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Assessment of information as regards the toxicity of T-2 and HT-2 toxin for ruminants

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Abstract

In 2011, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of T-2 (T2) and HT-2 (HT2) toxin in food and feed. No observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were derived for different animal species. In ruminants a LOAEL was established for the sum of T2 and HT2 of 0.3 mg/kg body weight (bw) per day, based on studies with calves and lambs. The CONTAM Panel noted that the effects observed in nutritionally challenged heifers and ewes give rise to the assumption that rumen detoxification of T2 may not always be complete and therefore effective to prevent adverse effects in ruminants. However, the limited data on the effects of T2 on adult ruminants did not allow a conclusion. The European Commission requested EFSA to review the information regarding the toxicity of T2 and HT2 for ruminants and to revise, if necessary, the established Reference Point (RP). Adverse effect levels of 0.001 and 0.01 mg T2/kg bw per day for, respectively, sheep and cows, were derived from case studies, estimated to correspond to feed concentrations of 0.035 mg T2/kg for sheep and 0.6 mg T2/kg for cows. RPs for adverse animal health effects of 0.01 mg/kg feed for sheep and 0.2 mg/kg feed for cows were established. For goats, the RP for cows was selected, in the absence of data that they are more sensitive. Based on mean exposure estimates performed in the previous Opinion, the risk of adverse health effects of feeds containing T2 and HT2 was considered a concern for lactating sheep. For milking goats, a comparison performed between dietary exposure and the RP derived for cows, indicates a potential risk for adverse health effects. For dairy cows and fattening beef, the risk is considered low.

Keywords: T-2 toxin, HT-2 toxin, animal health, feed, exposure, toxicity, ruminants

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Background

In 2011, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA established for the sum of T2 and HT2 in ruminants a LOAEL (Lowest Observed Adverse Effect Level) of 0.3 mg/kg bw per day for calves.

Information was more recently provided to the Commission services concluding that the Reference Point for adverse animal health effects for T2 and HT2 in ruminants established by EFSA in the abovementioned Opinion should be lower, based on an assessment of available scientific information.

The Commission has requested EFSA to assess this information to verify if the reference point for adverse animal health effects established for T2 and HT2 in ruminants can be confirmed or needs to be updated.

Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission (EC) asks EFSA to assess the information on the adverse animal health effects for T2 and HT2 in ruminants, taking into account the information submitted to the Commission.

The information on the T2 and HT2 adverse effects on animal health submitted by European Commission is summarized on Table 1 below.

Table 1: Selection of research studies to be (re)assessed, as submitted by the EC

Animal species	Studies to be (re)assessed
Ruminants	Hsu et al., 1972 Huszenicza et al., 2000 Kégl and Ványi, 1991 Pier et al., 1976

1.2. Additional information

1.2.1. Chemistry

T-2 and HT-2 toxin (T2 and HT2) belong to the trichothecenes, the largest group of *Fusarium* toxins. The chemistry of T2 and HT2 has been described previously by EFSA in detail (EFSA Panel, 2011, 2017). Briefly, trichothecenes have a common tetracyclic ring system and are divided into four groups (A–D) according to their chemical functionalities. T2 and HT2 belong to group A, characterised by an ester function at the C8 position. They are both soluble in most organic solvents but have very poor solubility in water, due to their low polarity.

1.2.2. Previous animal health risk assessments

In 2011, EFSA published a Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxins in food and feed (EFSA CONTAM Panel, 2011). No observed adverse effect levels (NOAELs) and LOAELs were derived for different animal species. Pigs were identified as the most sensitive species, with the main endpoints being immunological or haematological effects. It was concluded that in calves and lambs, exposure to 0.3 mg T2/kg bw per day or more resulted in gastrointestinal lesions, altered serum proteins and haematological alterations. Therefore, 0.3 mg T2/kg bw per day was considered as a LOAEL based on the available data. However, a NOAEL in young ruminants could not be identified and investigations using practically relevant concentrations of T2 were missing. The CONTAM Panel noted that the effects observed in nutritionally challenged heifers and ewes give rise to the assumption that rumen detoxification of T2 may not always be complete and therefore effective to prevent adverse effects in ruminants. However, the limited data on the effects of T2 on adult ruminants did not allow a conclusion.

