SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 13 July 2022

doi: 10.2903/j.efsa.2022.7534

Assessment of information as regards the toxicity of fumonisins for pigs, poultry and horses

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Dieter Schrenk, Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Jean-Charles Leblanc, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand, Tanja Schwerdtle, Christiane Vleminckx, Heather Wallace, Sven Daenicke, Carlo Stefano Nebbia, Isabelle P Oswald, Elena Rovesti, Hans Steinkellner and Laurentius (Ron) Hoogenboom

Abstract

In 2018, 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 fumonisins, their modified forms and hidden forms in feed. A no observed adverse effect level (NOAEL) of 1 mg/kg feed was established for pigs. In poultry a NOAEL of 20 mg/kg feed and in horses a reference point for adverse animal health effect of 8.8 mg/kg feed was established, referred to as NOAEL. The European Commission (EC) requested EFSA to review the information regarding the toxicity of fumonisins for pigs, poultry and horses and to revise, if necessary, the established NOAELs. The EFSA CONTAM Panel considered that the term reference point (RP) for adverse animal health effects better reflects the uncertainties in the available studies. New evidence which had become available since the previous opinion allowed to revise an RP for adverse animal health effects for poultry from 20 mg/kg to 1 mg/kg feed (based on a LOAEL of 2.5 mg/kg feed for reduced intestinal crypt depth) and for horses from 8.8 to 1.0 mg/kg feed (based on case studies on equine leukoencephalomalacia (ELEM)). For pigs, the previously established NOAEL was confirmed as no further studies suitable for deriving an RP for adverse animal health effects could be identified. Based on exposure estimates performed in the previous opinion, the risk of adverse health effects of feeds containing FB1–3 was considered a concern for poultry, when taking into account the RP of 1 mg/kg feed for intestinal effects. For horses and other solipeds, the risk is considered low, although a large uncertainty associated with exposure was identified. The same conclusions apply to the sum of FB1–3 and their hidden forms.

Keywords: fumonisins, exposure, toxicity, animal health risk assessment, horses, poultry, pigs

Requestor: European Commission

Question number: EFSA-Q-2021-00696

Correspondence: feedco@efsa.europa.eu

Panel members: Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Jean-Charles Leblanc, Carlo Stefano Nebbia, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand, Dieter Schrenk, Tanja Schwerdtle, Christiane Vleminckx and Heather Wallace.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Leblanc JC, Nielsen E, Ntzani E, Petersen A, Sand S, Schwerdtle T, Vleminckx C, Wallace H, Daenicke S, Nebbia CS, Oswald IP, Rovesti E, Steinkellner H and Hoogenboom LR, 2022. Scientific Opinion on the assessment of information as regards the toxicity of fumonisins for pigs, poultry and horses. EFSA Journal 2022;20(8):7534, 26 pp. https://doi.org/10.2903/j.efsa.2022.7534

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

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

Background

In 2018, EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. EFSA established for fumonisins in pigs (No Observed Adverse Effect Level (NOAEL) of 1 mg/kg feed), in poultry (NOAEL of 20 mg/kg feed) and in horses (NOAEL of 8.8 mg/kg feed).

Information was more recently provided to the Commission services concluding that the reference points for adverse animal health effects for fumonisins in pigs, poultry and horses 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 points for adverse animal health effects established for fumonisins in pigs, in poultry and in horses can be confirmed or need to be updated. In case the reference points for pigs, poultry and horses were updated, the risks to these farm animals in relation to the presence of fumonisins in feed, will be assessed using the exposure assessment included in the EFSA's 2018 opinion (EFSA CONTAM Panel, 2018a).

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 fumonisins in pigs, poultry and horses, and, if necessary, to update the scientific opinion on the risks to animal health related to the presence of fumonisins, their modified forms and hidden forms in feed, taking into account:

- information submitted to the Commission, and
- the exposure assessment of the previous opinion (EFSA CONTAM Panel, 2018a).

The information on the fumonisins adverse effects on animal health for submitted by European Commission are summarised in Table 1 below.

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

Animal species	Studies to be (re)assessed
Pigs	Rotter et al. (1996, 1997), Zomborszky-Kovács et al. (2002a)
Poultry	Benlasher et al., 2012 (ducks and turkeys) Grenier et al., 2015 (chickens) Henry et al., 2000 (chickens) Masching et al., 2016 (turkeys) Tardieu et al., 2007 (turkeys)
Horses	Jovanović et al. (2015), Vendruscolo et al. (2016)

1.2. Additional information

1.2.1. Chemistry

Fumonisins can be produced by various species of *Fusarium*, but predominantly by *F. verticillioides* and *F. proliferatum*. These moulds can also produce other mycotoxins. The chemistry of fumonisins is described by EFSA (EFSA CONTAM Panel, 2018b). Several classes of fumonisins have been identified. For the B-type, being assessed in this Opinion, FB_1 is the most widely occurring, followed by FB_2, FB_3 and FB_4. Fumonisins have a backbone resembling the sphingoid bases. They are highly polar and water soluble. There are also a number of modified forms of fumonisins. Furthermore, fumonisins can be physically entrapped (hidden forms), based on poor recovery during the extraction.

1.2.2. Previous animal and human health risk assessments

In 2005, EFSA published a Scientific Opinion related to fumonisins as undesirable substances in animal feed (EFSA, 2005). No observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were derived for different animal species based on fumonisin B_1 (FB_1), considered to be the most prevalent and most toxic derivative. Pigs and horses were identified as the most sensitive species to FB_1 with an LOAEL of 0.2 mg/kg body weight (bw) per day for FB_1 for both animal species based on an increased ratio of sphinganine/sphingosine (Sa/So) levels in serum. An LOAEL of 2 mg/kg bw per day for FB_1 was identified for poultry based on increased Sa levels and Sa/So ratios in liver (EFSA, 2005).

In 2014, the EFSA CONTAM Panel developed a Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed (EFSA CONTAM Panel, 2014). Risk characterisation was done by comparing exposure scenarios with the NOAELs/LOAELs for the parent compounds. The CONTAM Panel identified several uncertainties and data gaps for 'modified mycotoxins'[1] and recommended reassessing the animal health effects of zearalenone and fumonisins.

In 2018, the CONTAM Panel assessed the appropriateness to set a group health-based guidance value (HBGV) for fumonisins and modified forms (EFSA CONTAM Panel, 2018b). The CONTAM Panel established a tolerable daily intake (TDI) for FB_1 of 1.0 μg/kg bw per day based on increased incidences of megalocytic hepatocytes found in a chronic study with mice. The Panel also found it appropriate to include FB_2, FB_3 and FB_4 in a group TDI with FB_1, based on structural similarity and the limited data available indicating similar toxic profile and potencies. The group TDI was derived from a $BMDL_{10}$ of 0.1 mg/kg bw per day and using an uncertainty factor (UF) of 100 to account for intra- and interspecies variability. Regarding the modified forms, the Panel did not include these in the group TDI due to the limited data available. Nevertheless, the Panel indicated that the toxic potency of the modified forms is considered lower than the parent compounds, although not quantifiable (EFSA CONTAM Panel, 2018b). Non-covalently bound (hidden) forms should not be considered as modified forms and are thus included in the TDI.

In 2018, EFSA published a Scientific Opinion on the risks to animal health for the presence of fumonisins, their modified and hidden forms in feed (EFSA CONTAM Panel, 2018a). The CONTAM Panel concluded that the risk of adverse effects for the presence of fumonisins $FB_{1\text{-}3}$ in feed was of potential concern for pigs, was low for horses, rabbits, fish and poultry and very low for ruminants. The levels for the sum of $FB_{1\text{-}3}$ and its hidden forms were (assumed to be 1.6-fold higher than the sum of $FB_{1\text{-}3}$) only. Based on the calculated exposure, the risk of adverse effects to pigs was identified to be of concern. The relevant adverse effects observed in pigs, poultry and horses and the NOAELs/LOAELs are summarised in Table 2. It was concluded that a change in Sa/So ratio in serum or tissues by itself was not regarded as adverse since there was no relation with the adverse effects observed in various species. As a result, the LOAEL of 40 μg/kg bw for pigs derived by EFSA (2005) became an NOAEL. For horses, an NOAEL and LOAEL of, respectively, 0.01 and 0.05 mg/kg bw per day were derived from a study with *i.v.* exposure and converted to oral exposure assuming 5% bioavailability (EFSA, 2005). These were converted to feed levels based on default values (450 kg bw and feed intake of 9 kg dry matter (dm) per day or 10.2 kg 88% dm; EFSA CONTAM Panel, 2018a).

[1] Fumonisins modified forms: In the EFSA CONTAM Panel (2014) opinion, modified forms included both covalently and non-covalently (i.e. physically entrapped) bound forms (Covalent binding to food and feed matrix (hidden forms)). In the CONTAM opinion on appropriateness to set a group health-based guidance value for fumonisins and modified forms (EFSA CONTAM Panel, 2018b), the 2018 opinion on risks to animal health (EFSA CONTAM Panel, 2018a) and in the present opinion, non-covalently bound forms (hidden forms) are not considered as modified forms. Modified forms of FBs are phase I and phase II metabolites formed in fungi or infested plants or food or feed products of animal origin as well as forms arising from food or feed processing including covalent adducts with matrix constituents.

Table 2: NOAELs/LOAELs derived for each animal species in the EFSA 2018 opinion (EFSA CONTAM Panel, 2018a) and relevant toxicity studies

Species	No observed adverse effect level (NOAEL)	Lowest observed adverse effect level (LOAEL)	Adverse effects observed (type of study)	References
Pigs	1 mg FB_1/kg feed (corresponding to 40 μg/kg bw per day)	5 mg FB_1/kg feed corresponding to 200 μg/kg bw per day	Mild pulmonary lesions in 1 animal at 1 mg FB_1/kg feed (NOAEL). At 5 mg FB_1/kg feed increase in the weight of the lungs, pathological and histopathological chronic pulmonary changes in the lung and liver (LOAEL)	Zomborszky-Kovács et al. (2002a)
Chickens	20 mg FB_1/kg feed (corresponding to 2.6 mg/kg bw per day)	40 mg FB_1/kg feed (4.7 mg/kg bw per day)	Decreased liver lipids (from 40 mg/kg). Increased ratio GOT: AST (from 80 mg/kg). No effect on body weight gain, serum cholesterol, ALP and LDH	Henry et al. (2000)
Turkeys	20 mg FBs (FB_1 + FB_2)/ kg feed (corresponding to 0.9 mg FBs/kg bw per day)		No macroscopic lesions were detected in any tissues and histopathological examinations of liver and kidneys did not reveal any alterations. No effects on body weight gain, relative organ weights or feed conversion but a slight but statistically significant increase in feed consumption reported at 20 mg/kg feed	Tardieu et al. (2007)
Ducks	8 mg FB_1/kg feed	32 mg FB_1/kg feed	Serum biochemistry, indicative of liver damage	Tardieu et al. (2006)
Horses	8.8 mg/kg feed (0.2 mg FB_1/kg bw per day)	44 mg kg/feed (1 mg FB_1/kg bw per day)	Neurological abnormalities, cardiovascular effects (observed in an *i.v.* study, converted to oral exposure by assuming 5% oral bioavailability)	Foreman et al. (2004)

1.3. Legislation

Directive 2002/32/EC[2] on undesirable substances in animal feed, aimed to limit undesirable substances (i.e. chemical contaminants) in feed, includes, within Annex I, a list of substances which are tolerated in products intended for animal feed, subject to certain conditions. Fumonisins are not included in Annex I.

