6 | 20 | AFRC (1993) |
| Fattening cattle: cereal beef | 400 | 1.4 kg/day | 8.4 | 85 | AFRC (1993) |
| Sheep: lactating | 80 | Feeding twin lambs | 2.8 | 35 | AFRC (1993) |
| Goats: milking[(b)] | 60 | 6 kg milk/day | 3.4 | 75 | NRC (2007a) |
| Goats: fattening | 40 | 0.2 kg/day | 1.5 | 40 | NRC (2007a) |

(a): housed castrate cattle, medium maturing breed; (b): months 2-3 of lactation.

[33] Forages may include whole-crop cereals. While these may include T-2 and HT-2 toxins there are no data available on levels in these feeds.

### C1.2. Pigs, poultry and fish

Data for feed intake and live weight of pigs, poultry and fish from EFSA (2009a) and of ducks from Leeson and Summers (2008) are used in this Scientific Opinion (Table C2).

**Table C2:** Live weights and feed intake for pigs, poultry and fish (EFSA, 2009a) and ducks (Leeson and Summers, 2008).

| | Live weight (kg) | Feed intake (kg dry matter/day) |
|---|---|---|
| Pigs: piglets | 20 | 1.0 |
| Pigs: fattening pigs | 100 | 3.0 |
| Pigs: lactating sows | 200 | 6.0 |
| Poultry: broilers[(a)] | 2 | 0.12 |
| Poultry: laying hens | 2 | 0.12 |
| Turkeys: fattening turkeys | 12 | 0.40 |
| Ducks: fattening ducks | 3 | 0.14 |
| Salmonids | 2 | 0.04 |

(a): Chickens for fattening

### C1.3. Rabbits

A daily intake of 75 g/kg b.w. for a 2 kg rabbit is used in this Scientific Opinion to estimate exposure (based on Carabano and Piquer, 1998).

### C1.4. Companion animals

#### C1.4.1. Dogs and cats

The daily feed daily intakes of dogs and cats vary considerably, depending particularly on body weight, level of activity, pregnancy/lactation, diet composition and the nutrient composition of the food. In order to estimate the exposure of dogs and cats to T-2 and HT-2 toxins, daily intakes of 360 g/day for a 25 kg dog and a daily intake of 60 g/day for a 4 kg cat have been assumed, which reflect mature animals with a moderate level of activity for the species (NRC, 2006).

#### C1.4.2. Horses

In this Scientific Opinion, it is assumed that a mature horse (450 kg live weight) with a moderate level of activity has a dry matter intake of 9 kg/day, of which half is non-forage feeds (NRC, 2007b).

### C2. Diet composition and concentration estimates

Many livestock in the European countries are fed proprietary commercial compound feeds consisting of a range of feed materials. However, in the absence of any reliable data on levels of T-2 and HT-2 toxins in compound feeds provided by the European countries (Appendix B, Table B1), estimates of exposure have been made using estimated example diets for each of the livestock species and the mean concentrations of the sum of T-2 and HT-2 toxins in the individual feeds (Appendix B, Table B1). As discussed in Section C1.1, levels of these toxins in forage crops are low and it has been assumed that they make minimal or no contribution to the exposure. Therefore, for cattle, sheep, goats and horses exposure has only been estimated for non-forage feeds. The compositions of the estimated example rations are in Tables C4, C5 and C8.

## C2.1. Cattle, sheep and goats

Estimated example non-forage feed contents in the diets for cattle, sheep and goats are given in Table C4, together with the calculated mean LB and UB levels of the sum of T-2 and HT-2 toxins in these diets.

**Table C4:** Estimated example diet compositions of non-forage feed for cattle, sheep and goats, and the calculated mean lower-bound and upper-bound levels of the sum of T-2 and HT-2 toxins in these diets.

| Feeds | Dairy cow Early lactation | Beef cattle Cereal beef | Beef cattle Fattening | Sheep Lactating | Goats Dairy | Goats Fattening |
|---|---|---|---|---|---|---|
| Wheat (%) | 15 | | | 14 | | |
| Barley (%) | 20 | 60 | 40 | 18 | 25 | 20 |
| Oats (%) | | | | | 35 | 40 |
| Soybean meal (%) | 5 | | | 5 | 10 | 10 |
| Rapeseed meal (%) | 20 | 5 | 20 | 10 | 10 | 10 |
| Sunflower meal (%) | | 5 | | 5 | | |
| Beans (%) | 5 | | | 10 | | |
| Maize gluten feed (%) | 10 | 10 | 11 | | | |
| Wheat feed (%)[a] | 10 | 4 | 10 | 15 | 10 | 10 |
| Sugar beet pulp (%)[b] | 8 | 10 | 12 | 15 | | 2 |
| Molasses (%)[b] | 3 | 3 | 3 | 4 | 4 | 3 |
| Vegetable oils (%)[b] | 1 | 1 | 1 | 1 | 2 | 2 |
| Minerals, vitamins etc (%)[b] | 3 | 3 | 3 | 3 | 4 | 3 |
| **Sum of T-2 and HT-2 toxins[c]** | | | | | | |
| Lower-bound (µg/kg) | 13 | 19 | 17 | 11 | 63 | 70 |
| Upper-bound (µg/kg) | 24 | 38 | 30 | 22 | 79 | 84 |

(a): Product of flour or malting manufacture obtained from screened grains of wheat or dehusked spelt. It consists principally of fragments of the outer skins and of particles of grain from which less of the endosperm has been removed than in wheat bran[34]; (b): No data on T-2 and HT-2 toxin concentrations available, and therefore the contribution to the sum of T-2 and HT-2 toxin is assumed to be zero; (c): Concentrations calculated by using the mean concentrations of the sum of T-2 and HT-2 toxins reported for the individual feeds in Appendix B, Table B1.

While compound feeds are widely used, a significant proportion of dairy farmers mix and feed the non-forage feeds on the farm. The report from France described typical rations for dairy cows fed diets based on different forages with non-forage feeds and milk yields (AFSSA, 2009) (Table C5).

[34] Commission Regulation (EU) No 575/2011 of June 2011 on the Catalogue of feed materials OJ L 159, 17.6.2011, p. 25-65.

**Table C5:** Feed intakes of dairy cows fed diets based on different forages with non-forage feeds adjusted for milk yield (From AFSSA, 2009, modified).

| Type of forage | Milk production (kg/day) | Quantities of feed consumed (kg dry matter/day) | | |
|---|---|---|---|---|
| | | Forage[a] | Maize grain | Soybean meal |
| **Maize silage** | 30 | 15.3 | 4.4 | 2.3 |
| | 40 | 15.0 | 9.5 | 2.8 |
| | 50 | 14.7 | 8.8 | 4.4 |
| **Grass silage** | 30 | 17.4 | 3.5 | 0.78 |
| | 40 | 16.8 | 9.2 | 0.8 |
| | 50 | 16.5 | 12.5 | 0.82 |
| **Hay** | 30 | 16.3 | 8.0 | 0.19 |
| | 40 | 16.3 | 12.6 | 0.73 |
| | 50 | 15.8 | 11.0 | 2.3 |
| **Grazed grass** | 30 | 19.9 | 1.2 | 0 |
| | 40 | 18.8 | 7.2 | 0 |
| | 50 | 18.2 | 12.8 | 0 |

(a): Levels of T-2 and HT-2 toxins in forage crops are low and it has been assumed that they make no contribution to the sum of T-2 and HT-2 concentrations (see Section C.1.1.).