1.3. Legislation

Directive 2002/32/EC[1] on undesirable substances in animal feed, aimed to limit undesirable substances (i.e. chemical contaminants) in feed, includes, within Annex A, a list of substances which are tolerated in products intended for animal feed, subject to certain conditions. T2 and HT2 are not included in Annex A.

A guidance value for T2 and HT2 concentrations in feed are provided in Commission Recommendation 2016/1319/EC[2], limited to compound feed for cats. In particular, the Recommendation provides a guidance value of T2 and HT2 in a feeding stuff for cats with a moisture content of 12%, being 0.05 mg/kg.

2. Methodologies

The current assessment was developed applying a structured methodological approach, which implied developing a priori the protocol, or strategy, of the risk assessment and performing each step of the risk assessment in line with the strategy and documenting the process. The protocol in Annex A to this Opinion contains the method that was proposed for all the steps of the assessment process, including any subsequent refinements/changes made, if applicable.

2.1. Methodology for data collection and study appraisal

In 2021, the CONTAM Panel received from the European Commission the mandate for an assessment of information on the adverse animal health effects for T2 and HT2 in ruminants. A number of research studies were submitted by the Commission to inform the assessment and potentially derive a lower Reference Point compared to the previous EFSA Opinion (EFSA CONTAM Panel, 2011).

In addition to the papers provided as part of the mandate, the Working Group (WG) performed a literature search to obtain further evidence on ruminants which might have become available since the previous Opinion (EFSA CONTAM Panel, 2011). Three search strings were designed to identify potentially relevant studies published between 1/1/2010 (based on the year of publication of the EFSA CONTAM Panel, 2011) and 30/3/2022, the date when the actual search was performed (see Appendix A). After removal of duplicates and applying inclusion/exclusion criteria, potentially relevant references were identified. The total number of publications identified were 236, and the number of publications identified as potentially relevant were 5. The abstracts considered as potentially relevant were screened by the experts of the WG and were used in the assessment if considered relevant for the scope of the mandate by applying expert judgement. In addition to the literature search and the use of the papers submitted by the European Commission, a 'forward snowballing' approach[3] was applied by the WG members in order to potentially obtain further papers published up to 30/3/2022.

2.2. Methodology for risk characterisation

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by WHO/IPCS (2009).

EFSA guidance relevant for the present assessment has been duly considered (see Appendix B for the EFSA guidance applied).

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics

The fate of T2 in ruminants has been the subject of several reviews (Li et al., 2011; Yang et al., 2017), which were published after publication of the CONTAM Opinion of 2011. Results of *in vivo* studies with

[1] Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed. OJ L140, 30.5.2002, pp. 10–21.
[2] Commission Recommendation (EU) 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regardsdeoxynivalenol, zearalenone and ochratoxin A in pet food. OJ L 208, 2.8.2016, pp. 58–60.
[3] Identifying articles that have been cited in articles found in a search.

cows point to a rapid absorption, an extensive biotransformation to several less toxic metabolites and a rapid excretion of the parent compound and its metabolites, with negligible tissue accumulation and transfer to milk. Metabolism involves predominantly hydrolysis (liver, rumen, blood), cytochrome P450 (CYP)-mediated hydroxylation (liver), glucuronidation (liver) and de-epoxidation (rumen). The main metabolites generated are HT2, the hydroxylated derivatives of T2 (3'-OH-T-2) and HT2 (3'-OH-HT-2) and neosolaniol, as well as the glucuronic acid conjugates of HT2, 3'-OH-T-2 and 3'-OH-HT-2. Ruminal microbiota plays a key role in T2 detoxification (Li et al., 2011) and factors negatively affecting the viability of rumen microorganisms, including rumen acidosis, have the potential to impair the detoxification of T2 and several other mycotoxins (Debevere et al., 2020). Rumen acidosis arises when bovines are fed with diets rich in rapidly fermentable energy and poor in rumination stimulating fibre; it is a very common digestive disorder of cattle and occurs mostly in its subacute form (subacute rumen acidosis, SARA). Kleen et al. (2009, 2013) reported a prevalence of SARA of around 14 and 20% in dairy cows on Dutch and German farms, respectively. Morgante et al. (2007) showed SARA in 3 out of 10 herds of dairy cows on Italian farms, with more than 33% of the animals affected. The impact of SARA and other forms of ruminal acidosis on T2/HT2 degradation and toxicity is unclear.