Guidance values for fumonisin concentrations in feed are provided in Commission Recommendation 2016/1319/EC[3]. In particular, the recommendation provides guidance values of fumonisins B_1 + B_2 in a feedingstuff with a moisture content of 12%, being 5 mg/kg for pigs and horses and 20 mg/kg for poultry.

2. Data and methodologies

2.1. Data

EFSA commenced the collection of data on fumonisins in food and feed in December 2010 with a call for an annual collection of chemical contaminant occurrence data in food and feed. By the end of July 2017, a total of 18,273 analytical results from 8,057 samples had been submitted on occurrence of fumonisins in feed and made available in the EFSA database. Data received up to that date were

[2] 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, p. 10–21.
[3] Commission Recommendation (EU) 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food. OJ L 208, 2.8.2016, p. 58–60.

included in the estimated dietary exposure of the 2018 EFSA Opinion on Fumonisins (EFSA CONTAM Panel, 2018a), the methodology of which is briefly summarised in this section. For the full details on data collection, the 2018 Opinion on the risk posed by fumonisins in feed to animal health should be consulted (EFSA CONTAM Panel, 2018a).

2.2. Methodologies

2.2.1. 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 fumonisins in pigs, poultry and horses, and, if necessary, an update to the Scientific Opinion on the risks to animal health related to the presence of fumonisins, their modified forms and hidden forms in feed (EFSA CONTAM Panel, 2018a). A number of research studies were submitted by the European Commission to inform the assessment and to potentially derive a lower reference point (RP) for adverse effect on animal health compared to the previous EFSA Opinion (EFSA CONTAM Panel, 2018a).

In addition to the papers provided as part of the mandate, the working group (WG) on mycotoxins in feed decided to perform a limited literature search to obtain further evidence which might have become available since the previous Opinion (EFSA CONTAM Panel, 2018a). Five search strings were designed to identify potentially relevant studies published between 1 January 2017 (based on the year of publication of the EFSA CONTAM Panel, 2018a) and 2 January 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. Exclusion criteria related to papers not concerning toxicity in poultry, horses and pigs, papers on co-exposure with other mycotoxins or detoxification compounds, papers relating to exposure other than oral. The total number of publications identified, and the number of publications identified as potentially relevant for each of the animal species was as follows: 105/8 for horses, 350/46 for pigs, 236/32 for poultry. The abstracts considered as potentially relevant were screened by the experts of the working group and were used in the assessment if considered relevant for the scope of the mandate, by applying expert judgement. In addition to the limited literature search and the use of the papers submitted by the European Commission, a 'forward snowballing' approach[4] was applied by the WG members in order to obtain further papers published up to 2 January 2022.

2.2.2. Methodology applied for dietary exposure assessment, hazard and risk characterisation

In the 2018 Opinion (EFSA CONTAM Panel, 2018a), two approaches were followed. Where information was available, the level of fumonisin contamination in compound feed was used to estimate exposure. In cases where this information was not available, the fumonisin concentrations of individual feed materials were taken into account, together with example diets, to estimate P95 and mean exposure both at LB and UB.

For the full details on the dietary exposure assessment performed for fumonisins, the 2018 Opinion should be consulted (EFSA CONTAM Panel, 2018a).

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by the WHO/IPCS (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by the 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 toxicokinetics (TK) of fumonisins in pigs, poultry and horses has been summarised by EFSA (EFSA CONTAM Panel, 2018a). In general, fumonisins are characterised by a low bioavailability, due to

[4] Identifying articles that have been cited in articles found in a search.

their charged structure (three negative charges) and by the likely poor expression of a specific transporter at the enteric level (Shier, 2000). Although qualitative and quantitative species-related differences have been reported, in general fumonisins are subjected to consecutive hydrolytic reactions mainly occurring in the enteric tract giving rise first to partially hydrolysed fumonisin A and B (pHF1a and pHF1b) and ultimately to HFB_1 (also referred to as aminopentol, AP). In exposed animals, both the parent compound and the metabolites may be found in liver and kidney and, to a lesser extent, in muscle. Faecal elimination largely outweighs the urinary one.

3.1.1.1. Avian species

Scant information is available on fumonisin TK in chickens, ducks and turkeys (Guerre, 2015). Oral bioavailability is lower than in pigs (see Section 3.1.1.2) and in the order turkeys > ducks > chickens; this has been related to a general lower sensitivity of the avian species compared to mammalian species. Evidence for a very low bioavailability (i.e. plasma levels around 2.5 ng/mL (LOQ) after oral dosing has been recently provided also for HFB_1 (Antonissen et al., 2020). Kinetic studies indicate a rapid elimination of the toxins, particularly in chickens and ducks with very limited tendency to tissue accumulation. Accordingly, the exposure to feed levels around the EU guidance values[3] resulted in measurable fumonisin concentrations only in liver.

3.1.1.2. Pigs

As recently reviewed by Schelstraete et al. (2020), a low oral bioavailability (3–4%) has been calculated, with rapid absorption as reflected by plasma peaks occurring a few hours after exposure. The available literature points to extensive biotransformation, with hydrolytic reactions seemingly occurring along the entire enteric tract, and to biliary excretion and notable enterohepatic circulation. The excretion is via faeces and, to a much lesser extent, urine. There is evidence of a slow elimination of fumonisins and their hydrolysed metabolites, with measurable amounts still being detectable in excreta and tissues (liver and kidneys) 9–10 days after the withdrawal of a contaminated diet.

3.1.1.3. Solipeds

No information on fumonisins TK could be retrieved for horses or other solipeds.

3.1.2. Mode of action

Fumonisins are known to be inhibitors of ceramide synthase enzymes (CerS), which are essential for the production of sphingolipids. Based on Wang et al. (1991), this is a competitive inhibition, due to the structural analogy of fumonisins with free sphingolipid bases. These sphingolipids are highly bioactive compounds and important components in cellular membranes. The inhibition results in increased levels of sphingosine and its phosphate, and in particular of sphinganine, and its phosphate. As a result, there is also an increase of the sphinganine/sphingosine (Sa/So) ratio in tissues and blood, as observed in humans and various animal species. In the Opinion on setting an HBGV for fumonisins and modified forms (EFSA CONTAM Panel, 2018b), the EFSA CONTAM Panel concluded that 'the disruption of the sphingolipid metabolism is linked at an early stage with fumonisin-induced pathologies including porcine pulmonary oedema, ELEM, liver and kidney toxicity, tumour promotion, carcinogenicity and NTDs in animal studies'. In the Opinion on fumonisins in feed (EFSA CONTAM Panel, 2018a), EFSA's CONTAM Panel conclude that *'a critical reappraisal of the literature, however, revealed that in pigs the increase in serum Sa/So may occur even in the absence of other biochemical changes or tissue lesions (Riley et al., 1993) and shows a clear time- and dose dependence (Zomborszky-Kovács et al.,* 2002a,b*). In other species (e.g. ducks), the increase in serum Sa/So seems to occur only in an early phase and could not be related to decrease in body weight or tissue lesions (Tran et al., 2006). Therefore, the CONTAM Panel considers it necessary to derive reference points for fumonisins based on endpoints other than the sole alteration of sphingolipid ratio in serum or organs'*. In addition to the effects on sphingolipid metabolism, various *in vitro* and *in vivo* studies show effects that point at oxidative stress. However, it is unclear to what extent this results in the observed adverse effects in farm animals.

3.1.3. Adverse effects in poultry, pigs and horses

The sections below describe the critical studies from the 2018 Opinion for poultry, pigs and horses, as well as newly identified studies, not evaluated in that assessment. For horses, it was decided to also

review the case studies again since more studies were identified to increase the body of evidence and regarding the fact that the previously derived NOAEL was based on an intravenous (*i.v.*) study with assumption of a low oral bioavailability, introducing considerable uncertainty in the NOAEL. For the scope of this opinion, it was considered that a reference point (RP) for adverse animal health effects better reflects the uncertainties in the studies available, which were often not suitable to derive the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group, as per definition of NOAEL. It should also be noted that for farm animals, it is more common to express the RP (or NOAEL) per kg feed rather than per kg bw per day.

3.1.3.1. Poultry

In 2005, EFSA derived an LOAEL of 2 mg/kg bw per day for poultry (EFSA, 2005). It was also concluded that the LOAELs for other poultry species were higher, being 5 mg/kg bw per day for Mallard ducks, 17 mg/kg bw per day for Peking ducklings and 9 mg/kg bw per day for turkeys.

In the EFSA 2018 Opinion, the CONTAM Panel concluded on an NOAEL of 20 mg FB_1[5]/kg feed (2 mg FB_1/kg bw per day) for chickens, based on the decrease in liver lipid content observed by Henry et al. (2000) in broiler chicks.

An NOAEL of 8 mg FB_1/kg feed was identified in ducks according to changes in serum liver enzymes indicative of liver damage; an NOAEL of 20 mg FB_1/kg feed was derived for turkeys, being the top dosage in a dose–response study in which no adverse effects were noticed at any FB_1 concentration.

A number of new studies were identified and are described below, as well as studies brought to the attention of the Panel by competent authorities.