## C2.2. Pigs and poultry

Pig and poultry diets consist predominantly of cereals (wheat or maize) and vegetable proteins. Pig diets may also include more fibrous feeds, particularly for older animals. The estimated example feed compositions in the diets for pigs and poultry are presented in Table C6 together with the calculated mean LB and UB concentrations of the sum of T-2 and HT-2 toxins in these diets.

**Table C6:** Estimated example diet composition for pigs and poultry, and the calculated mean lower-bound and upper-bound levels of the sum of T-2 and HT-2 toxins in these diets.

| Feeds | Piglets | Pigs for fattening | Lactating sow | Broilers | Laying hens | Turkeys for fattening | Ducks for fattening |
|---|---|---|---|---|---|---|---|
| Wheat (%) | 48 | 48 | 50 | 38 | 30 | 30 | 45 |
| Barley (%) | 20 | 20 | 11 | | | 35 | 15 |
| Maize (%) | | | | 38 | 35 | | |
| Soybean meal (%) | 22 | 11 | 16 | 15 | 22 | 15 | 28 |
| Rapeseed meal (%) | 3 | 4 | | | | | |
| Lucerne meal (%) | | | | | 4 | 9 | 5 |
| Wheat feed (%)[a] | | 9 | 14 | 1 | | | 7 |
| Molasses (%)[b] | 3 | 4 | 4 | 3 | 3 | 3 | |
| Vegetable oils (%)[b] | 1 | 1 | 2 | 1 | 2 | 4 | |
| Minerals, vitamins etc (%)[b] | 3 | 3 | 3 | 4 | 4 | 4 | |
| **Sum of T-2 and HT-2 toxins[c]** | | | | | | | |
| Lower-bound (µg/kg) | 5 | 9 | 10 | 16 | 8 | 8 | 8 |
| Upper-bound (µg/kg) | 25 | 29 | 28 | 29 | 26 | 28 | 26 |

(a): Product of flour or malting manufacture obtained from screened grains of wheat or dehusked spelt. It consists principally of fragments of the outer skins and of particles of grain from which less of the endosperm has been removed than in wheat bran[34]; (b): No data on T-2 and HT-2 toxin concentrations available, and therefore the contribution to the sum of T-2 and HT-2 toxin is assumed to be zero; (c): Concentrations calculated by using the mean concentrations of the sum of T-2 and HT-2 toxins reported for the individual feeds in Appendix B, Table B1.

## C2.3. Rabbits

In a typical French commercial rabbit compound feed, the main ingredients were sunflower meal (20 %), dried lucerne (19.1 %), wheat/maize bran (18.3 %), barley (17.6 %), sugar beet pulp (11.9 %)[35] and beans (10.4%)[35] (T. Gidenne, 2011, personal communication).

## C2.4. Fish

A wide range of diets is used for commercially farmed fish in Europe. However, the salmon feed composition described in Table C7 has been used as being representative of commercial feed producers (Berntssen et al., 2010). The estimated example feed composition in the diet for farmed fish is presented in Table C7 together with the calculated mean LB and UB concentrations of the sum of T-2 and HT-2 toxins.

**Table C7:** Estimated example feed composition in the diet for growing salmon (Berntssen et al., 2010) and the calculated mean lower-bound and upper-bound levels of the sum of T-2 and HT-2 toxins in this diet.

| Feeds | % |
|---|---|
| Fishmeal[(a)] | 30.5 |
| Wheat | 13.2 |
| Soybean meal | 12.3 |
| Maize gluten feed | 11.5 |
| Fish and vegetable oils[(a)] | 31.9 |
| Minerals, vitamins etc[(a)] | 0.6 |
| **Sum of T-2 and HT-2 toxins[(b)]** | |
| Lower-bound (µg/kg) | 5 |
| Upper-bound (µg/kg) | 9 |

(a): No data on T-2 and HT-2 toxin concentrations available, and therefore the contribution to the sum of T-2 and HT-2 toxin is assumed to be zero; (b): Concentrations calculated by using the mean concentrations of the sum of T-2 and HT-2 toxins reported for the individual feeds in Appendix B, Table B1.

## C2.5. Companion animals

### C2.5.1. Dogs and cats

A 'typical' ration for dogs and cats is difficult to define, since it depends on many factors such as the type of breed, the energy requirements for their physiological status, etc. Pet foods are formulated to meet these specific requirements and therefore different products will be used for different requirements. This inevitably results in a range of daily rations covering different conditions (G. Simone, 2011, personal communication). In addition, many pet food manufacturers produce food of different quality (e.g. premium and standard), which may reflect different proportions of cereals and non-cereal ingredients.

For the purpose of this Scientific Opinion, the CONTAM Panel have estimated daily intake using data compiled from 6 and 7 different brands for dog and cat food, respectively, available on the French market (obtained from pet food stores and veterinary clinics) (J-M Fremy, 2011, personal

[35]No data on T-2 and HT-2 toxin concentrations available, and therefore the contribution to the sum of T-2 and HT-2 toxin is assumed to be zero.

communication). In these samples, the cereals used were wheat, maize, barley, rice and maize gluten feed. The amounts of cereals in the premium and standard quality dog food were 45 % and 65 %, respectively, and in cat foods 40 % in premium quality and 55 % in the standard quality (B.M. Paragon, 2011, personal communication). The exact proportions of cereals used are not stated, but for the purposes of this Scientific Opinion it has been assumed that they are included in equal proportions of wheat, barley, maize and maize gluten feed (no data on T-2 toxin or HT-2 toxin concentrations in rice were available), and that cereals represent 55 % of the food. Based on these assumptions, the LB and UB concentrations for the sum of T-2 and HT-2 toxins in dog and cat food are 15 and 25 µg/kg, respectively.

### C2.5.2. Horses

The following estimated feed composition of a blend of non-forage feeds has been used to calculate the mean LB and UB levels of the sum of T-2 and HT-2 toxins in a horse diet (Table C8). The calculated mean UB and LB concentrations of the sum of T-2 and HT-2 toxins in this diet are also presented in Table C8.

**Table C8:** The estimated example diet composition of a blend of non-forage feeds for horses, and the calculated lower bound and upper bound levels of the sum of T-2 and HT-2 toxins in this diet.

| **Feeds** | **%** |
|---|---|
| Oats | 40 |
| Beans[(a)] | 10 |
| Wheat feed | 30 |
| Oat feed[(b)] | 12 |
| Molasses[(a)] | 5 |
| Minerals, vitamins etc[(a)] | 3 |
| **Sum of T-2 and HT-2 toxins[(c)]** | |
| Lower-bound (µg/kg) | 111 |
| Upper-bound (µg/kg) | 120 |

(a): No data on T-2 and HT-2 toxin concentrations available, and therefore the contribution to the sum of T-2 and HT-2 toxin is assumed to be zero; (b): Product obtained during the processing of screened, dehusked oats into oat groats and flour. It consists principally of oat bran and some endosperm;[34] (c): Concentrations calculated by using the mean concentrations of the sum of T-2 and HT-2 toxins reported for the individual feeds in Appendix B, Table B1.

## D. SUMMARY STATISTICS OF THE CHRONIC DIETARY EXPOSURE TO THE INDIVIDUAL T-2 AND HT-2 TOXINS AND THE DETAILED MEAN AND 95TH PERCENTILE CHRONIC DIETARY EXPOSURES TO THE SUM OF T-2 AND HT-2 TOXINS, T-2 TOXIN AND HT-2 TOXIN

Summary statistics of the chronic dietary exposure to the individual T-2 and HT-2 toxins (ng/kg b.w. per day) for total population across the dietary surveys are presented in Tables D1 and D2). Detailed mean and 95th percentile chronic dietary exposure estimates for the sum of T-2 and HT-2 toxins, T-2 toxin and HT-2 toxin (ng/kg b.w. per day) for total population in upper-bound (UB) and lower-bound (LB) for each dietary surveys are presented in Tables D3-D8.