3.1.2. Mode of action

The modes of actions of T2 and HT2 have been described in detail in the previous CONTAM Opinion on T2 and HT2 in feed (EFSA CONTAM Panel, 2011) and in the Opinion on setting a health-based guidance value (HBGV) for T2/HT2 and its modified forms (EFSA CONTAM Panel, 2016). Most of the data available concern T2 while there is little information available on HT2 (to which T2 is rapidly converted).

Briefly, T2 induces ribotoxic and oxidative stress and inhibits DNA, RNA and protein synthesis. T2 has been shown to cause apoptosis and lipid peroxidation, affecting cell membrane integrity. Recent investigations also suggest that T2/HT2 induces anorexia/emesis via alteration of pro-inflammatory cytokines and satiety hormones (Wu et al., 2013, 2016; Gaigé et al., 2014).

3.1.3. Adverse effects in ruminants

In 2011, the CONTAM Panel reviewed the existing literature and concluded on a LOAEL of 0.3 mg/kg bw per day for calves and lambs (EFSA CONTAM Panel, 2011). This was based on Pier et al. (1976), showing bloody faeces at doses of 0.32 and 0.64 mg/kg bw per day, and studies showing decreased levels of serum proteins and immunoglobulins at a dose of 0.6 mg/kg bw per day (Mann et al., 1983), and reduced response to the stimulation of lymphocytes by phytohaemagglutinin at repeated doses of 0.3 (Mann et al., 1984) and 0.6 mg/kg bw per day (Buening et al., 1982). In the latter studies only one dose was tested. In lambs dosed with 0.3 and 0.6 mg T2/kg bw for 21 days, all animals developed focal hyperaemia and dermatitis at the mucocutaneous junction of the commissure of the lips, diarrhoea, leucopenia, lymphopenia and lymphoid depletion of the mesenteric lymph nodes and spleen (Friend et al., 1983).

The CONTAM Panel concluded at that time that adverse effects in calves and lambs occur at doses of 0.3 mg/kg bw per day and higher (identified as a LOAEL), but that there was no information to derive a NOAEL.

The Opinion also included a study by Huszenicza et al. (2000), showing effects on ovulation in heifers in which rumen acidosis was induced by meal feeding of large amounts of concentrate feed, was supposed to reduce the detoxification in the rumen. Ovarian malfunction was induced by a daily dose of purified T-2 toxin of 0.025 mg/kg bw per day for 20 days under these conditions. The authors also investigated the effects on ovarian function in ruminal non-acidotic and acidotic ewes receiving daily doses of 0.005 and 0.015 mg/kg bw per day for 21 days, showing effects in 3 out of 4 and 3 out of 3 acidotic ewes dosed with 0.005 and 0.015 mg T-2 toxin/kg bw per day, respectively. The CONTAM Panel concluded from this study that T-2 toxin metabolism in the rumen is an important factor in ruminants determining T2 toxicity, but did not take this study into account when deriving the LOAEL.

After the 2011 Opinion, several studies were brought to the attention of the European Commission by Member States and thus reviewed in the current Opinion. Two new studies on sheep and goats were identified in the literature search.

For the scope of this Opinion, it was decided that establishing Reference Points (RPs) for adverse effects for animal health was preferable to identifying NOAELs or LOAELS. RPs for adverse effects for animal health better reflect the uncertainties in the studies evaluated, because in these studies it was often not possible to identify a dose that causes a statistically significant increase in an adverse effect as compared to the control group. This is particularly the case in field studies where all animals received the contaminated feed.

Studies to be reassessed

The case study by Hsu et al. (1972) reported effects in cows exposed to maize shown to contain 2 mg T2/kg. Seven out of 35 lactating Holstein cows died over a 5-month period after prolonged ingestion of a diet containing 60% of the ground mouldy corn. Post-mortem examination revealed extensive haemorrhages on the serosal surface of all viscera. The authors concluded that the nature of the observed effects suggests a 'major causal relationship'. The CONTAM Panel noted that an intake of 15 kg dry matter of which 60% being the corn, would result in daily ingestion of 18 mg T2/day or 0.03 mg/kg bw per day for a cow of 600 kg.