Broilers

Studies to be reassessed

In the study by Henry et al. (2000), broiler chickens were given feed containing 0, 20, 40 or 80 mg pure FB_1/kg for 21 days. FB_1 did not affect body weight or growth in this study, but reduced feed conversion ratio at the highest tested dose. There was a dose-dependent increase in liver sphinganine levels and the Sa/So ratio in all groups. In serum, the ratio was only increased at the highest dose. Total liver lipids were decreased in chickens given 40 or 80 mg FB_1/kg feed and serum glutamate oxaloacetate aminotransaminase/aspartate aminotransferase (SGOT/ASP) ratio and feed conversion ratio were increased at 80 mg FB_1/kg feed. Serum cholesterol, triglycerides, uric acid, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were not affected by any treatment. The effects on sphingolipids were not considered as adverse in the absence of a clear relation with the adverse effects. Instead, EFSA considered the decrease in liver lipid as an adverse effect and identified an NOAEL of 20 mg/kg feed, which would correspond to 2 mg/kg bw per day.

In addition to the study by Henry et al. (2000), EFSA was asked to review the paper by Grenier et al. (2015). In this study, 1-day-old Ross broiler chickens (six replicate cages, six chicks/cage) were fed diets containing 0 (control), 5.6, 11.3, 17.5, 47.8 or 104.8 mg/kg of the sum of FB_1 and FB_2 from fungal cultures for 20 days. At 10-day intervals, six birds/treatment were sacrificed, and tissue samples were collected for subsequent analysis. Body weight and feed intake were not affected by the treatment. A dose-related increase in the Sa/So ratio was detected in liver (from 11.3 mg/kg diet at d10) and in some extrahepatic tissues, particularly in caecum (from 11.3 mg/kg diet at d20). Furthermore, contrasting effects (decrease at day (d) 10 and increase at d20) on the gene expression of proinflammatory cytokine genes were observed in the small intestines. The upregulation recorded at d20 was not dose-dependent and the largest increase was found in chickens exposed to 11.3 mg/kg feed. The effects on Sa/So ratio and gene expression of cytokines observed in this study are not considered as adverse, and hence, this study is not useful for deriving an NOAEL.

New studies

Several new studies have been published since the last Opinion (EFSA CONTAM Panel, 2018a) and are described below.

A 14-day feeding trail was conducted by Grenier et al. (2017) with 7-day-old male Ross 708 broilers (n = 36/group), which were exposed to a diet contaminated with 0.02 (control) or 11 mg FB/kg (8.2 mg FB_1 + 2.8 mg FB_2) for 14 days. The treatment had no effect on body weight gain, feed intake

[5] Pure toxin.

or feed conversion ratio. Conversely, FB-exposed birds showed an increased Sa/So ratio in both serum and liver. In addition, significantly higher gene expression of the intestinal (mid jejunum) cytokines IL-8 and IL-10 was found in the FB group, with potential negative effects on gut immunity.

Metayer et al. (2019) fed 1-day-old male Ross PM3 chicks (n = 14/group) with a diet containing 0 or 20 mg FB_1 + FB_2/kg. Individual body weight and feed consumption were measured weekly. Blood sampling was performed on the 35th day of age and animals were thereafter stunned and necropsied. Samples of heart, liver, spleen, thymus, pancreas, kidneys, testicles duodenum, jejunum, ileum, caecum, caeca tonsils and bursa of *Fabricius* were collected. Fumonisin-treated chicks did not show changes in body weight and feed consumption or alterations in weight and gross or microscopic pathology of the collected organs. Likewise, no treatment-related changes in blood haematology and serum and tissue biochemistry (oxidative stress parameters) were detected other than an increase in the liver Sa/So ratio.

In the frame of a study aimed at evaluating the TK of hydrolysed fumonisin B_1 (HFB_1), 16 male Ross 308 broiler chicks were fed for 14 days either a control or a fumonisin-contaminated diet at concentrations below the EU guidance level (10.8 mg FB_1, 3.3 mg FB_2 and 1.5 mg FB_3/kg feed). None of the chicks showed signs of toxicity during the trial or changes in feed intake and final body weight (Antonissen et al., 2020).

In another study (Galli et al., 2020), male 1-day-old Cobb500 chicks (n = 10/group) were administered a diet containing 0 or 600 mg fumonisin/kg for 10 days. Compared to controls, fumonisin exposure resulted in lower body weight and weight gain, as well as in changes in serum biochemistry such as an increase in cholesterol, uric acid and transaminases. In liver, treated birds displayed an increase in reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS) and glutathione S-transferase (GST) and a decrease in catalase and superoxide dismutase.

Sousa et al. (2020) treated 1-day-old male Cobb 500 broiler chicks (n = 20/group) with 0.26 (control), 2.5 (group 1), 5 (group 2) or 10 (group 3) mg fumonisin/kg diet for 10 days, starting at d12 of life. Fumonisins were extracted from corn inoculated with *Fusarium verticilloides* isolates and measured with a liquid chromatography – fluorescence method following immunoaffinity clean-up and post-column derivatisation. No other mycotoxins were analysed. After 5 days of treatment (d17) and at the end of the treatment (d21), 10 birds from each group were sacrificed for blood and tissue collection. At d21 but not d17, there was a dose-related decrease in body weight reaching statistical significance in birds exposed to the highest dosage (10 mg/kg diet). No histological lesions were detected in lung, spleen and liver from all challenged broilers, but changes related to oxidative stress were detected in groups 2 and 3. In particular, there was a rise in liver ROS and lipid peroxide (LPO) content along with a fall in GSH-Px and GST activity (group 3 only). A similar trend was observed in serum, treated birds (groups 2 and 3) showing an increase in ROS and GST and a decrease in GSH-Px, while LPO levels were actually decreased. Despite the absence of histological changes in the gut (unspecified tracts), villus height (group 3) and crypt depth (all treated groups) were decreased after treatment for 10 days but not 5 days. Taken together, an LOAEL of 2.5 mg fumonisin/kg diet (estimated to correspond to 0.4 mg/kg bw per day) could be derived based on reduced intestinal crypt depth. For the reduction in weight gain, an NOAEL of 5 mg/kg feed was identified.

The effects of fumonisins at their EU guidance value in feed (20 mg/kg) on gut health and integrity was tested on 1-day-old male Ross 308 broilers (n = 126 per group) by Paraskeuas et al. (2021). Fumonisins were commercially produced by *Fusarium verticillioides* and subsequently purified; the final concentrations in the experimental feed[6] liquid chromatography – mass spectroscopy (LC–MS/MS) technique. At the end of the treatment (21 days), animals were sacrificed, and samples of duodenum, jejunum, ileum and ceca were collected. Compared to untreated controls, fumonisins caused a decrease in both body weight gain and feed intake. In addition, treated birds displayed site-specific significant changes in gene expression pointing to a worsening of the antioxidant response and barrier integrity.

Laying hens

Studies to be reassessed

No studies for laying hens were submitted for assessment within the supporting documentation.

[6] FUM starter diet = 20,002 ± 2002 μg FB_1 and 6,183 ± 742 μg FB_2/kg; FUM grower diet = 14,748 ± 1,475 μg FB_1 and 5,995 ± 720 μg FB_2/kg; and FUM finisher diet = 8,134 ± 976 _ μg FB_1 and 5,959 _ 715 _ μg FB2/kg.

In the only retrieved study by EFSA 2018 (Siloto et al., 2013), 37-week-old Hisex Brown laying hens were offered either a control diet or a diet containing 25 mg FB_1 + FB_2/kg feed for 56 days (two cycles of 28 days, six replicates, with four birds per replicate for each treatment group). The treatment, corresponding to 1.6 mg/kg bw per day, did not affect performance, blood lipids or plasma lipid cholesterol but caused a shortening of the small intestines (1.37 vs. 1.57 m) and a sharp decrease (around 50%) in liver fat content.

New studies

A study by Tomaszewska et al. (2021) was performed with 32 9-week-old Isa Brown chickens reared for laying, which were daily dosed (intra-crop) with 0 (53 μg/kg feed), 1.0 mg/kg bw, 4.0 mg/kg bw or 10.9 mg/kg bw of an FB_1 + FB_2 (73:27) containing extract for up to 21 days. Fumonisins were synthetised on maize grain inoculated with *Fusarium moniliforme* and measured with an LC-fluorescence method. The 1.0 mg/kg bw dose corresponded to a feed concentration of 20 mg/kg (EU guidance value), the 4.0 mg/kg bw/day (80 mg/kg feed) to twice the NOAEL calculated by EFSA for broiler chicks (2 mg/kg bw, EFSA CONTAM Panel, 2018a) and the 10.9 mg/kg bw one (218 mg/kg feed) was the median LD_{50} calculated from a preliminary trial lasting 21 days and involving chickens of the same age and breed. At the end of the study, all hens were sacrificed and blood samples were collected. Liver, duodenum, jejunum and both the tibiae were also collected. Treated birds had lower body weight ($p < 0.001$) irrespective of the FB_1-FB_2 dosage. No consistent dose-related changes were observed concerning basal blood haematology and serum biochemical parameters. For example, AST was unchanged, while ALT and ALP were increased only at the highest dosage and Ca decreased in the 1.0 mg/kg bw group only; a decrease in Cu and Mg was noted in the 4.0 and 10.9 mg/kg bw groups. By contrast, fumonisins damaged the epithelial integrity of the duodenum and jejunum in all treated groups, as assessed by a number of histomorphometric parameters (villi and crypt height and width, and their ratios) and the disruption of the villi epithelium in histological preparations. Liver changes included an increase in the number of apoptotic hepatocytes (1.0 mg/kg bw group), swollen hepatocytes with ballooning degeneration (4.0 mg/kg group) and cytoplasmic vacuolisation and signs of cirrhosis (10.9 mg/kg bw group). Finally, changes in bone structure and density and a general increase in the amount of immature collagen fibres were observed in tibiae of treated hens, which may be interpreted as an intensified bone turnover. In conclusion, the 21-day exposure of chicken reared for laying to fumonisin at 20 mg/kg feed (LOAEL) resulted in a number of adverse effects including a decrease in body weight along with disruption of enteric epithelial integrity as well as in liver and bone histological and structural changes. Table 3 summarises the new studies on adverse effects in poultry.