**Table D1:** Summary statistics of the chronic dietary exposure to T-2 toxin (ng/kg b.w. per day) for total population across the dietary surveys.

| Age class | Summary statistics of exposure (ng/kg b.w. per day) | | | | | |
|---|---|---|---|---|---|---|
| | Minimum | | Median | | Maximum | |
| | LB | UB | LB | UB | LB | UB |
| **Mean dietary exposure in total population** | | | | | | |
| Infants | 2.0 | 6.0 | -(a) | -(a) | 2.1 | 11 |
| Toddlers | 4.6 | 16 | 5.7 | 23 | 7.4 | 34 |
| Other children | 4.2 | 14 | 4.9 | 22 | 5.9 | 33 |
| Adolescents | 2.1 | 10 | 2.8 | 15 | 3.3 | 17 |
| Adults | 0.9 | 5.1 | 1.5 | 10 | 2.1 | 11 |
| Elderly | 0.8 | 5.1 | 1.1 | 8.2 | 2.0 | 10 |
| Very elderly | 0.8 | 6.8 | 1.0 | 8.4 | 2.1 | 11 |
| **95th percentile dietary exposure in total population(b)** | | | | | | |
| Infants | 7.4 | -(c) | -(c) | -(c) | -(c) | 39 |
| Toddlers | 10 | 34 | 14 | 37 | 17 | 56 |
| Other children | 8.8 | 24 | 12 | 38 | 16 | 64 |
| Adolescents | 5.1 | 22 | 7.1 | 29 | 9.9 | 34 |
| Adults | 2.2 | 10 | 3.9 | 18 | 5.7 | 21 |
| Elderly | 2.0 | 10 | 2.5 | 16 | 5.2 | 19 |
| Very elderly | 1.8 | 14 | 1.9 | 15 | 5.3 | 21 |

b.w.: body weight; LB: lower-bound; UB: upper-bound.
(a): Not calculated; estimates available only from two dietary surveys; (b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table; (c): Not calculated; estimates available only from one dietary survey.

**Table D2:** Summary statistics of the chronic dietary exposure to HT-2 toxin (ng/kg b.w. per day) for total population across the dietary surveys.

| Age class | Summary statistics of exposure (ng/kg b.w. per day) | | | | | |
|---|---|---|---|---|---|---|
| | Minimum | | Median | | Maximum | |
| | LB | UB | LB | UB | LB | UB |
| **Mean dietary exposure in total population** | | | | | | |
| Infants | 3.8 | 10 | -[(a)] | -[(a)] | 5.1 | 15 |
| Toddlers | 7.8 | 29 | 11 | 35 | 21 | 43 |
| Other children | 6.6 | 22 | 8.0 | 29 | 11 | 41 |
| Adolescents | 2.6 | 14 | 4.7 | 19 | 5.6 | 23 |
| Adults | 1.8 | 8 | 2.5 | 12 | 3.8 | 14 |
| Elderly | 1.6 | 8.8 | 2.0 | 10 | 3.6 | 13 |
| Very elderly | 1.4 | 9.2 | 2.1 | 10 | 2.4 | 13 |
| **95$^{th}$ percentile dietary exposure in total population[(b)]** | | | | | | |
| Infants | 14 | -[(c)] | -[(c)] | -[(c)] | -[(c)] | 49 |
| Toddlers | 16 | 50 | 27 | 61 | 48 | 69 |
| Other children | 14 | 40 | 20 | 51 | 30 | 76 |
| Adolescents | 7.1 | 29 | 11 | 36 | 16 | 42 |
| Adults | 3.8 | 17 | 6.3 | 23 | 9.5 | 26 |
| Elderly | 3.2 | 15 | 4.9 | 19 | 9.0 | 22 |
| Very elderly | 3.0 | 18 | 3.9 | 19 | 6.9 | 24 |

b.w.: body weight; LB: lower-bound; UB: upper-bound.
(a): Not calculated; estimates available only from two dietary surveys; (b): The 95$^{th}$ percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table; (c): Not calculated; estimates available only from one dietary survey.

**Table D3:** Mean and 95th percentile (P95) chronic dietary exposure to the sum of T-2 and HT-2 toxins (ng/kg b.w. per day) for total population in lower-bound (LB) scenario for each dietary survey.

| Dietary survey[a] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P95 |
| BE/1 | | | | | | | 8.5 | 23 | 5.9 | 16 | 3.8 | 9.9 | 3.3 | 8.7 |
| BE/2 | | | 16 | 53[b] | 16 | 35 | | | | | | | | |
| BG | 5.9 | 19 | 13 | 27 | 11 | 22 | | | | | | | | |
| CY | | | | | | | 9.2 | 20 | | | | | | |
| CZ | | | | | 12 | 26 | 8.3 | 19 | 8.3 | 25 | | | | |
| DK | | | | | 16 | 34 | 8.9 | 20 | 6.5 | 15 | 5.7 | 12. | 6.4 | 14[b] |
| FI/1 | | | 28 | 65 | 16 | 34 | | | | | | | | |
| FI/2 | | | | | | | | | 5.6 | 14 | 5.8 | 13 | | |
| FI/3 | | | | | 11 | 21 | | | | | | | | |
| FR | | | | | 14 | 32 | 7.9 | 20 | 4.3 | 11 | 3.3 | 7.9 | 2.8 | 5.3 |
| DE/1 | | | 15 | 32 | 16 | 33 | | | | | | | | |
| DE/2 | | | 16 | 34 | 16 | 36 | | | | | | | | |
| DE/3 | | | 16 | 34 | 15 | 32 | | | | | | | | |
| DE/4 | | | | | | | 6.9 | 18 | 6.1 | 16 | 5.7 | 14 | 5.2 | 12 |
| GR | | | | | 14 | 31 | | | | | | | | |
| HU | | | | | | | | | 4.1 | 9.1 | 3.6 | 6.7 | 3.8 | 6.5 |
| IE | | | | | | | | | 9 | 22 | | | | |
| IT | 6.2 | 24[b] | 17 | 33[b] | | | | | 5 | 9.6 | 4.2 | 7.5 | 4.1 | 7 |
| LV | | | | | 15 | 44 | 8.3 | 25 | 5.1 | 14 | | | | |
| NL/1 | | | | | | | | | 6.1 | 19 | | | | |
| NL/2 | | | 12 | 23 | 11 | 21 | | | | | | | | |
| ES/1 | | | | | | | | | 4.3 | 11 | | | | |
| ES/2 | | | | | | | 4.4 | 12 | 3.4 | 7.2 | | | | |
| ES/3 | | | | | 11 | 22 | 7.3 | 16 | | | | | | |
| ES/4 | | | 18 | 79[b] | 10 | 27 | 5.9 | 15 | | | | | | |
| SE/1 | | | | | | | | | 5.4 | 11 | | | | |
| SE/2 | | | | | 11 | 23 | 7.5 | 15 | | | | | | |
| UK | | | | | | | | | 7.5 | 18 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

**Table D4:** Mean and 95th percentile (P95) chronic dietary exposure to the sum of T-2 and H-2 toxins (ng/kg b.w. per day) for total population in upper-bound (UB) scenario for each dietary survey.