In another case report from Hungary (Kégl and Ványi, 1991), dairy cows showed decreased feed intake, bloody diarrhoea, reduced milk production and absence of visible oestrus. Concentrations of T2 were detected in the feed given to the cows under intensive milk production at levels of 0.6 mg/kg. The animals got better once the contaminated diet was removed, with a return to normal feed intake and a gradual improvement of the diarrhoea. The ovarian functions and milk production normalised after 9–11 days. Considering that the animals had a fodder ration of 10.5 kg/day, the CONTAM Panel calculated that it would result in an ingestion of 0.01 mg T2/kg bw per day for a cow of 600 kg.

In the study by Pier et al. (1976), calves were exposed orally to T2 in capsules, at doses of 0.08, 0.16, 0.32 and 0.64 mg/kg bw per day (n = 1 per dose, 3 controls). Mild enteritis was observed at all doses tested, while the animal exposed to the highest dose died at day 20. Bloody faeces were observed at 0.32 and 0.64 mg/kg bw per day. Due to the limitations of the study design, a LOAEL cannot be derived.

Huszenicza et al. (2000) carried out a study with heifers fed a starch-rich diet to induce rumen acidosis (n = 4) followed by oral exposure to 0.025 mg T2/kg bw per day for 20 days. A control group was used with the same diet but without T2 (n = 3). In T2 treated heifers, ovulation occurred later and plasma progesterone level remained low (<3 nmol/L) for a longer period as compared to the controls. Another study was performed on ewes with half of the animals receiving a starch-rich diet to induce rumen acidosis (n = 15) and half of the animals receiving a regular diet (n = 15). Within each diet group, animals were orally exposed to 0, 0.005 or 0.015 mg T2/kg bw per day for 3 weeks (n = 5/subgroup). Due to damage to the catheter, samples from some animals were lost. In the two subgroups that received the starch-rich diet and 0.005 or 0.015 mg T2/kg bw, the following ovarian malfunctions were observed in, respectively, three out of four, and in three out of three animals, i.e. lower progesterone peak concentration in the midluteal phase, shortening of the corpus luteum lifespan and prolonged follicular phases. In the subgroup receiving regular diet and the mid-dose of T2 no ovarian malfunction was observed. In the controls (starch-rich or regular diet) no ovarian malfunctions were observed. In the subgroup receiving the regular diet (no acidosis) and the high dose of T2, a lower progesterone peak concentration was observed in one out of four ewes. The other four and three animals receiving the mid and high dose showed no abnormalities.

New studies

A study on goats and a case study on sheep were published since the last Opinion (EFSA CONTAM Panel, 2011), providing further information on adverse effects in ruminants when exposed orally to T2 and HT2.

In Nayakwadi et al. (2020), 18 juvenile goats (2–3 months of age) were divided into three groups (n = 6 each) and exposed to T2 concentrations of 0 (control), 10 or 20 mg/kg feed. Half of the animals of each group were euthanised at 15 days, the other half at 30 days. Observations revealed reduced feed intake, reduced weight gain and lethargy in both treatment groups, with a significant weight gain reduction in group 2 (20 mg/kg of feed) from day 15. Diarrhoea was observed in both treatment groups, together with widespread pathological lesions in the gastrointestinal tract in group 2. Haematological changes observed in treated kids included reduction of haemoglobin, total leucocyte and thrombocyte counts. A number of histological alterations were observed by the authors, in particular, but not limited to liver and intestine, for both the animals in Group 1 and Group 2. The lower concentration used in this study (10 mg/kg feed) could be considered an adverse effect concentration and corresponds to a dose level of 1.1 mg/kg bw per day, based on 0.7 kg feed per day and 6.4 kg bw (Martínez Marín et al., 2010). This is higher than the LOAEL established in the 2011 EFSA Opinion (0.3 mg/kg bw per day derived for calves and lambs). No NOAEL could be established from this study.