Table 3: New studies on adverse effects on poultry which have become available since the 2018 Opinion (EFSA CONTAM Panel, 2018a)

N/group, breed gender	Dosage and duration	Endpoint(s)	NOAEL/LOAEL (mg/kg feed)	Reference
10, male Cobb 500	0, 600 mg/kg diet 10 days	BW, WG↓ serum and liver oxidative stress	LOAEL 600 mg/kg feed	Galli et al. (2020)
36, male Ross 708 broilers	0, 11 mg/kg diet for 14 days	BW, WG, FCR unchanged Sa/So ratio in serum and liver ↑ Intestinal cytokines ↑	TBD	Grenier et al. (2017)
20, male Cobb 500	0, 2.5, 5 or 10 mg FB_1/kg diet from day 12 to day 21 (10 days)	Feed intake, weight gain↓ Serum and liver oxidative stress parameters↑ Liver, gut, spleen and lung histology Villi height and crypt depth↓	LOAEL 2.5 mg/kg feed for decreased crypt depth; NOAEL 5 and LOAEL 10 mg/kg feed for decreased weight gain	Sousa et al. (2020)
126, 1-day-old male Ross 308 broilers	0, 20 mg FB_1/kg diet (+ FB_2) for 21 days with 3 different diets (starter, grower, finisher)	Body weight gain and feed intake ↓ GENE expression of antioxidant response, stress, inflammation, and integrity of different enteric segments ↓	LOAEL 20 mg/kg feed	Paraskeuas et al. (2021)

N/group, breed gender	Dosage and duration	Endpoint(s)	NOAEL/LOAEL (mg/kg feed)	Reference
8, 9-week-old Isa Brown hens (chicken reared for laying)	0, 1.0, 4 or 10.9 mg/kg bw of the FB_1 + FB_2 extract for up to 21 days (intracrop) 1 mg/kg bw à 20 mg/kg diet, 4 mg/kg bw à 80 mg/kg diet 10.9 mg/kg bw à 218 mg/kg diet	WG ↓ enteric villi and crypt height ↓ liver histological changes, changes in bone structure and composition	LOAEL 20 mg/kg feed	Tomaszkievska et al. (2021)

N: Number; LOAEL: Lowest Observed Adverse Effect Level; NOAEL: No Observed Adverse Effect Level; Sa/So: Sphinganine/ Sphingosine; BW: body weight; WG: weight gain; FCR: feed conversion ratio.

Turkeys

Studies to be reassessed

Two of the three studies provided in the supplementary file were already evaluated by EFSA in 2018. These studies (Benlasher et al., 2012; Tardieu et al., 2007) showed only an effect on the sphingolipid ratio, which is not considered as adverse. The study by Masching et al. (2016) aimed at investigating the effect of adding carboxylesterase FumD to the feed, only evaluating the effect on the sphingolipid ratio in serum. This ratio was increased by the fumonisins (15 mg/kg FB_1 + FB_2), but is not considered as adverse.

New studies

The limited literature search described in Section 2.2.1 did not identify any new studies on toxic effects of fumonisins in feed in turkeys.

Conclusions on poultry

- For chicken new studies were identified, some showing effects at lower feed levels than the previously derived NOAEL of 20 mg/kg feed.
- Based on reduced weight gain, an NOAEL of 5 mg/kg feed was derived.
- In addition, also effects on intestines were observed, in particular a decrease in crypt depth with an LOAEL of 2.5 mg/kg feed, being the lowest dose applied. Applying a default UF of 3, the CONTAM Panel derived an RP for adverse animal health effects ('NOAEL') of 1 mg/kg feed.
- The reviewed studies for turkeys that were previously assessed did not result in a change in NOAELs.

3.1.3.2. Pigs

In the previous assessment, EFSA (EFSA CONTAM Panel, 2018a) concluded that porcine pulmonary oedema syndrome is the specific effect produced by FB_1 in pigs and that cardiovascular effects of FBs could play a role in the development of this abnormality. Increased sphinganine/sphingosine (Sa/So) ratio in serum and tissues, liver and kidney toxicity, delay in sexual maturity and reproductive functionality alterations, impairment of innate and acquired immune response, histological lesions in internal organs as well as alterations of brain physiology have been reported in many studies irrespective of the FBs concentration. An NOAEL of 1 mg FBs/kg feed and an LOAEL of 5 mg/kg feed could be identified for pigs based on lung lesions observed in a study by Zomborszky-Kovács et al. (2002a).

Studies to be reassessed

Rotter et al. (1996) exposed 16 female and 16 castrated-male pigs for 8 weeks to feed contaminated with pure FB_1 at 0, 0.1, 1 and 10 mg/kg. In males fed increasing concentrations of FB_1, an almost significant linear decrease in average daily gain was observed. In this sex, a significant

difference among the diets was only observed at week 1. In females, no significant differences in weight gain were observed throughout the experiment.

In a further study (Rotter et al., 1997), the authors exposed 32 castrated-male pigs to feed contaminated with pure FB_1 at 0, 0.11, 0.33 and 1 mg/kg feed from 1 week post-weaning until market weight. There were no significant dose-related differences in growth rate, feed consumption and feed efficiency among different diets. In addition, no significant differences were observed among treatments for any of the carcass quality parameters. However, at the highest dose (1 mg/kg feed), a variable feed consumption and an increased variability in carcass quality characteristics (fat/lean deposition in loin and ham) as compared with controls were observed.

Zomborszky-Kovács et al. (2002a) used fungal culture of *Fusarium moniliforme* to prepare diets and exposed barrows for 4 weeks to feeds contaminated at 0, 10, 20 and 40 mg/kg. They also exposed barrows for 8 or 20 weeks to feeds contaminated at 0, 1, 5 and 10 mg/kg. The authors did not report the exact concentration of toxins in the final diet. No significant effect on feed consumption, body weight gain and feed conversion were observed whatever the concentration or the duration of the exposure. The highest doses (20 and 40 mg/kg feed) induced a time- and dose-dependent increase in the serum AST activities. After 8 days of exposure to diet contaminated with 10, 20 or 40 mg FB_1/kg, a dose-dependent increase in the Sa/So ratio was observed in the serum of the animals, and in the second part of the study after 15 days for the doses of 5 and 10 mg/kg feed. Mild to severe pulmonary oedema was observed in animals exposed to 10–40 mg FB_1/kg feed. In addition, a dose-related increase in macroscopic alterations in the lung was observed, starting already at 1 mg/kg feed but without other clinical signs. No statistical analysis was performed and controls were not shown. For the low doses, the incidence increased with time. The authors themselves concluded that an NOAEL of 1 mg/kg feed could be derived from their study.

New studies

New studies have been published since the last CONTAM Panel Opinion (EFSA CONTAM Panel, 2018a). Table 4 summarises these new studies on adverse effects in pigs.

Terciolo et al. (2019) fed for 28 days weaned castrated male piglets with a diet containing 0, 3.7, 8.1 and 12.2 mg/kg of FB_1 + FB_2. Dietary exposure to 3.7 mg FBs/kg feed significantly increased the plasma Sa/So ratio and induced histological alterations in the heart and the intestine. Exposure to 8.1 and 12.2 mg FBs/kg feed did not significantly increase the Sa/So ratio but induced histological alterations in the heart, the intestine, the kidney and for the highest dose also in the lung, lymphoid organs and the liver. This last dose also induced an increase of plasma triglyceride and urea concentrations.

A 4-week feeding trial (Regnier et al., 2017) was conducted with 12 castrated male pigs (n = 6/group) which were exposed to a control diet or a diet contaminated with 14.2 mg FBs/kg feed (10.2 mg FB_1 + 2.5 mg FB_2 + 1.5 mg FB_3/kg). Exposure to the toxin decreased body weight gain, increased the Sa/So ratio and altered the transcriptome and the phosphorylation of proteins in the intestine and the liver. The authors also observed a decreased antibody response upon vaccination with *Mycoplasma hyopneumoniae*. The same trial also revealed an alteration of the intestinal microbiota with a decrease in the diversity index, and shift and a constrain of the structure and the composition of the bacterial community.

An alteration of the intestinal microbiota was also observed in piglets receiving feed contaminated with 17 mg FB_1 /kg for 9 days (Dang et al., 2019).

In another study (Szabo et al., 2020), weaned piglets received for 21 days a control diet or a diet contaminated with 15 or 30 mg FBs (FB_1 + FB_2 + FB_3) /kg feed. When compared to control, final body weight was higher in the 30 mg/kg group. The authors also observed that exposure to the toxin affected the activity of the red cell membrane sodium pump and the lipid profile of these cells.

Rao et al. (2020) provided dietary fumonisin (FB_1 + FB_2) concentrations of 7.2, 14.7, 21.9, 32.7 and 35.1 mg/kg feed to 350 pigs for 28 days; no control group was included. Increasing toxin concentration decreased average daily gain, average daily feed intake and gain/feed ratio. Increasing FB_1 + FB_2 concentrations also increased the serum Sa/So ratio on days 14 and 28 of the trial.

Conclusions on pigs

- The older studies that have been reassessed did not warrant a change of the previously derived NOAEL of 1 mg/kg feed.
- No further dose–response studies suitable for deriving a lower NOAEL could be identified from the papers published since the last EFSA opinion in 2018.
- Therefore, the RP for adverse animal health effects in pigs remains 1 mg/kg feed.

Table 4: New studies on adverse effects in pigs which have become available since the 2018 Opinion (EFSA CONTAM Panel, 2018a)

N/group, breed gender	Dosage and duration	Endpoint(s)	Notes	NOAEL/LOAEL (mg/kg feed)	Reference
6, castrated males	0, 3.7, 8.1 and 12.2 mg/kg diet for 28 days	Sa/So ratio and biochemistry in plasma. Histological analysis of intestine, heart, lung, lymph node, spleen, kidney and liver		LOAEL 3.7 mg/kg feed based on Sa/So ratio and histological observation of the intestine and the heart	Terciolo et al. (2019)
6,castrated males	0, and 14.2 mg FB_1 + FB_2 + FB_3/kg diet for 28 days	Weight gain, villus height in the intestine, Sa/So ratio. Antibody response to a vaccinal antigen, biochemistry in the plasma, gene expression in the liver and the intestine		One very high dose (14.2 mg FB_1 + FB_2 + FB_3 /kg diet) with effect	Regnier et al. (2017)
6, castrated males	0, and 14.2 mg FB_1 + FB_2 + FB_3/kg diet for 28 days	Microbiota analysis		One very high dose (14.2 mg FB_1 + FB_2 + FB_3/kg diet) with effect	Mateos et al. (2018)
70 pigs	7.2, 14.7, 21.9, 32.7 and 35.1 mg/kg diet for 28 days	Weight gain, feed intake, Sa/so ratio	No control	Impossible to conclude as no controls were included in the experiment	Rao et al. (2020)
6, pigs	0, 15 and 30 mg/kg diet for 21 days	Animal and organ weight gain, red cell membrane sodium pump activity, red cell fatty acid profile, red cell antioxidant status and lipid peroxidation		One very high dose (15 mg/kg diet) with effect	Szabo et al. (2020)
6, pigs	0, 17 mg/kg diet for 9 days	Intestinal microbiota		One very high dose (17 mg/kg diet) with effect	Dang et al. (2019)

N: Number; LOAEL: Lowest Observed Adverse Effect Level; NOAEL: No Observed Adverse Effect Level; Sa/So: Sphinganine/ Sphingosine.