| Dietary survey[(a)] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P9 | Mean | P95 |
| BE/1 | | | | | | | 18 | 37 | 14 | 28 | 11 | 22 | 10 | 19 |
| BE/2 | | | 42 | 73[(b)] | 38 | 68 | | | | | | | | |
| BG | 16 | 51 | 42 | 65 | 39 | 66 | | | | | | | | |
| CY | | | | | | | 22 | 40 | | | | | | |
| CZ | | | | | 32 | 61 | 24 | 47 | 18 | 39 | | | | |
| DK | | | | | 36 | 64 | 21 | 38 | 14 | 26 | 13 | 22 | 13 | 25[(b)] |
| FI/1 | | | 43 | 91 | 32 | 58 | | | | | | | | |
| FI/2 | | | | | | | | | 14 | 26 | 14 | 26 | | |
| FI/3 | | | | | 26 | 45 | | | | | | | | |
| FR | | | | | 30 | 56 | 19 | 37 | 13 | 24 | 12 | 23 | 11 | 17 |
| DE/1 | | | 30 | 48 | 31 | 59 | | | | | | | | |
| DE/2 | | | 33 | 59 | 30 | 54 | | | | | | | | |
| DE/3 | | | 32 | 65 | 31 | 53 | | | | | | | | |
| DE/4 | | | | | | | 14 | 29 | 12 | 24 | 10 | 21 | 10 | 19 |
| GR | | | | | 35 | 67 | | | | | | | | |
| HU | | | | | | | | | 15 | 26 | 13 | 23 | 15 | 25 |
| IE | | | | | | | | | 17 | 32 | | | | |
| IT | 11 | 39[(b | 40 | 86[(b)] | 35 | 71 | 23 | 41 | 16 | 28 | 14 | 25 | 14 | 25 |
| LV | | | | | 27 | 59 | 19 | 42 | 11 | 26 | | | | |
| NL/1 | | | | | | | | | 14 | 29 | | | | |
| NL/2 | | | 30 | 54 | 27 | 44 | | | | | | | | |
| ES/1 | | | | | | | | | 11 | 24 | | | | |
| ES/2 | | | | | | | 13 | 30 | 10 | 20 | | | | |
| ES/3 | | | | | 29 | 51 | 20 | 36 | | | | | | |
| ES/4 | | | 34 | 108[(b | 27 | 56 | 18 | 38 | | | | | | |
| SE/1 | | | | | | | | | 13 | 24 | | | | |
| SE/2 | | | | | 27 | 47 | 19 | 33 | | | | | | |
| UK | | | | | | | | | 15 | 28 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

**Table D5:** Mean and 95th percentile (P95) chronic dietary exposure to T-2 toxin (ng/kg b.w. per day) for total population in lower-bound (LB) scenario for each dietary survey.

| Dietary survey[a] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P9 | Mean | P95 |
| BE/1 | | | | | | | 2.8 | 7.4 | 1.6 | 4.5 | 0.90 | 2.5 | 0.8 | 2.2 |
| BE/2 | | | 5.0 | 15[b] | 5.2 | 13 | | | | | | | | |
| BG | 2.1 | 7.4 | 5.9 | 14 | 5.1 | 14 | | | | | | | | |
| CY | | | | | | | 3.0 | 7.0 | | | | | | |
| CZ | | | | | 4.2 | 12 | 3.0 | 7.1 | 1.5 | 4.4 | | | | |
| DK | | | | | 4.3 | 8.8 | 2.5 | 5.8 | 1.2 | 3.0 | 0.80 | 2.0 | 1.0 | 2.7[b] |
| FI/1 | | | 7.4 | 17 | 4.7 | 10 | | | | | | | | |
| FI/2 | | | | | | | | | 1.4 | 3.3 | 1.5 | 3.5 | | |
| FI/3 | | | | | 4.9 | 9.4 | | | | | | | | |
| FR | | | | | 5.4 | 12 | 3.0 | 7.3 | 1.6 | 3.9 | 1.1 | 2.5 | 0.80 | 1.8 |
| DE/1 | | | 5.6 | 12 | 5.9 | 13 | | | | | | | | |
| DE/2 | | | 5.8 | 14 | 5.8 | 14 | | | | | | | | |
| DE/3 | | | 5.7 | 13 | 5.8 | 12 | | | | | | | | |
| DE/4 | | | | | | | 2.7 | 7.7 | 2.1 | 5.7 | 2.0 | 5.2 | 2.1 | 5.3 |
| GR | | | | | 5.3 | 12 | | | | | | | | |
| HU | | | | | | | | | 0.9 | 2.2 | 0.90 | 2.0 | 0.90 | 1.9 |
| IE | | | | | | | | | 1.7 | 4.0 | | | | |
| IT | 2.0 | 6.2[b] | 5.5 | 11[b] | 4.2 | 9.0 | 2.4 | 5.1 | 1.4 | 2.8 | 1.1 | 2.4 | 1.1 | 1.9 |
| LV | | | | | 5.1 | 16 | 3.3 | 9.9 | 1.7 | 5.2 | | | | |
| NL/1 | | | | | | | | | 1.9 | 5.3 | | | | |
| NL/2 | | | 4.6 | 10 | 4.4 | 9.9 | | | | | | | | |
| ES/1 | | | | | | | | | 1.2 | 3.3 | | | | |
| ES/2 | | | | | | | 2.1 | 7.9 | 1.2 | 3.3 | | | | |
| ES/3 | | | | | 4.4 | 9.6 | 2.8 | 7.0 | | | | | | |
| ES/4 | | | 7.0 | 39[b] | 4.2 | 12 | 2.4 | 7.0 | | | | | | |
| SE/1 | | | | | | | | | 1.5 | 3.3 | | | | |
| SE/2 | | | | | 4.4 | 9.4 | 2.8 | 6.6 | | | | | | |
| UK | | | | | | | | | 1.9 | 4.5 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

**Table D6:** Mean and 95th percentile (P95) chronic dietary exposure to T-2 toxin (ng/kg b.w. per day) for total population in upper-bound (UB) scenario for each dietary survey.

| Dietary survey[a] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P9 | Mean | P95 |
| BE/1 | | | | | | | 13 | 25 | 10 | 20 | 8.2 | 16 | 8.1 | 15 |
| BE/2 | | | 32 | 60[b] | 28 | 48 | | | | | | | | |
| BG | 11 | 39 | 34 | 56 | 33 | 63 | | | | | | | | |
| CY | | | | | | | 17 | 33 | | | | | | |
| CZ | | | | | 22 | 42 | 17 | 34 | 10 | 20 | | | | |
| DK | | | | | 22 | 35 | 13 | 23 | 8.0 | 14 | 6.7 | 12 | 6.8 | 14[b] |
| FI/1 | | | 16 | 34 | 14 | 24 | | | | | | | | |
| FI/2 | | | | | | | | | 5.1 | 10 | 5.1 | 10 | | |
| FI/3 | | | | | 23 | 44 | | | | | | | | |
| FR | | | | | 22 | 38 | 14 | 26 | 10 | 19 | 9.4 | 18 | 8.7 | 15 |
| DE/1 | | | 19 | 36 | 20 | 34 | | | | | | | | |
| DE/2 | | | 20 | 38 | 19 | 34 | | | | | | | | |
| DE/3 | | | 19 | 36 | 19 | 31 | | | | | | | | |
| DE/4 | | | | | | | 10 | 22 | 8 | 17 | 7.0 | 14 | 7.2 | 16 |
| GR | | | | | 31 | 64 | | | | | | | | |
| HU | | | | | | | | | 10 | 18 | 9.1 | 16 | 10 | 18 |
| IE | | | | | | | | | 10 | 18 | | | | |
| IT | 6.0 | 24[b] | 28 | 57[b] | 26 | 50 | 17 | 31 | 11 | 20 | 10 | 19 | 11 | 21 |
| LV | | | | | 18 | 37 | 15 | 31 | 8.5 | 21 | | | | |
| NL/1 | | | | | | | | | 10 | 19 | | | | |
| NL/2 | | | 23 | 41 | 20 | 37 | | | | | | | | |
| ES/1 | | | | | | | | | 8.4 | 18 | | | | |
| ES/2 | | | | | | | 11 | 28 | 8.3 | 17 | | | | |
| ES/3 | | | | | 23 | 38 | 16 | 28 | | | | | | |
| ES/4 | | | 23 | 72[b] | 22 | 40 | 16 | 33 | | | | | | |
| SE/1 | | | | | | | | | 10 | 19 | | | | |
| SE/2 | | | | | 22 | 42 | 16 | 29 | | | | | | |
| UK | | | | | | | | | 9 | 16 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