In a case study, Ferreras et al. (2013) described the adverse effects to a flock of 1,000 sheep which had accidentally been exposed to feed contaminated with T2, in Spain. Approximately 19% of the affected animals died during the acute phase. In this phase, the animals showed reduced feed and

water consumption, ruminal atony and apathy. The case study described also the stage of the intoxication between 2 and 4 months after the start of the outbreak, when the surviving animals showed reduced body weight gain, frequent abortions and wool loss. In the acute phase, two animals out of the affected flock which were submitted to pathological assessment, showed signs of rumenitis, ulceration of the abomasum, myocarditis, necrosis of the pancreas, and oedema of the brain and skin. In the subsequent phase, the four animals submitted to pathological assessment showed oral lesions, myocardial fibrosis, a certain degree of serum biochemical alterations and opportunistic infections. The case study provides concentrations of T-2 toxin in various samples of the contaminated feed, ranging from 0.015 to 0.056 mg/kg feed (average 0.035 mg/kg). Table 2 summarises the new studies on adverse effects in ruminants.

Table 2: Studies on adverse effects on ruminants. Doses were converted to feed levels and vice versa based on information provided in the study or default values used by the CONTAM Panel. None of the studies allowed derivation of a NOAEL

N/group, breed gender	Dosage and duration	Endpoint(s)	Adverse effect concentration (mg/kg feed)*	Adverse effect level (mg/kg bw per day)*	Reference
N = 5, (plus N = 5 control), Holstein calves	0, 0.3 mg T2/kg bw per day, for 56 days	Neutrophil function and reaction to cutaneously injected phytohaemagglutinin		0.3	Mann et al. (1984)
N = 6, (plus N = 6 control), Holstein calves	0, 0.5 mg T2/kg bw per day, for 28 days	Number of B lymphocytes and the response of the B-cell enriched fraction to phytohaemagglutinin increased		0.5	Mann et al. (1984)
N = 4 (plus N = 3 control), heifers	0, 0.025 mg T2/ kg bw per day (with a diet that induced **acidification** of the rumen)	Late ovulation, decreased increase of progesterone levels in plasma.	*1.2 (based on 8.4 kg for fattening beef of 400 kg bw)*	0.025	Huszenicza et al. (2000)
N = 4, 4, 3 with acidosis, N = 3, 5, 4 without acidosis, ewes	0, 0.005 or 0.015 mg T2/kg bw per day (partly with a diet that induced **acidification** of the rumen)	Lower progesterone peak concentration in the midluteal phase, shortening of the corpus luteum lifespan and prolonged duration follicular phases (no effects in sheep without rumen acidification)	*0.14 (based on 2.8 kg for sheep of 80 kg bw)*	0.005 (with diet that induced acidification of the rumen)	Huszenicza et al. (2000)
N = 35, lactating Holstein cows	2 mg/kg corn, 5 months (case study)	Serosal surface of all viscera	1.2 (based on 60% corn)	*0.03 (based on 15 kg feed per day and 600 kg bw)*	Hsu et al. (1972)
N = 220, Dairy cows	0.6 mg/kg feed (case study)	Feed intake ↓, diarrhoea, milk production ↓, Absence of visible oestrus. Body weight gain ↓, frequent abortions and wool loss	0.6	*0.01 (based on consumption of 10.5 kg/day and 600 kg bw)*	Kégl and Ványi (1991)

N/group, breed gender	Dosage and duration	Endpoint(s)	Adverse effect concentration (mg/kg feed)*	Adverse effect level (mg/kg bw per day)*	Reference
N = 1, calves, 3 controls	0, 0.08, 0.16, 0.32 and 0.64 mg/kg bw per day	Mild enteritis and loose faeces at all doses, Acute enteric response with bloody faeces at 0.32 and 0.64 mg/kg bw. Animal with highest dose died.			Pier et al. (1976)
N = Approx. 1,000 sheep	0.015 to 0.056 mg/kg feed, case study	Feed and water consumption ↓, Ruminal atony and apathy. 84 of 440 ewes (19%) died	0.035 (mean)	*0.001 (based on 2.8 kg feed per day and 80 kg bw)*	Ferreras et al. (2013)
N = 6, Barbari breed Juvenile goats (2–3 months)	0, 10 and 20 mg/kg of feed, 15 and 30 days	Feed intake, weight gain ↓. Haemoglobin, total leucocyte, thrombocyte counts ↓. Oxidative stress parameters ↑. Serum and tissue catalase and superoxide dismutase ↓. Liver and intestinal histological changes	10	*1.1 (based on 0.7 kg feed per day and 6.4 kg bw)*	Nayakwadi et al. (2020)

bw: body weight.
*: Adverse effects concentrations or levels derived via conversion by the CONTAM Panel are indicated in italics.