3.1.3.3. Solipeds

No controlled study is available from which an NOAEL can be derived for solipeds orally exposed to fumonisins. Case reports showing the general sensitivity of solipeds to fumonisins were already discussed by EFSA in 2005 and 2018. Due to the lack of information on feed consumption, case reports were not considered suitable for deriving an LOAEL or NOAEL for horses and other solipeds (EFSA 2005, EFSA CONTAM Panel 2018a). Hence, the pivotal dose–response study reported by Smith et al. (2002) and Foreman et al. (2004) was used to derive an NOAEL of 0.01 mg/kg bw per day, translated to an oral NOAEL of 0.2 mg/kg bw per day, based on an oral bioavailability of 5% which has to be considered as a source of uncertainty. Using a feed intake of 9 kg per day and body weight of 450 kg, this was translated to a RP for adverse animal health effects, referred to as NOAEL in the previous opinion, of 8.8 mg/kg feed.

Literature research revealed no further controlled dose–response experiments since the last opinion of EFSA in 2018. For these reasons, some of the older case reports and experiments already highlighted by EFSA in 2005 and 2018 from a different perspective were reviewed again together with some new case reports published since 2018. Table 5 summarises the case reports and controlled experiments on adverse effects in solipeds.

In general, both case reports and controlled experiments often lack information on feed intake (Ross et al., 1991; Thiel et al., 1991; Sydenham et al., 1992) or roughage intake, mostly offered as hay for ad libitum consumption (Wilson et al., 1992), making it impossible to calculate the total FB concentration of the entire diet. However, it might be justified to conclude that the toxicologically

effective doses were probably several factors lower in the total ration than in the suspected feedstuffs. This conclusion is further supported by the fact that intake of roughage is substantially higher than that of concentrate feed. In the previous opinion, a minimum of 50% roughage intake was assumed by EFSA CONTAM Panel (2018a) to estimate the exposure of horses. Further uncertainty is introduced by the different materials identified as the source of fumonisins that were not fully characterised or characterisable for the entire panel of fumonisins and their modified forms and other mycotoxins.

In one of the few controlled experiments, Wilson et al. (1992) used corn screenings as a source of FB_1 which were offered to ponies together with the compound feed proportion of the diet while alfalfa hay was offered freely. Thus, the reported FB_1 levels referred to the feed concentrate only. Consequently, the FB_1 level of 8 mg/kg feed inducing histopathological brain lesions in the ponies typically for equine leucoencephalomalacia (ELEM) would have been lower when expressed on a whole ration including hay. This feed level was calculated to correspond to a dose of 0.13 mg/kg bw per day in phase 2 of the study when the corn screenings were fed at 1.6% of the body weight. Although FB_2 was reported to be consistently present in the corn screenings at 32%, the doses were solely reported as FB_1. A second group of ponies were exposed to FB_1 intermittently whereby feed FB_1 concentrations of < 1 mg and 22 mg/kg were alternated irregularly. One pony of this group died of ELEM suggesting the 22 mg/kg feed (0.18 mg FB_1/kg bw per day) to be effective in inducing ELEM under this specific dosing regimen.

In another controlled study, two horses, a filly and a colt weighing 150 and 190 kg, respectively, were exposed to daily FB_1 doses from 0.625 to 2 mg/kg bw, and 0.95 to 3.8 mg/kg bw (Kellerman et al., 1990). These daily doses were administered over periods of 33 and 29 days distributed to a total of 21 and 20 doses irregularly spread over the experimental periods. Both horses developed clinical signs of ELEM which were confirmed by corresponding pathological and histopathological findings. Assuming a total feed intake of 3.5% of the body weight (EFSA CONTAM Panel, 2018a) for growing horses, the daily FB_1 doses corresponded to dietary FB_1 concentrations of 18–57 mg/kg and 29–114 mg/kg for the filly and the colt, respectively. Although the dosing regimen mimicked a more practical scenario, i.e. varying diet concentrations of FB_1, which can be expected when longer feeding periods are considered, the lack of a strict dose–response design does not allow to derive an NOAEL from this controlled experiment.

Considering case studies, Thiel et al. (1991) described a survey of cases of ELEM in the USA confirmed by clinical, pathological and histopathological findings. The suspected feed included 10 commercial feed and four corn samples. The sum of FB_1 and FB_2 concentrations ranged between 1.4 and 39.6 mg/kg (FB_1 proportion = 80–96%) suggesting the possibility of doses lower than the current guidance value of 5 mg FB_1 + FB_2/kg to be effective in inducing ELEM. Similarly, Ross et al. (1991) identified concentrations between 1 and 126 mg FB_1/kg in sweet feeds (a type of concentrate feed containing molasses and a blend of pellets, corn, oats, corn screenings and/or additives) and pure corn that were effective in induction of ELEM, confirmed by high morbidity and even higher mortality in diseased animals, together with pathological and histopathological findings.

Twelve cases of confirmed ELEM in Brazil were associated with FB_1 and FB_2 concentrations in a range between 0.2–38.5 mg/kg and 0.1–12.0 mg/kg, respectively, in various feed sources (corn, corn residues, straw, black oats, mixed feed) (Sydenham et al., 1992), further supporting the view that ELEM might be related to FB_1 + FB_2 levels lower than the current guidance value and NOAEL.

The FB_1 concentrations that caused the death of 100 donkeys from various regions of Mexico ranged between 0.67 and 28.5 mg/kg (Rosiles et al., 1998). Three out of these 100 donkeys were examined for pathological confirmation of ELEM.

These reports (Ross et al., 1991; Thiel et al., 1991; Sydenham et al., 1992; Rosiles et al., 1998) included several cases from various geographical regions with a corresponding variation in FB_1/FB_2 concentrations in the suspected feedstuffs, thus allowing to draw some conclusions on feedstuff levels effective in inducing ELEM. In contrast, individual case reports are characterised by specific FB_1/FB_2 concentrations representative for the actual case only. Consequently, those individual case reports are less suited to estimate minimum effective doses but can be arranged in the overall range covered in the literature.

An individual case was reported where four horses died of ELEM that had freely access to native grass and were fed 2 kg of corn per animal per day shown to contain 46 and 53 mg FB_1/kg (Mallmann et al., 1999). Pereira dos Santos et al. (2013) identified a feedstuff concentration of 6.6 mg/kg to be related to confirmed cases of ELEM of six horses and four mules. Seven of 60 horses grazing at pastures and fed additionally a mixture of corn and wheat bran ad libitum were affected by ELEM whereby the concentrate feed supplement contained 12.5 mg FB_1/kg and 5.3 mg FB_2/kg (Giannitti

et al., 2011). Cases of ELEM were reported for a farm in Brazil which was associated with the feeding of freshly harvested immature corn with a fumonisin concentration of 2 mg/kg (Echenique et al., 2019). No details on the analysis were provided and fumonisins were not further specified. Additional uncertainty arises from the feeding practice in that freshly harvested grains are usually not fed to livestock because of possibly causing digestive disorders.

Six horses from three locations in Mexico that had been fed diets containing 50% corn stover contaminated by a total fumonisin concentration of 1.80, 5.23 and 4.54 mg/kg were affected by ELEM, as clinically, pathologically and histopathologically confirmed (Reyes-Velázquez et al., 2018). Based on 50% corn stover in the ration, the total dietary fumonisin concentrations were estimated at 0.90, 2.62 and 2.27 mg/kg. Taking into account the feed consumption and body weight of the animals, the corresponding exposure varied between 0.02 and 0.09 mg/kg bw per day. Interestingly, the lowest estimated oral exposure of 0.02 mg fumonisins/kg bw per day causing ELEM was 10-fold lower than the NOAEL of 0.2 mg FB_1/kg bw per day derived by EFSA CONTAM Panel (2018a).

Based on the described case reports and controlled studies, the range of the lowest concentrations of FBs in compound feed shown to be effective in inducing ELEM are summarised in Table 5. Assuming a maximum intake of 50% compound feed of complete feed intake, these values were transformed to minimum and maximum adverse effect concentrations of complete feed. Taking these minimum and the maximum adverse effect concentrations into consideration, medians (min-max) of 3.3 (0.1–29) and 19 (1–114) mg/kg complete feed would suggest the range of adverse effect concentrations to be expected. It has to be considered that a contribution of 50% compound feed to the total ration is not a conservative assumption. These proportions are probably markedly lower than 50% in the majority of practical feeding situations resulting in putatively lower adverse effect concentrations.

Table 5: Characterisation of case reports and controlled experiments with regard to fumonisin concentrations in compound feed or feed materials shown to be responsible for cases of ELEM in solipeds, as confirmed by histopathological findings

Compound feed/feed material	Fumonisins	Affected Equidae	Lowest/highest concentration in feed (mg/kg as fed)	Adverse effect concentration[(a)] (mg/kg diet)	Type of study	Reference
Corn Screenings	FB_1 + FB_2	18 horses	39–145 (sum) (0.6–2.1 mg FB_1/ kg bw per day)	20–73	Case report	Wilson et al. (1990)
Corn Screenings (2 groups)	FB_1	4 ponies 5 ponies	15–22 8 (two %bw rations) (0.06–0.18 mg/kg bw per day)	4	Controlled experiment	Wilson et al. (1992)
(Pure toxin p.o.)	FB_1	2 horses	0.625–2 mg/kg bw[(b)] 0.95–3.8 mg/kg bw[(b)]	18–57[(c)] 29–114[(c)]	Controlled experiment	Kellerman et al. (1990)
Commercial feed (n = 10), corn (n = 4)	FB_{1+2}	14 horses	1.4–23.3 (feed) 8.4–39.6 (corn)	0.7–20	Case report (cases not described)	Thiel et al. (1991)
Sweet feed, corn	FB_1	45 horses	1–126	0.5–63	Case report	Ross et al. (1991)
Corn, corn residues, straw, black oats, mixed feed	FB_{1+2}	12 horses	0.2–38.5 0.2–50.5 FB1 + FB2	0.1–19	Case report	Sydenham et al. (1992)
Corn	FB_1	> 100 donkeys	0.67–13.3	0.3–7	Case reports	Rosiles et al. (1998)
Corn	FB_1	4 horses	46–53	23–27	Case report	Mallmann et al. (1999)

Compound feed/feed material	Fumonisins	Affected Equidae	Lowest/highest concentration in feed (mg/kg as fed)	Adverse effect concentration[(a)] (mg/kg diet)	Type of study	Reference
Corn animal feed used as food supplement	FB (no description analysis)	6 horses, 4 mules	6.6	3.3	Case report	Pereira dos Santos et al. (2013)
Corn, wheat bran	FB_{1+2}	7 of 60	17.8	9		Giannitti et al. (2011)
Immature corn	FB (no description analysis)	2 horses	2	1	Case report	Echenique et al. (2019)
Corn stover (at least 50% of ration)	FB	1 pony, 5 horses	1.8 (pony) 4.5 and 5.2 (horses) (0.02–0.09 mg/kg bw per day)	0.9 2.3 and 2.6	Case report (with more details on bw and feed intake)	Reyes-Velázquez et al. (2018)

N: Number.
(a): Estimated adverse effect concentration for complete feed assuming a proportion of compound feed of 50% of the whole diet.
(b): Irregular dosing regimen.
(c): Assuming a feed intake level of 3.5% of body weight.