**Table D7:** Mean and 95th percentile (P95) chronic dietary exposure to HT-2 toxin (ng/kg b.w. per day) for total population in lower-bound (LB) scenario for each dietary survey.

| Dietary survey[a] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P9 | Mean | P95 |
| BE/1 | | | | | | | 5.4 | 15 | 3.0 | 8.2 | 1.6 | 4.8 | 1.5 | 4.1 |
| BE/2 | | | 11 | 38[b] | 11 | 25 | | | | | | | | |
| BG | 3.8 | 14 | 8.6 | 19 | 7.7 | 17 | | | | | | | | |
| CY | | | | | | | 5.2 | 12 | | | | | | |
| CZ | | | | | 7.2 | 20 | 5.0 | 13 | 2.4 | 5.8 | | | | |
| DK | | | | | 10 | 24 | 5.2 | 13 | 2.6 | 7.3 | 2.0 | 5.7 | 2.3 | 7.2[b] |
| FI/1 | | | 21 | 48 | 11 | 24 | | | | | | | | |
| FI/2 | | | | | | | | | 2.9 | 7.7 | 3.6 | 9.0 | | |
| FI/3 | | | | | 6.6 | 14 | | | | | | | | |
| FR | | | | | 9.8 | 24 | 5.6 | 15 | 2.6 | 6.6 | 1.8 | 4.9 | 1.4 | 3.0 |
| DE/1 | | | 10 | 28 | 10 | 24 | | | | | | | | |
| DE/2 | | | 11 | 29 | 10 | 25 | | | | | | | | |
| DE/3 | | | 11 | 26 | 9.8 | 23 | | | | | | | | |
| DE/4 | | | | | | | 3.9 | 10 | 2.5 | 6.9 | 2.3 | 6.2 | 2.4 | 6.9 |
| GR | | | | | 7.7 | 20 | | | | | | | | |
| HU | | | | | | | | | 1.8 | 3.8 | 1.6 | 3.2 | 1.9 | 3.4 |
| IE | | | | | | | | | 3.8 | 9.4 | | | | |
| IT | 5.1 | 17[b] | 11 | 23[b] | 8.0 | 16 | 4.5 | 8.3 | 2.6 | 5.2 | 2.3 | 4.2 | 2.2 | 3.9 |
| LV | | | | | 9.2 | 30 | 4.8 | 16 | 2.3 | 6.3 | | | | |
| NL/1 | | | | | | | | | 2.4 | 5.7 | | | | |
| NL/2 | | | 7.8 | 16 | 7.0 | 15 | | | | | | | | |
| ES/1 | | | | | | | | | 2.1 | 5.8 | | | | |
| ES/2 | | | | | | | 2.6 | 7.1 | 1.8 | 4.4 | | | | |
| ES/3 | | | | | 7.2 | 16 | 4.5 | 10 | | | | | | |
| ES/4 | | | 14 | 68[b] | 7.0 | 20 | 3.8 | 10 | | | | | | |
| SE/1 | | | | | | | | | 2.2 | 5.4 | | | | |
| SE/2 | | | | | 7.3 | 16 | 4.4 | 10 | | | | | | |
| UK | | | | | | | | | 3.6 | 9.5 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

**Table D8:** Mean and 95th percentile (P95) chronic dietary exposure to HT-2 toxin (ng/kg b.w. per day) for total population in upper-bound (UB) scenario for each dietary survey.

| Dietary survey[a] | Infants | | Toddlers | | Other children | | Adolescents | | Adults | | Elderly | | Very elderly | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | P95 | Mean | P95 | Mean | P9 | Mean | P95 | Mean | P9 | Mean | P9 | Mean | P95 |
| BE/1 | | | | | | | 18 | 34 | 13 | 26 | 10 | 20 | 10 | 18 |
| BE/2 | | | 43 | 82[b] | 38 | 64 | | | | | | | | |
| BG | 15 | 49 | 42 | 69 | 41 | 74 | | | | | | | | |
| CY | | | | | | | 23 | 42 | | | | | | |
| CZ | | | | | 28 | 56 | 22 | 42 | 12 | 25 | | | | |
| DK | | | | | 30 | 52 | 17 | 32 | 11 | 19 | 8.8 | 15 | 9.2 | 18[b] |
| FI/1 | | | 31 | 67 | 22 | 40 | | | | | | | | |
| FI/2 | | | | | | | | | 8.4 | 17 | 8.9 | 17 | | |
| FI/3 | | | | | 28 | 51 | | | | | | | | |
| FR | | | | | 29 | 51 | 18 | 35 | 12 | 23 | 11 | 22 | 11 | 19 |
| DE/1 | | | 29 | 50 | 29 | 50 | | | | | | | | |
| DE/2 | | | 31 | 63 | 29 | 49 | | | | | | | | |
| DE/3 | | | 29 | 55 | 29 | 48 | | | | | | | | |
| DE/4 | | | | | | | 14 | 29 | 10 | 21 | 9.1 | 18 | 9.4 | 19 |
| GR | | | | | 41 | 76 | | | | | | | | |
| HU | | | | | | | | | 12 | 22 | 11 | 19 | 13 | 22 |
| IE | | | | | | | | | 13 | 24 | | | | |
| IT | 10 | 40[b | 38 | 75[b] | 34 | 64 | 21 | 39 | 14 | 25 | 13 | 22 | 13 | 24 |
| LV | | | | | 25 | 54 | 18 | 41 | 11 | 25 | | | | |
| NL/1 | | | | | | | | | 13 | 23 | | | | |
| NL/2 | | | 35 | 60 | 31 | 51 | | | | | | | | |
| ES/1 | | | | | | | | | 10 | 21 | | | | |
| ES/2 | | | | | | | 14 | 33 | 10 | 20 | | | | |
| ES/3 | | | | | 30 | 49 | 20 | 37 | | | | | | |
| ES/4 | | | 35 | 120[b | 29 | 55 | 20 | 40 | | | | | | |
| SE/1 | | | | | | | | | 12 | 22 | | | | |
| SE/2 | | | | | 27 | 50 | 19 | 34 | | | | | | |
| UK | | | | | | | | | 12 | 23 | | | | |

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; P95: 95th percentile.

(a): Original acronyms of the dietary surveys and the number of subjects is given in Table 12; (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

## E. DOSE-RESPONSE MODELLING

The details of the benchmark dose (BMD) analysis for the risk characterisation of T-2 and HT-2 toxins are presented in this Appendix. The BMD approach was applied to the dose-response data of the study of Rafai et al. (1995a,b) and tentatively to the concentration-response data of the study of Meissonnier et al. (2008a).