Conclusions

Previously, the CONTAM Panel concluded that in calves and lambs daily doses of 0.3 mg T2/kg bw per day were shown to result in adverse effects. Since this was the lowest dose applied in controlled studies, it was concluded that a NOAEL could not be established (EFSA CONTAM Panel, 2011).

The case studies by Hsu et al. (1972) and Kégl and Ványi (1991) with bovine animals imply that a dose of 0.03 and 0.01 mg T2/kg bw per day, i.e. 10- to 30-fold lower than the LOAEL of 0.3 mg/kg bw per day, may cause serious adverse effects, including death of part of the animals.

A case study with sheep reported adverse effects, including death of 19% of the ewes, at an average feed concentration of 0.035 mg/kg feed, estimated to correspond to a dose level of 0.001 mg T2/kg bw per day (Ferreras et al., 2013).

Rumen acidosis, a relatively common situation in ruminants, leads to higher susceptibility to these toxins, showing reproductive effects in heifers at a dose of 0.025 mg T2/kg bw per day and in ewes at 0.005 mg T2/kg bw per day (Huszenicza et al., 2000). The higher susceptibility is likely to result from decreased detoxification of the toxin in the rumen.

For goats, a controlled study showed an adverse effect concentration of 10 mg T2/kg feed, being the lowest dose tested. This was estimated to correspond to 1.1 mg/kg bw per day (Nayakwadi et al., 2020) and is higher than the previously established LOAEL of 0.3 mg/kg feed for calves and lambs.

Regarding the adversity of the effects observed in the case studies, the CONTAM Panel decided to derive RPs based on these studies, even though only a few studies were available. The low effect levels are supported by the study by Huszenicza et al. (2000) showing effects in animals with acidosis induced by the specific feeding regimen. Hence, adverse effect levels of 0.001 and 0.01 mg T2/kg bw per day for, respectively, sheep and cows, is considered more appropriate, corresponding to feed concentrations of 0.035 mg T2/kg for sheep and 0.6 mg T2/kg for bovines. Since these levels cause adverse effects, applying a UF of 3 results in Reference Points for adverse animal health effects of 0.01 mg/kg feed for sheep and 0.2 mg/kg feed for bovines. In the absence of data showing that goats are more sensitive than bovines, the CONTAM Panel decided to apply the same RP as for bovines.

3.2. Feed occurrence data

The collection of new potentially available occurrence data on feed concentrations was outside the remit of the present mandate. With the aim of revising the risk characterisation, and in view of the revised RPs for ruminants, the CONTAM Panel referred to the feed occurrence data included in the 2011 Opinion, which should be consulted for further detail.

In the 2011 Opinion, T-2 and HT-2 toxins were identified mainly in grains. The highest concentrations were identified, in particular, in oats and oat products. Processing of grains to obtain compound feedstuffs was shown not to affect H2 and HT2 levels.

For the full details on feed occurrence data underpinning the exposure assessment of the 2011 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2011).

3.3. Exposure assessment

In the 2011 Opinion (EFSA CONTAM Panel, 2011), due to the limited number of compound feed samples available for the assessment, example diets were derived on the basis of general principles and practices for animal feeding. The example diets for the various livestock categories were used in conjunction with the feed intakes and mean occurrence data provided by the Member States to derive the mean LB and UB concentrations for different animal species.

For the full details on the exposure assessment performed for T2 and HT2 in the 2011 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2011).

3.4. Risk characterisation

For the scope of the present mandate, the newly derived RPs for adverse animal health effects/ adverse effect concentration in feed for cows and sheep have been compared against the respective exposure values derived in the 2011 Opinion. The comparison is included in Table 3 below. Exposure estimates, UB mean, are presented together with RP/adverse effect level for cows and sheep, as revised by the Panel in the present scientific Opinion. For goats, the same RP and adverse effect level as for bovines was applied. The estimates of exposure to the sum of T2 and HT2 are presented in Section 6.2 of the 2011 Opinion (EFSA CONTAM Panel, 2011).