Conclusions in solipeds

- No further controlled dose–response studies, suitable for deriving an oral NOAEL, could be identified since the last EFSA opinion in 2018.
- Both older and recent case reports strongly suggest that ELEM might be induced by fumonisin concentrations lower than the current NOAEL of 8.8 mg/kg diet (EFSA CONTAM Panel, 2018a) and the current guidance value of 5 mg FB_1 + FB_2/kg diet (European Commission, 2006).
- Estimated adverse effect doses expressed per kg bw per day, based on body weight and feed intake, in some of the studies are in a similar range as the NOAEL and LOAELs of, respectively, 0.01 and 0.05 mg/kg bw per day observed after i.v. dosing in the study used by EFSA to derive an NOAEL in the previous opinion. In some case studies, they are lower than the oral NOAEL of 0.2 mg/kg bw per day derived by EFSA based on a bioavailability of 5%.
- Also other case studies, where the feed consumption was not clear, show effective concentrations clearly below 8.8 mg/kg feed or feed material. Therefore, case reports should be taken into account in the overall risk assessment.
- The presence of modified forms of fumonisins in naturally contaminated feedstuffs might be a source of uncertainty and may have contributed to low fumonisin concentrations being effective in inducing ELEM. In addition, other mycotoxins produced by *Fusarium* species may have been present in the feed although a role in inducing ELEM is currently unclear.
- Both controlled experiments and case reports often lack information on total feed intake and fumonisin concentrations reported for suspected feedstuffs, which makes extrapolation to the daily ration and actual exposure of the animals difficult.
- The minimum fumonisin concentrations in individual feedstuffs which were connected to ELEM in case reports should be divided by at least 2 to provide an estimate for the concentration of the whole daily ration. The factor of 2 accounts for a minimum roughage proportion of 50% of the daily ration (EFSA CONTAM Panel, 2018a) for those studies where no feed intake was recorded.
- Regarding the uncertainty in the reported adverse effect concentrations in these case studies, it was decided not to use the lowest reported adverse effect concentrations but the median of 3.3 mg/kg feed of the minimum reported levels in the various studies. Applying a default uncertainty factor of 3, the CONTAM Panel derived a reference point for adverse animal health effects of 1 mg/kg feed.

3.2. Feed occurrence data

The collection of new potentially available data on feed occurrence was outside the remit of the present mandate. With the aim of revising the risk characterisation, in view of the revised NOEALs for horses and poultry, the Panel referred to the feed occurrence data included in the 2018 Opinion, which should be consulted for further detail.

In the 2018 Opinion, fumonisins and their modified forms were reported to be predominantly found in cereal crops, cereal grains and by-products of cereal processing, which are widely used as feed for livestock. Fumonisins and modified forms were also identified in certain forages, in particular maize silage, which for certain livestock are often the sole feed. Hence, intake of forages was also estimated to assess the likely exposure.

For the full details on feed occurrence data underpinning the exposure assessment of the 2018 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2018a).

3.3. Exposure assessment

In the 2018 Opinion (EFSA CONTAM Panel, 2018a), two approaches were followed. Where information was available, the mean and P95 levels of fumonisin contamination in compound feed were used to estimate mean and high exposure. This was for example applied for horses. In cases where this information was not available, the fumonisin concentrations of individual feed materials were taken into account, together with example diets, to estimate P95 and mean exposure, both LB and UB.

For horses, the exposure was based on concentrations in 115 samples of compound feed for horses with very few detected levels of FB_1 and FB_2 (98 and 96% LC, respectively). This compound feed was assumed to contribute 50% to the feed intake, the other 50% coming from dried hay. There was a 10-fold difference between UB and LB exposure of horses, both at the mean and P95 exposure, indicating a large uncertainty. This is caused by the rather low levels in feed and hay, resulting in a high fraction of LC data.

For chickens, insufficient data on levels in compound feed were available and levels in feed were calculated based on example diets and levels in individual feed materials. Differences between LB and UB exposure were less than twofold, indicating much less uncertainty than for horses.

For the full details on the exposure assessment performed for fumonisins in the 2018 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2018a).

3.4. Risk characterisation

In 2018, the EFSA Panel estimated for a number of farm livestock and companion animal categories the chronic exposure to fumonisins (expressed as the sum of FB_1, FB_2 and FB_3) in feed at the mean and 95th percentile concentrations in animal diets based on expected feed intakes and, if required, example diets. The exposure to the sum of fumonisins and hidden forms was derived using a multiplying factor of 1.6. The risks for a number of livestock categories were characterised by comparing the reference points (NOAELs or LOAELs) against the calculated exposure (please refer to the EFSA CONTAM Panel, 2018a for further details).

For the scope of the present mandate, the newly derived RP for adverse animal health effects/ adverse effect concentration in feed for horses and chickens have been compared against the respective exposure values derived in the 2018 Opinion. The comparison is included in Tables 6 and 7 below. Exposure estimates, both UB mean and 95th percentile, are presented together with RP/ adverse effect concentration for solipeds (horses) and poultry (chickens), which were revised by the Panel in the present scientific Opinion. The estimates of exposure to FBs and the sum of FBs and their hidden forms are presented in Section 3.3 of the 2018 Opinion (EFSA CONTAM Panel, 2018a).

Table 6: Comparison of estimated FBs exposure levels and RP/adverse effect concentration for chickens and horses

Animal species	RP (mg FBs/ kg feed)	Adverse effect concentration (mg FBs/kg feed)	Estimated exposure (mg FBs/kg feed)[(a)]		Estimated exposure, % of RP/adverse effect concentration	
			P95 (UB)	Mean (UB)	P95 (UB)	Mean (UB)
Chickens[(b)]	1	2.5	1.54	0.51	154/62	51/20
Horses	1	3.3	0.20	0.18	20/6	18/5

RP: Reference point (for adverse animal health effects); FB: fumonisin B; UB: upper bound; –: not available.
(a): Exposures have been calculated from dietary concentrations expressed on a fresh weight (88% dry matter) basis to make them comparable with the data from which RP/adverse effect concentration have been derived.
(b): For both the mean and P95 exposure, the highest exposure values were used. For the mean, it corresponds to species-specific compound feed and for the P95 to a maize silage-based diet.

For **chickens,** the estimated exposure to FBs at the UB mean or 95th percentile was 51% and 154% of the NOAEL derived based on a decrease in intestinal crypt depth, indicating a concern.

For **horses**, the calculated chronic exposures at the UB mean and UB 95th percentile were 18% and 20% of the identified reference point for adverse animal health effect, respectively, indicating that the risk for chronic adverse health effects from feed containing FBs is low for horses.

Table 7: Comparison of estimated FBs + hidden forms exposure levels and NOAELs/LOAELs or RP/adverse effect concentration for chickens and horses

Animal species	RP (mg toxins/ kg feed)	Adverse effect concentration (mg toxins/kg feed)	Estimated exposure (mg FBs/kg feed)[(a)]		Estimated exposure, % of RP/adverse effect concentration	
			P95 (UB)	Mean (UB)	P95 (UB)	Mean (UB)
Chickens[(b)]	1	2.5	2.46	0.81	246/98	81/32
Horses	1	3.3	0.31	0.29	31/9	29/9

RP: Reference point; FB: fumonisin B; UB: upper bound; –: not available.
(a): Exposures have been calculated from dietary concentrations expressed on a fresh weight (88% dry matter) basis to make them comparable with the data from which NOAELs/LOAELs (or RP/adverse effect concentration) have been derived.
(b): For both the mean and P95 exposure, the highest exposure values were used. For the mean, it corresponds to species-specific compound feed and for the P95 to a maize silage-based diet.

Risk characterisation for FBs and their hidden forms (Table 7) was based on UB exposure. The estimated exposures were compared with the RP/adverse effect concentration identified for FBs, as hidden forms can be disrupted leading to FBs.

For **chickens,** the estimated exposure of FBs and their hidden forms at the UB mean or 95th percentile was 81% and 246% of the RP derived based on a decrease in intestinal crypt depth, indicating a concern.

For **horses**, the calculated chronic exposure at the UB mean and UB 95th percentile was 29% and 31% of the identified reference point for adverse animal health effect, respectively, indicating that the risk for chronic adverse health effects from feed containing FBs is low for horses.

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, its limited scope and the short deadline provided for its adoption, only a brief evaluation could be carried out focusing on the particular uncertainties in 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:

- Qualitative and quantitative species-related differences have been reported for TK of fumonisins; however, scant information is available for chickens, while no information could be retrieved for horses or other solipeds.

- Toxicity data were often obtained by using naturally contaminated material which may contain also modified forms and other mycotoxins.
- Example animal diets were used to calculate animal exposure (e.g. horses). In practice, there is a high variability of feedstuffs used and feeding systems for livestock.
- Uncertainty on the representativity of the samples and hence the levels that caused the effects in case studies.
- No robust toxicological data were available for horses or other solipeds.
- The estimated LB levels in the diet were 10-fold lower than the UB levels, indicating a large uncertainty in the exposure assessment for horses.

The overall uncertainty incurred with the present assessment is high.

4. Conclusions

Adverse effects in farm animals

- The CONTAM Panel confirmed the previous conclusion that a change in the Sa/So ratio should not be regarded as adverse by itself.

Poultry

- New studies were identified that showed adverse effects on the intestine at feed levels lower than the previously established NOAEL of 20 mg/kg feed.
- An LOAEL of 2.5 mg/kg feed was established for chickens based on reduced intestinal crypt depth.
- Applying an uncertainty factor of 3, the CONTAM Panel derived a RP for adverse animal health effects ('NOAEL') of 1 mg/kg feed for chickens.
- For turkeys and ducks, the NOAEL remained as before, i.e. 20 and 8 mg/kg feed, respectively.