Rafai et al. (1995a,b) reported dose-response data for three time points (7, 14 and 21 days) for a control and four dose groups of 0.5, 1.0, 2.0 and 3.0 mg/kg of purified T-2 toxin (90 % purity) in feed to 9-10 pigs per group (see Section 7.4.2.). The average daily intakes of toxin by the pigs and their exposures were 0.38, 0.81, 1.24 and 1.43 mg, and 29, 62, 105 and 129 µg/kg b.w. per day, respectively.

The Panel on Contaminants in the Food Chain (CONTAM Panel) identified the specific antibody response, anti-horse globulin available as A-HG titre(log2) values observed at day 21 as the critical effect for a BMD analysis, see Table 2 in Rafai et al. (1995b). This choice is supported by the respective dose-response of anti-ovalbumin reported by Meissonnier et al. (2008a) for 5 time points (1, 7, 14, 21 and 28 days) for a control and three dose groups of 0.54, 1.32, and 2.10 mg/kg of highly purified T-2 toxin (> 98 % purity) (see Section 7.4.2.).[36] Information on average daily intakes of T-2 toxin by the pigs and the intake doses in µg/kg bodyweight were however not available in the study of Meissonnier et al. (2008a), and therefore dose-response analysis directly comparable to that for the Rafai et al. (1995a,b) study was not possible.

In the absence of statistical or toxicological considerations supporting deviation from the default value proposed by EFSA (2009), the CONTAM Panel chose the benchmark response (BMR) of 5 % when applying the BMD approach on the dose- (antibody) response data available as anti-horse globulin titre values of the study of Rafai et al. (1995a,b). For reasons of comparison the CONTAM Panel also calculated the BMD, the 95 % lower confidence limit for the benchmark dose response (BMDL) and the 95 % upper confidence limit for the benchmark dose response (BMDU) values also for a BMR of 10 % (not reported in Tables below).

The BMD analysis was based on means and standard deviations available from the study of Rafai et al. (1995a,b), and on means and standard deviations calculated for the available data for day 21 of Meissonnier et al., (2008a). The PROAST software (version 26.0 under R 2.10.2) was used, following advice given in EFSA (2011).

Tables E1 and E2 present the results of fitting the two nested families of the Exponential (E) and the Hill models (E) implemented in PROAST to the data of Rafai et al. (1995a,b) and Meissonnier et al. (2008a), respectively. Model E1 denotes the reduced model for both families. Whereas the response described by the Exponential models E2 and E3, and Hill models H2 and H3, tends to zero with increasing doses, it is allowed to tend to non-zero values in the Exponential models E4 and E5, and Hill models H4 and H5, thus allowing an additional model parameter describing such a positive response at high doses. Therefore, the numbers of model parameters is 2, 3, 3 and 4 for the models E2, E3, E4, and E5, and H2, H3, H4, and H5, respectively (not reported in Tables E1 and E2). At first, the models of the two nested families were fitted and the best fitting models were identified using the implemented algorithms of the software. In a second analysis also each single model was fitted and the outcomes reported. Not all models fitted well and in a number of models no BMDL could be calculated (indicated as not available (n.a.) in Tables E1 and E2). Usually this would indicate that the BMDL is very low and the model fit would be therefore not acceptable due to too wide confidence intervals of the BMD. In some cases no convergence of the fitting algorithm or unstable outcomes were observed (indicated as not calculated (n.c.) because of non-convergence in Tables E1 and E2).

[36] The individual anti-ovalbumin data on the day 21 were provided for the CONTAM Panel by I. Oswald, the principal investigator of the study of Meissonnier et al. (2008a).

Tables E1 and E2 present for both studies analysed the specific models fitted, the log-likelihood values, the characterisation of the model fit, the $BMD_{05}$ values, and the $BMDL_{05}$ and $BMDU_{05}$ for a BMR of 5 %. Since the BMDL is the lower 95 % confidence bound of the BMD and the BMDU is the upper 95 % confidence bound of the BMD, the interval BMDL-BMDU represents the 90 % confidence interval of the BMD. This provides a descriptive measure of the accuracy of the BMD, which for an acceptable BMDL value should not be larger than one order of magnitude, i.e. the BMD/BMDL or the BMDU/BMDL ratio should not be considerably larger than about 5 or 10, respectively. The best fitting models when using the algorithm implemented in PROAST software for determining the optimal model in the nested family are identified in Table E1 (see also Figures E1 and E2).

**Table E1:** The benchmark dose (BMD), the 95 % benchmark dose lower confidence limit (BMDL) and 95 % benchmark dose upper confidence limit (BMDU) values for anti-horse globulin (A-HG) titre values (on log2 base) of Rafai et al. (1995a,b) calculated for benchmark response (BMR) of 5 % in units of µg/kg b.w. per day.

| Model | Log-likelihood | Model fit[a] | $BMD_{05}$ | 90 % CI, two-sided[b] | |
|---|---|---|---|---|---|
| | | | | $BMDL_{05}$ µg/kg b.w. per day | $BMDU_{05}$ µg/kg b.w. per day |
| Full | 14.23 | | | | |
| Reduced (null) | -0.14 | | | | |
| **Exponential nested family** | | | | | |
| E2 | 8.89 | not selected | 18.1 | 13.4 | 28.2. |
| E3 | 11.09 | selected (p=0.04) | 0.08 | n.c. | n.c. |
| E4 | 9.92 | selected (p=0.01) | 1.77 | n.a. | 21.8 |
| E5 | 11.09 | not selected | 0.08 | n.a. | 9.3 |
| **Hill nested family** | | | | | |
| H2 | 9.14 | selected (p=0.02) | 15.0 | 10.3 | 24.7 |
| H3 | 11.05 | not selected | n.c. | n.c. | n.c. |
| H4 | 10.55 | not selected | 2.4 | n.a. | 14.9 |
| H5 | 11.05 | not selected | 0.12 | n.a. | 9.9 |

E: Exponential model; H: Hill model; CI: confidence interval; BMD: benchmark dose; BMDL: the 95 % benchmark dose lower confidence limit; BMDU: 95% benchmark dose upper confidence limit; b.w.: body weight; p: p-value.
(a): p-value of the comparison with the full model; (b): BMDL and BMDU are one-sided confidence bounds at the level of 0.95; n.a.: not available. BMDL could not be calculated by PROAST. This indicates that BMDL is near to 0.0 and thus the model is not acceptable based on EFSA (2009) due to too wide CI; n.c.: not calculated because of non-convergence of fitting algorithm or unstable outcome.

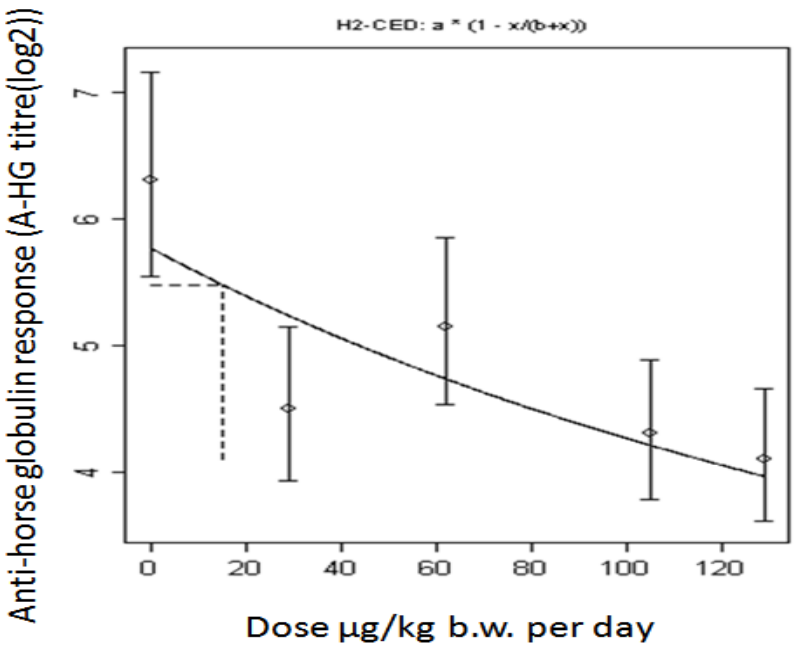


**Figure E1:** Fitted Hill family model H2 to the Rafai et al. (1995a,b) data on anti-horseglobulin response (A-HG titre(log2)) with $BMDL_{05}$. The dotted vertical line indicates the $BMDL_{05}$.