Table 3: Comparison of estimated exposure to the sum of T2 and HT2 and RP/adverse effect level for cows and sheep

Animal species	RP (µg/kg bw per day)	Adverse effect level (µg/kg bw per day)	Estimated exposure (µg/kg bw per day)	Estimated exposure, % of RP/adverse effect level	
			Mean LB/UB	Mean LB/UB	
				% of RP	% of adverse effect concentration
Dairy cows	3.3	10	0.16/1.7	4.8/51	1.6/17
Cereal beef	3.3	10	0.39/0.76	12/23	3.9/7.6
Lactating sheep	0.33	1	0.26/0.51	79/155	26/51
Milking goats	3.3*	10*	2.7/3.3	82/100	27/33
Fattening goats	3.3*	10*	0.91/1.2	28/36	9.1/10

RP: Reference Point (for adverse animal health effects); bw: body weight; LB: lower bound; UB: upper bound; –: not available.
*: For goats, the same RP and adverse effect level as for bovines was applied.

For **Dairy cows**, the estimated exposure to the sum of T2 and HT2 at the upper bound (UB) mean was 51% of the RP. The UB exposure is 17% of the dose causing serious adverse effects reported in case studies, including death of part of the animals, whereas the lower bound (LB) exposure is ten-fold lower. It is deemed that this indicates a low risk for adverse health effects on dairy cows.

Similarly, for **Fattening beef** the estimated exposure to the sum of T2 and HT2 at the UB mean was 23% of the RP and 7.6% of the dose causing serious adverse effects reported in case studies, including death of part of the animals, indicating a low risk for adverse health effects on fattening beef.

For **Lactating sheep,** the estimated exposure to the sum of T2 and HT2 at the UB mean was 154% of the RP and 51% of the dose reported to cause serious adverse health effects, indicating a potential risk for adverse health effects.

For **Milking goats,** a comparison of the estimated UB mean exposure to the sum of T2 and HT2 against the RP derived for bovines, shows that the two numbers are in the same order of magnitude. This indicates a potential risk for adverse health effects for milking goats, assuming them as sensitive as bovines.

For **Fattening goats,** a comparison of the estimated UB mean exposure to the sum of T2 and HT2 against the RP derived for bovines indicates no health concern.

3.5. Uncertainty analysis

The evaluation of uncertainty in the present assessment was performed following the principles laid down in the guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). However, considering the specific nature of this Opinion and its limited scope, only a brief evaluation could be carried out, focusing on the particular uncertainties in the design of the studies evaluated and on uncertainties occurring in such studies. A full quantification of these uncertainties was not carried out based on the reasons explained above.

Particular uncertainties of the studies used for this assessment are as follows:

- Controlled studies were performed with a limited number of doses and/or animals.
- Toxicity data were often obtained by using naturally contaminated material which may contain also other mycotoxins.
- Uncertainty on the representativity of the samples and hence the levels that caused the effects in case studies.
- Example animal diets were used to calculate animal exposure (e.g. goat kids). In practice there is a high variability of feedstuffs used and feeding systems for livestock.
- Feed levels are relatively old. Furthermore, only the mean exposure was estimated in the 2011 Opinion and not the P95 exposure which may be several-fold higher.
- No robust toxicological data are available for adult ruminants.
- In Huszenicza et al. (2000), rumen acidosis was induced. The detoxification capacity of the rumen might be impaired by this digestive disorder, which appears to be a common condition in specific feeding regimes. It is unclear if feeding scenarios inducing a certain form of acidosis played a role in the outcome of case studies, showing effects at similar low dose levels.

The overall uncertainty incurred with the present assessment is high.

4. Conclusions

Adverse effects in ruminants

- A LOAEL of 0.3 mg T2/kg bw per day was previously derived for calves and lambs from controlled studies. A NOAEL could not be established from the evaluated studies (EFSA CONTAM Panel, 2011).
- More weight was given to case studies, including one new report on sheep. These studies showed effects at lower exposure than the LOAEL of 0.3 mg T2/kg bw per day.
- One study with sheep and cows with diet-induced acidosis showed adverse effects at low dose levels. Particularly SARA is a common form of rumen acidosis with a reported prevalence of approximately 20%, but its relevance for rumen capacity for T2 metabolism and consequently toxicity requires further research. Adverse effect levels of 0.001 and 0.01 mg T2/kg bw per day for, respectively, sheep and cows, were derived from the case studies, estimated to correspond to feed levels of 0.035 for sheep and 0.6 mg T2/kg feed for cows.
- Applying a UF of 3, Reference Points (RPs) for adverse animal health effects of 0.01 mg/kg feed for sheep and 0.2 mg/kg feed for cows were established.
- For goats, no RP could be established based on available studies. The CONTAM Panel decided to apply the RP for bovines in the absence of studies that would indicate that goats are more sensitive.