Pigs

- Information submitted as supporting documentation of the mandate has been reassessed and did not warrant a change in the NOAEL/LOAEL.
- No further dose–response studies suitable for deriving a lower NOAEL could be identified since the last EFSA opinion in 2018.
- Therefore, the RP for adverse animal health effects for pigs remains 1 mg/kg feed.

Solipeds

- For horses and other solipeds, more weight was given to case studies, including several new reports.
- For the various cases of equine leucoencephalomalacia (ELEM), adverse effect concentrations as low as 0.2 mg/kg feed material were identified. However, there is uncertainty, like the possible co-occurrence of other mycotoxins. Therefore, the median of the minimum reported levels of 3.3 mg/kg feed was used as the RP (adverse effect concentration).
- Applying an uncertainty factor of 3 to this adverse effect concentration, the previously established RP for adverse animal health effect (referred to as NOAEL in the previous opinion) of 8.8 mg/kg feed, derived from a study with *i.v.* dosing, is replaced by 1 mg/kg feed.

Risk characterisation

When comparing the estimated mean and P95 levels of the sum of fumonisins and hidden forms with the new RPs for adverse animal health effects ('NOAEL') for chickens and horses the following could be concluded.

Poultry

- The estimated UB mean and P95 exposure to FBs plus their hidden forms for chickens are 0.81 and 2.46 mg/kg feed, respectively, corresponding to 81% and 246% of the NOAEL of 1 mg/kg feed thereby indicating a health concern.

Solipeds

- The estimated UB mean and P95 exposure to FBs plus hidden forms for horses are 0.29 and 0.31 mg/kg feed, respectively, corresponding to 29% and 31% of the RP for adverse animal health effects of 1 mg/kg feed, indicating a low risk for adverse health effects in horses and thus indicate no health concern.

5. Recommendations

- Further information on the TK of fumonisins is required for all animal species.
- More information on the mode of action for the adverse effects is needed, also in relation to the effect on sphingolipid metabolism for all animal species.
- To reduce uncertainties, controlled experimental studies would be necessary, including mechanistic studies, to address the issue of sensitivity of solipeds.
- Analytical methods should be improved to reduce the uncertainty in the estimated exposure for horses.

References

Antonissen G, De Baere S, Novak B, Schatzmayr D, den Hollander D, Devreese M and Croubels S, 2020. Toxicokinetics of hydrolyzed fumonisin B(1)after single oral or intravenous bolus to broiler chickens fed a control or a fumonisins-contaminated diet. Toxins, 12.

Benlasher E, Geng X, Nguyen NT, Tardieu D, Bailly JD, Auvergne A and Guerre P, 2012. Comparative effects of fumonisins on sphingolipid metabolism and toxicity in ducks and turkeys. Avian Diseases, 56, 120–127. https://doi.org/10.1637/9853-071911-Reg.1

Commission Recommendation (EU) 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food (Text with EEA relevance) (OJ L 208 02.08.2016, p. 58, CELEX). Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32016H1319

Dang HU, Zsolnai A, Kovács M, Bóta B, Mihucz G, Pósa R, Marosi K, Kachlek M and Szabó-Fodo J, 2019. The effect of fumonisins producing Fusarium verticillioides on the microbiota in pig caecum. Acta Veterinaria Brno, 88, 65–71.

Echenique JVZ, Estima-Silva P, Pereira DB, Marques LS, Ribeiro LS and Schild AL, 2019. Leukoencephalomalacia in horses associated with immature corn consumption. Ciencia Rural, 49, 3.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to fumonisins as undesirable substances in animal feed. EFSA Journal 2005;3(7):235, 32 pp. https://doi.org/10.2903/j.efsa.2005.235

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(6):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), Hart A, Maxim L, Siegrist M, Von Goetz N, da Cruz C, Merten C, Mosbach-Schulz O, Lahaniatis M, Smith A and Hardy A, 2019. Guidance on Communication of Uncertainty in Scientific Assessments. EFSA Journal 2019;17(1):5520, 73 pp. https://doi.org/10.2903/j.efsa.2019.5520

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen H-K, Alexander J, Barregard L, Bignami M, Bruschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Dall'Asta C, Eriksen G-S, Taranu I, Altieri A, Roldan-Torres R and Oswald IP, 2018a. Scientific opinion on the risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. EFSA Journal 2018;16(5):5242, 144 pp. https://doi.org/10.2903/j.efsa.2018.5242

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen H-K, Barregard L, Bignami M, Bruschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Dall'Asta C, Gutleb AC, Humpf H-U, Galli C, Metzler M, Oswald IP, Parent-Massin D, Binaglia M, Steinkellner H and Alexander J, 2018b. Scientific opinion on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms. EFSA Journal 2018;16(2):5172, 75 pp. https://doi.org/10.2903/j.efsa.2018.5172

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. EFSA Journal 2014;12(12):3916, 107 pp. https://doi.org/10.2903/j.efsa.2014.3916

EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Martino L, Merten C, Mosbach-Schulz O and Hardy A, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123

European Commission, 2006. Commission recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal of the European Union, 229, 7–9.

Foreman JH, Constable PD, Waggoner AL, Levy M, Eppley RM, Smith GW, Tumbleson ME and Haschek WM, 2004. Neurologic abnormalities and cerebrospinal fluid changes in horses administered fumonisin B1 intravenously. J Vet Intern Med., 18, 223–230.

Galli GM, Griss LG, Fortuoso BF, Silva AD, Fracasso M, Lopes TF, Schetinger MRS, Gundel S, Ourique AF, Carneiro C, Mendes RE, Boiago MM and Da Silva AS, 2020. Feed contaminated by fumonisin (Fusarium spp.) in chicks has a negative influence on oxidative stress and performance, and the inclusion of curcumin-loaded nanocapsules minimizes these effects. Microbial Pathoglogy, 148, 104496. https://doi.org/10.1016/j.micpath.2020.104496

Giannitti F, Diab SS, Pacin AM, Barrandeguy M, Larrere C, Ortega J and Uzal FA, 2011. Equine leukoencephalomalacia (ELEM) due to fumonisins B1 and B2 in Argentina. Pesquisa Veterinaria Brasileira, 31, 407–412.

Grenier B, Schwartz-Zimmermann HE, Gruber-Dorninger C, Dohnal I, Aleschko M, Schatzmayr G, Moll WD and Applegate TJ, 2017. Enzymatic hydrolysis of fumonisins in the gastrointestinal tract of broiler chickens. Poultry Science, 96, 4342–4351.

Grenier B, Schwartz-Zimmermann HE, Caha S, Moll WD, Schatzmayr G and Applegate TJ, 2015. Dose-dependent effects on sphingoid bases and cytokines in chickens fed diets prepared with fusarium verticillioides culture material containing fumonisins. Toxins, 7, 1253–1272. https://doi.org/10.3390/toxins7041253

Guerre P, 2015. Fusariotoxins in avian species: yoxicokinetics. Metabolism and Persistence in Tissues. Toxins (Basel)., 7, 2289–2305. https://doi.org/10.3390/toxins7062289

Henry MH, Wyatt RD and Fletchert OJ, 2000. The toxicity of purified fumonisin B1 in broiler chicks. Poultry Science, 79, 1378–1384. https://doi.org/10.1093/ps/79.10.1378

Jovanović M, Trailovic D, Kukolj V, Nesic S, Marinkovic D, Nedeljkovic-Trailovic J, Jakovac Strajn B and Milicevic D, 2015. An outbreak of fumonisin toxicosis in horses in Serbia. World Mycotoxin Journal, 8, 387–391.

Kellerman TS, Marasas W, Thiel PG, Gelderblom W, Cawood M and Coetzer J, 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. The Onderstepoort Journal of Veterinary Research, 57, 269–275.

Mallmann CA, Santurio JM and Dilkin P, 1999. Equine leukoencephalomalacia associated with ingestion of corn contaminated with fumonisin B1. Revision Microbiology, 30, 249–252.

Masching S, Naehrer K, Schwartz-Zimmermann HE, Sărăndan M, Schaumberger S, Dohnal I, Nagl V and Schatzmayr D, 2016. Gastrointestinal degradation of Fumonisin B_1 by carboxylesterase FumD prevents fumonisin induced alteration of sphingolipid metabolism in Turkey and Swine. Toxins, 8, 84. https://doi.org/10.3390/toxins8030084

Mateos I, Combes S, Pascal G, Cauquil L, Barilly C, Cossalter AM, Laffitte J, Botti S, Pinton P and Oswald IP, 2018. Fumonisin-exposure impairs age-related ecological succession of bacterial species in weaned pig gut microbiota. Toxins (Basel), 10, 230. https://doi.org/10.3390/toxins10060230

Metayer JP, Travel A, Mika A, Bailly JD, Cleva D, Boissieu C, Guennec JL, Froment P, Albaric O, Labrut S, Lepivert G, Marengue E, Tardieu D and Guerre P, 2019. Lack of toxic interaction between fusariotoxins in broiler chickens fed throughout their life at the highest level tolerated in the European Union. Toxins (Basel), 11, 455. https://doi.org/10.3390/toxins11080455

Paraskeuas V, Griela E, Bouziotis D, Fegeros K, Antonissen G and Mountzouris KC, 2021. Effects of deoxynivalenol and fumonisins on broiler gut cytoprotective capacity. Toxins (Basel), 13, 729. https://doi.org/10.3390/toxins13100729

Pereira dos Santos CE, Medeiros de Souto FS, Santurio JM and Marquez LC, 2013. Leucoencefalomalácia em equídeos da região Leste de Mato Grosso. Acta Scientiae Veterinariae, 41, 1119.

Rao ZX, Tokach MD, Woodworth JC, DeRouchey JM, Goodband RD, Calderón HI and Dritz SS, 2020. Effects of Fumonisin-contaminated corn on growth performance of 9 to 28 kg nursery pigs. Toxins, 12, 604. https://doi.org/10.3390/toxins12090604

Régnier M, Gourbeyre P, Pinton P, Napper S, Laffite J, Cossalter AM, Bailly JD, Lippi Y, Bertrand-Michel J, Bracarense APFRL, Guillou H, Loiseau N and Oswald IP, 2017. Identification of signaling pathways targeted by the food contaminant FB_1: transcriptome and kinome analysis of samples from pig liver and intestine. Molecular Nutrition & Food Research Res., 61, 1700433. https://doi.org/10.1002/mnfr.201700433

Reyes-Velázquez WP, Anguiano-Sevilla CN, Anguiano-Estrella R and Rojo FG, 2018. Association of acute equine leukoencephalomalacia (ELEM) with fumonisins concentrations in corn stover in an outbreak in the state of Jalisco Mexico. Austral Journal of Veterinary Sciences [Internet]. 50, 111–113. Available online: https://ajvs.cl/index.php/ajvs/article/view/109

Riley RT, An NH, Showker JL, Yoo HS, Norred WP, Chamberlain WJ, Wang E, Merrill AH Jr, Motelin G, Beasley VR and Haschek WM, 1993. Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker of exposure to fumonisin-containing feeds in pigs. Toxicology and Applied Pharmacology, 118, 105–112.