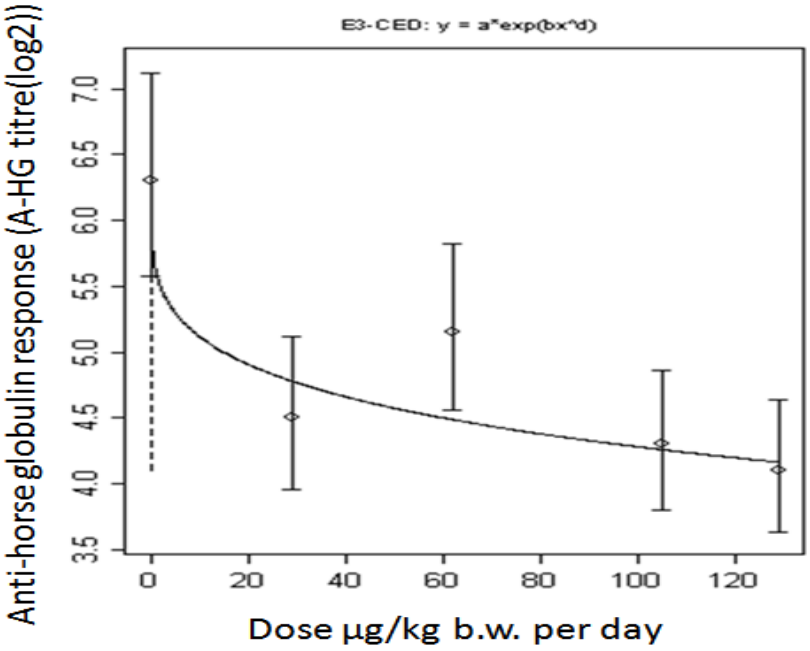


**Figure E2:** Fitted Exponential family model E3 to the Rafai et al. (1995a,b) data on anti-horseglobulin response (A-HG titre(log2)) with $BMDL_{05}$. The dotted vertical line indicates the $BMDL_{05}$.

The CONTAM Panel noted that model fitting to the data was difficult because of some non-monotonicity in the observed means of the data where the drop from the control at the lowest dose was

not seen at the next dose level. The fit of the models of the Exponential family was insufficient since either a model was not selected or the $BMDL_{05}$ of a selected model was not acceptable. In contrast, among the Hill family the model H2 was selected as the best model among the four models and provided a fit from which a $BMD_{05}$ = 15.0 and an acceptable $BMDL_{05}$ = 10.3 µg/kg b.w. per day could be calculated. Although it was noted that the outcome of the BMD analysis shows more modeling uncertainty than usually expected when applying the BMD approach to dose-response data, the result of the model H2 seems to be the best available description of these data (see Figure E1). The results of this model are supported by the outcome of model E2 for which both BDML and BMDU could be calculated. Increasing the BMR to 10 % would lead to $BMD_{10}$ = 31.6 and BMDL = 21.7 µg/kg b.w. per day, respectively, which is not surprising given that the fitting model H2 has a rather linear shape in the range of the experimental doses.

Although no intake data were reported in the study of Meissonnier et al. (2008a), the CONTAM Panel tentatively evaluated the concentration-response values available for anti-ovalbumin using the BMD approach, in the same way as for the data of Rafai et al. (1995a,b). A conversion factor of 53 derived from the feed intake data reported by Rafai et al. (1995a,b) was applied to approximate the intake dose for the T-2 and HT-2 toxin concentrations reported for feed by Meissonnier et al. (2008a). Overall, a larger variation of the BMD values (ranging between 6 and 29 µg/kg b.w. per day) and of BMDL values (ranging between 1 and 5 µg/kg b.w. per day) was observed and the BMDU/BMDL ratio exceeded a factor of 10 for all four models where both limits could be calculated (Table E2). Referring to this large modeling uncertainty, and the fact that the feed intake data were not available and could only be estimated based on the Rafai et al. (1995a,b) study, the CONTAM Panel noted that these data cannot be interpreted appropriately. However, they support the presence of antigen response in the region of the BMDL calculated from the Rafai et al. (1995a,b) data. Although not considered for the benchmark dose calculations, the data on the other endpoints studied by Rafai et al. (1995 a,b) (e.g. lymphocyte stimulation tests for anti-horse globulin, phytohaemagglutinin and concanavalin A), clearly supported the existence of dose-response effects at day 21 and were apparently supportive of the $BMDL_{05}$ of 10 µg/kg b.w. per day used by the CONTAM Panel for the risk characterisation of T-2 and HT-2 toxins.

**Table E2:** The benchmark dose (BMD), the 95 % benchmark dose lower confidence limit (BMDL) and 95 % benchmark dose upper confidence limit (BMDU) values for anti-ovalbumin response of Meissonnier et al. (2008a) calculated for benchmark response (BMR) of 5 % in units of µg/kg b.w. per day.

| Model | Log-likelihood | Model fit[a] | $BMD_{05}$ | 90 % CI, two-sided[b] | |
|---|---|---|---|---|---|
| | | | | $BMDL_{05}$ µg/kg b.w. per day[c] | $BMDU_{05}$ µg/kg b.w. per day[c] |
| Full | 13.20 | | | | |
| Reduced (null) | 17.91 | | | | |
| **Exponential nested family** | | | | | |
| E2 | 15.98 | selected (p>0.05) | 9.0 | 5.0 | 53.5 |
| E3 | 15.72 | not selected | n.c. | n.c. | n.c. |
| E4 | 15.33 | not selected | n.a. | n.a. | 22.3 |
| E5 | 14.19 | not selected | 28.1 | 1.4 | 68.9 |
| **Hill nested family** | | | | | |
| H2 | 15.73 | selected (p>0.05) | 5.8 | 2.5 | 33.9 |
| H3 | 15.66 | not selected | 2.1 | n.a. | 48.2 |
| H4 | 15.52 | not selected | 3.2 | n.a. | 26.0 |
| H5 | 14.19 | not selected | 28.6 | 2.4 | 64.1 |

E: Exponential model; H: Hill model; CI: confidence interval; BMD: benchmark dose; BMDL: the 95 % benchmark dose lower confidence limit; BMDU: 95% benchmark dose upper confidence limit; b.w.: body weight; p: p-value.
(a): p-value of the comparison with the full model; (b): BMDL and BMDU are one-sided confidence bounds at the level of 0.95; (c): The unit µg/kg b.w. per day was converted from mg/kg feed per day by using a conversion factor of 53 derived from the intakes reported in the study of Rafai et al. (1995a,b); n.a.: not available. BMDL could not be calculated by PROAST. This indicates that BMDL is near to 0.0 and thus the model is not acceptable based on EFSA (2009) due to too wide CI; n.c.: not calculated because of non-convergence of fitting algorithm or unstable outcome.