Risk characterisation

When comparing the estimated mean UB levels of the sum of T2 and HT2 with the new RPs for adverse animal health effects for ruminants the following could be concluded:

- The estimated LB and UB mean exposure to the sum of T2 and HT2 for dairy cows was estimated at 0.16 and 1.7 μg/kg bw per day, respectively, corresponding to 4.8 and 51% of the RP for adverse animal health effects of 3.3 μg/kg bw per day. This indicates a low risk for adverse health effects in dairy cows and thus no health concern.
- The estimated LB and UB mean exposure to the sum of T2 and HT2 for cereal beef was estimated at 0.39 and 0.76 μg/kg bw per day, corresponding to 12 and 23% of the RP for adverse animal health effects of 3.3 μg/kg bw per day. This indicates a low risk for adverse health effects in fattening beef and thus no health concern.
- The estimated LB and UB mean exposure to the sum of T2 and HT2 for lactating sheep is 0.26 and 0.51 μg/kg bw per day, corresponding to 79 and 155% of the RP for adverse animal health effects of 0.33 μg/kg bw per day, indicating a potential risk for adverse health effects.
- For Milking goats, a comparison performed between the estimated LB and UB mean exposure to the sum of T2 and HT2 of 2.7 and 3.3 μg/kg bw per day and the RP derived for bovines (3.3 μg/kg bw per day), indicates a potential risk for adverse health effects.
- For Fattening goats, a comparison performed between the estimated LB and UB mean exposure to the sum of T2 and HT2 of 0.91 and 1.2 μg/kg bw per day and the RP derived for bovines (3.3 μg/kg bw per day), indicates a low risk for adverse health effects and thus no health concern.

5. Recommendations

- Further information on the toxicokinetics of T2 and HT2 is required for ruminants.
- To reduce uncertainties, controlled experimental studies would be necessary, including mechanistic studies, to address the issue of sensitivity of ruminants in conditions of rumen impairment.
- Analytical methods should be improved to reduce the uncertainty in the estimated exposure.

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Abbreviations and/or acronyms

bw	body weight
CONTAM	Panel on Contaminants in the Food Chain
HBGV	health-based guidance value
HT2	HT-2 toxin
LB	lower bound
LOAEL	lowest observed adverse effect level
NOAEL	no observed adverse effect level
RP	Reference Point
SARA	subacute rumen acidosis
T2	T-2 toxin
UB	upper bound
UF	uncertainty factor
WHO	World Health Organization

Appendix A – Literature search for supporting information for the assessment

Web of Science

Timespan = from 1/1/2010 to 30/3/2022

Set	Query	Results	Comments
	#1 AND #2 AND #3	**236**[(a)]	WOS TOXICITY in **ruminants**
#1	Fusariotoxin T2 OR insariotoxin OR Mycotoxin T2 OR T-2 mycotoxin OR toxin T2 OR T2-toxin OR T2-trichothecene ORHT-2 OR Fusariotoxin HT-2 OR Mycotoxin HT-2 OR Toxin HT 2		**Main search WOS** Command word: TS
#2	cow* OR calves OR calf* OR bull* OR rumen OR ruminant* OR goat* OR sheep* OR lamb* OR deer OR caprine* OR ovin* OR bovine OR cattle OR heifers OR steer		**Farm animals - ruminants** Command words: TS
#3	tox* OR poison* OR cancer OR carcino* OR tumor* OR tumour* OR organ OR tissue OR immun* OR neuro* OR developmental OR teratogen* OR repro* OR liver OR kidney OR brain OR lung OR cardiovascular OR health OR clinical OR growth OR weight OR NOAEL OR LOAEL		**Toxicity** Command words: TS

(a): Having removed the duplicates.

Appendix B – EFSA guidance documents applied for the risk assessment

- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051
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Annex A – Protocol for the development of the opinion

Annex A is the protocol undertaken for the scientific development of this opinion and can be found in the online version of this output ('Supporting information' section) at: https://doi.org/10.2903/j.efsa.2022.7564