## ABBREVIATIONS

| | |
|---|---|
| 1-AN | 1-anthroylnitrile |
| ACE | Accelerated solvent extraction |
| ACN | Acetonitrile |
| AcOH | Acetic acid |
| AFRC | Agricultural and Food Research Council |
| AFSSA | French Food Safety Authority/Agence Française de Sécurité des Aliments |
| A-HG | Anti-horse globulin |
| ALT | Alanine aminotransferase |
| AOAC | Association of Official Analytical Chemists |
| APC | Antigen-presenting cell |
| APCI | Atmospheric pressure chemical ionisation |
| APPI | Atmospheric pressure photoionisation |
| AST | Aspartate aminotransferarse |
| ATA | Alimentary Toxic Aleukia |
| BAM | Federal Institute for Materials Research and Testing |
| BE | Belgium |
| BG | Bulgaria |
| BMD | Benchmark dose |
| BMDL | The 95 % benchmark dose lower confidence limit |
| BMDU | The 95 % benchmark dose upper confidence limit |
| BMR | Benchmark response |
| b.w. | Body weight |
| CAS | Chemical Abstracts Service |
| CCR7 | Chemokine receptor-7 |
| c.e. | Culture extract |
| CEEREAL | European Breakfast Cereal Association |
| $(CH_3)_2CO$ | Acetone |
| CD | Cytotoxicity dose |
| CD86 | Cluster of differentiation 86 |
| CK | Creatine kinase |
| CI | Confidence interval |
| c.m. | Culture material |
| ConA | Concanavalin A |
| CONTAM Panel | Panel on Contaminants in the Food Chain |
| CRM | Certified reference materials |
| CY | Cyprus |
| CZ | Czech Republic |
| DAD | Diode-array detector |
| DE | Germany |
| DK | Denmark |
| DM | Dry matter |
| DMSO | Dimethylsulfoxid |
| DON | Deoxynivalenol |
| DTH | Delayed-type hypersensitivity |
| EC | European Commission |
| ECD | Electron capture detection |
| EFSA | European Food Safety Authority |
| EIA | Enzyme immunoassay |
| ELIME-array | Enzyme-Linked-Immunomagnetic-Electrochemical array |
| ELISA | Enzyme-linked immunosorbent assay |
| ES | Spain |
| ESI | Electrospray ionization |

| | |
|---|---|
| EtOAc | Ethyl acetate |
| EU | European Union |
| EVIRA | The Finnish Food Safety Authority |
| EXPOCHI | Article 36 project 'Individual food consumption data and exposure assessment studies for children' |
| FAO/WHO | Food and Agriculture Organization of the United Nations/World Health Organization |
| FEFAC | European Feed Manufacturers Federation |
| FI | Finland |
| FID | Flame ionisation detection |
| FLD | Fluorescence detector |
| FPG | Formamidopyrimidine DNA glycosylase |
| FR | France |
| GC | Gas chromatography |
| GDG | Growth daily gain |
| GEMS/Food | Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme |
| GIT | Gastrointestinal tract |
| GMT | Gamma glutamyltransferase |
| GR | Greece |
| GSH | Glutathione |
| HCOOH | Formic acid |
| HeLa | Human cervix carcinoma cells |
| HepG2 | Human hepatoma cells |
| HFB | Heptafluorobutyrate |
| HF-LPME | Hollow fiber liquid-phase micro extraction |
| HLA-DR | Human leukocyte antigen complex |
| HPLC | High performance liquid chromatography |
| HPLC-FLD | High performance liquid chromatography coupled with fluorescence detection |
| HPTLC | High performance thin layer chromatography |
| HSV-1 | Herpes simplex virus-1 |
| HT-2 | HT-2 toxin |
| HU | Hungary |
| IA | Immunoaffinity |
| IARC | International Agency on Research on Cancer |
| $IC_{50}$ | 50 % inhibitory concentration |
| IE | Ireland |
| IFN- γ | Interferone gamma |
| Ig | Immunoglobulin |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| IGF | Insulin-like growth factor |
| IL-1β | Interleukin-1beta |
| INRA | Institut national de la recherche agronomique, France |
| *i.p.* | Intraperitoneal |
| IT | Italy |
| *i.v.* | Intravenously |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| JRC | Joint Research Centre |
| LB | Lower bound |
| LC | Liquid chromatography |
| LC-MS/MS | Liquid chromatography coupled to tandem mass spectrometry |
| LDH | Lactate dehydrogenase |

| | |
|---|---|
| LOAEL | Lowest-observed-adverse-effect level |
| LOD | Limit of detection |
| LOEL | Lowest-observed- effect level |
| LOQ | Limit of quantification |
| LPS | Lipopolysaccharide |
| LV | Latvia |
| MAPK | Mitogen-activated protein kinase |
| MCH | Haemoglobin amount per red blood cell |
| MCHC | Mean corpuscular haemoglobin concentration |
| MCV | Average red blood cell size |
| MDA | Malondialdehyde |
| MeOH | Methanol |
| MHC | Major histocompatibility complex |
| ML | Maximum level |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| MTT | Agrifood Research Finland |
| n.a. | Not applicable |
| N | Number of samples |
| ND | Not detected |
| NED | No-effect dose |
| NL | The Netherlands |
| NMR | Nuclear magnetic resonance |
| NOAEL | No-observed-adverse-effect level |
| NOEL | No-observed-effect level |
| NRC | National Research Council |
| NRL | National reference laboratory |
| OTA | Ochratoxin A |
| P450 1A | Cytochrome P450 1A |
| PBS | Phosphate buffered saline |
| PCV | Packed cell volume |
| PFPA | Pentafluoropropionic anhydride |
| PGF2$\alpha$ | Prosthaglandin F2 alpha |
| PHA | Phytohaemagglutinin |
| PMA | Phorbol 12-myristate 13-acetate |
| PMTDI | Provisional maximum tolerable daily intake |
| PROAST | PROAST software |
| RL | Reference laboratory |
| QuEChERS | Quick, easy, cheap, effective, rugged and safe |
| ROS | Reactive oxygen species |
| RSD | Relative standard deviation |
| $RSD_r$ | Relative standard deviation under repeatability conditions |
| $RSD_R$ | Relative standard deviation under reproducibility conditions |
| SAPK/JNK | Stress-activated protein kinase |
| *s.c.* | Subcutaneously |
| SCF | Scientific Committee on Food |
| SCOOP | Scientific co-operation |
| SE | Sweden |
| SPE | Solid phase extraction |
| SPR | Surface plasmon resonance |
| T-2 | T-2 toxin |
| TDI | Tolerable daily intake |
| TFAA | Trifluoroacetic acid anhydride |
| TGF | Transforming growth factor |

| | |
|---|---|
| TGF-β 1 | Transforming growth factor-beta1 |
| TLC | Thin layer chromatography |
| TNF-α | Tumor necrosis factor-alpha |
| t-TDI | Temporary tolerable daily intake |
| TUNEL | Terminal deoxynucleotidyl transferase dUTP nick end labeling |
| UB | Upper bound |
| UDP | Uridine 5'-diphospho-glucuronosyltransferase |
| UGT | UDP-Glucuronosyltransferase |
| UHPLC | Ultra high performance-liquid chromatograph |
| UK | United Kingdom |
| USSR | Union of Sovietic Socialist Republics |
| UV | Ultraviolet |
| v/v | volume/volume |