Rosiles MR, Bautista J, Fuentes VO and Ross F, 1998. An outbreak of equine leukoencephalomalacia at Oaxaca, Mexico, associated with fumonisin B1. Transbound. Emerging Disease, 45, 299–302.

Ross PF, Rice LG, Reagor JC, Osweiler GD, Wilson TM, Nelson HA, Owens DL, Plattner RD, Harlin KA and Richard JL, 1991. Fumonisin B1 concentrations in feeds from 45 confirmed equine leukoencephalomalacia cases. Journal of Veterinary Diagnostic Investigation, 3, 238–241.

Rotter BA, Thompson BK, Prelusky DB, Trenholm HL, Stewart B, Miller JD and Savard ME, 1996. Response of growing swine to dietary exposure to pure fumonisin B1 during an eight-week period: growth and clinical parameters. Natural Toxins, 4, 42–50. https://doi.org/10.1002/19960401nt6

Rotter BA, Prelusky BD, Fortin A, Miller JD and Savard ME, 1997. Impact of pure fumonisin B1 on various metabolic parameters and carcass quality of growing-finishing swine — preliminary findings. Canadian Journal of Animal Science, 77, 465–470. https://doi.org/10.4141/A96-105

Schelstraete W, Devreese M and Croubels S, 2020. Comparative toxicokinetics of Fusarium mycotoxins in pigs and humans. Food Chem Toxicology, 137, 111140. https://doi.org/10.1016/j.fct.2020.111140

Shier WT, 2000. The fumonisin paradox: a review of research on oral bioavailability of fumonisin B1, a mycotoxin produced by fusarium moniliforme. Journal of Toxicology Toxin Revenue, 19, 161–187.

Siloto EV, Oliveira EF, Sartori JR, Fascina VB, Martins BA, Ledoux DR, Rottinghaus GE and Sartori DR, 2013. Lipid metabolism of commercial layers fed diets containing aflatoxin, fumonisin, and a binder. Poultry Science, 92, 2077–2083. https://doi.org/10.3382/ps.2012-02777

Smith GW, Constable PD, Foreman JH, Eppley RM, Waggoner AL, Tumbleson ME and Haschek WM, 2002. Cardiovascular changes associated with intravenous administration of fumonisin B-1 in horses. American Journal of Veterinary Research, 63, 538–545.

Sousa MCS, Galli GM, Alba DF, Griss LG, Gebert RR, Souza CF, Baldissera MD, Gloria EM, Mendes RE, Zanelato GO, Gris A, Boiago MM, Stefani LM and da Silva AS, 2020. Pathogenetic effects of feed intake containing of fumonisin (Fusarium verticillioides) in early broiler chicks and consequences on weight gain. Microbial Pathogensis, 147, 104247. https://doi.org/10.1016/j.micpath.2020.104247

Sydenham EW, Marasas WFO, Shephard GS, Thiel PG and Hirooka EY, 1992. Fumonisin concentrations in Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicoses. Journal of Agricultural Food Chemistry, 40, 994–997.

Szabó A, Ali O, Lóki K, Balogh K, Mézes M, Bartók T, Horváth L and Kovács M, 2020. Orally administered fumonisins affect porcine red cell membrane sodium pump activity and lipid profile without apparent oxidative damage. Toxins, 12, 318. https://doi.org/10.3390/toxins12050318

Tardieu D, Bailly JD, Skiba F, Metayer JP, Grosjean F and Guerre P, 2007. Chronic toxicity of fumonisins in turkeys. Poultry Science, 86, 1887–1893. https://doi.org/10.1093/ps/86.9.1887

Tardieu D, Tran ST, Auvergne A, Babile R, Benard G, Bailly JD and Guerre P, 2006. Effects of fumonisins on liverand kidney sphinganine and the sphinganine to sphingosine ratio during chronic exposure in ducks. Chemico-Biological Interactions, 160, 51–60. https://doi.org/10.1016/j.cbi.2005.11.004

Terciolo C, Bracarense AP, Souto P, Cossalter AM, Dopavogui L, Loiseau N, Oliveira C, Pinton P and Oswald IP, 2019. Fumonisins at doses below EU regulatory limits induce histological alterations in piglets. Toxins, 11, 548. https://doi.org/10.3390/toxins11090548

Thiel PG, Shephard GS, Sydenham EW, Marasas W, Nelson PE and Wilson TM, 1991. Levels of fumonisins B1 and B2 in feed associated with confirmed cases of equine leukoencephalomalacia. Journal of Agricultural Food Chemistry, 39, 109–111.

Tomaszewska E, Rudyk H, Dobrowolski P, Donaldson J, Świetlicka I, Puzio I, Kamiński D, Wiącek D, Kushnir V, Brezvyn O, Muzyka V, Doraczyńska R, Muszyński S and Kotsyumbas I, 2021. Changes in the intestinal histomorphometry, the expression of intestinal tight junction proteins, and the bone structure and liver of pre-laying hens following oral administration of fumonisins for 21 days. Toxins, 13, 375. https://doi.org/10.3390/toxins13060375

Tran ST, Tardieu D, Auvergne A, Bailly JD, Babile R, Durand S, Benard G and Guerre P, 2006. Serum sphinganine and the sphinganine to sphingosine ratio as a biomarker of dietary fumonisins during chronic exposure in ducks. Chemico-Biological Interactions, 160, 41–50. https://doi.org/10.1016/j.cbi.2005.07.009

Vendruscolo CP, Frias NC, de Carvalho CB, de Sá LR, Belli CB and Baccarin RY, 2016. Leukoencephalomalacia outbreak in horses due to consumption of contaminated hay. Journal of Veterinary Internal Medicine, 30, 1879–1881. https://doi.org/10.1111/jvim.14588

Wang E, Norred WP, Bacon CW, Riley RT and Merrill AH Jr, 1991. Inhibition of sphingolipid biosynthesis byfumonisins. Implications for diseases associated withFusarium moniliforme. Journal of Biological Chemistry, 266, 14486–14490.

Wilson TM, Ross PF, Rice LG, Osweiler GD, Nelson HA, Owens DL, Plattner RD, Reggiardo C, Noon TH and Pickrell JW, 1990. Fumonisin B1 levels associated with an epizootic of equine leukoencephalomalacia. Journal of Veterinary Diagnostic Investigation, 2, 213–216.

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and 303 Methods for the Risk Assessment of Chemicals in Food, International Programme on Chemical Safety, 304 Environmental Health Criteria 240. Chapter 6: Dietary Exposure Assessment of Chemicals in Food 305. Available online: http://www.who.int/ipcs/food/principles/en/index1.html

Wilson TM, Ross PF, Owens DL, Rice LG, Green SA, Jenkins SJ and Nelson HA, 1992. Experimental reproduction of ELEM. A study to determine the minimum toxic dose in ponies. Mycopathologia, 117, 115–120.

Zomborszky-Kovács M, Kovács F, Horn P, Vetési F, Repa I, Tornyos G and Tóth A, 2002a. Investigations into the time- and dose-dependent effect of fumonisin B1 in order to determine tolerable limit values in pigs. Livestock Production Science, 76(3), 251–256. https://doi.org/10.1016/S0301-6226(02)00125-2

Zomborszky-Kovács M, Vetési F, Horn P, Repa I and Kovács F, 2002b. Effects of prolonged exposure to low-dose fumonisins B-1 in pigs. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health, 49, 197–201. https://doi.org/10.1046/j.1439-0450.2002.00519.x

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AP	aminopentol
ASP	Aspartate aminotransferase
AST	Aspartate transaminase
BW	body weight
CONTAM	Panel on Contaminants in the Food Chain
dm	dry matter
ELEM	leukoencephalomalacia
ECHA	European chemical agency
FB_{1-3}	Fumonisin B1-3
FCR	feed conversion ratio
GOT	glutamate oxaloacetate aminotransaminase
GSH	Glutathione
GST	glutathione S-transferase
HBGV	Health-based guidance value
HFB_1	hydrolysed Fumonisin B_1
HPLC	High Pressure Liquid Chromatography
IPCS	International Programme on Chemical Safety
i.v.	Intravenous
LC	Lethal Concentration
LC–MS	Liquid Chromatography – Mass Spectroscopy
LD_{50}	median Lethal Dose
LDH	Lactate dehydrogenase
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of detection
LOQ	Limit of quantification
LPO	lipid peroxide
MS	Mass Spectroscopy
NOAEL	No Observed Adverse Effect Level
ROS	reactive oxygen species
RP	Reference point
Sa/So	Sphinganine/Sphingosine
SGOT	Serum glutamic oxaloacetic transaminase
TBARS	Thiobarbituric acid reactive substances
TDI	tolerable daily intake
TK	Toxicokinetics
UF	uncertainty factor
USA	United States of America
WG	weight gain
WHO	World Health Organization
ww	wet weight

Appendix A – Literature search for supporting information for the assessment

Web of Science

Time span = from 2017 until 2 January 2022.

Set	Query	Results	Comments
	#1 AND #2 AND #5	**105**[(a)]	WOS TOXICITY in **horses**
	#1 AND #3 AND #5	**338**[(a)]	WOS TOXICITY in **pigs**
	#1 AND #4 AND #5	**236**[(a)]	WOS TOXICITY in **poultry**
#1	Fumonisin OR fumonisins OR FB OR HFB* OR PHFB* OR NDF-FB OR NDF/FB OR NCM-FB OR NCM/FB OR hidden fumonisin* OR masked fumonisin*		**Main search WOS** Command word: TS
#2	horse* OR stallion* OR mare* OR foal* OR equine		**Farm animals** – **horses** Command words: TS
#3	pig OR swine* OR sow* OR gilt* OR boar* OR porcine		**Farm animals** – **pigs** Command words: TS
#4	poultry OR chicken* OR hen OR cock* OR rooster* OR broiler* OR duck* OR goose OR geese OR geesling* OR turkey* OR quail* OR duckling		**Farm animals** – **poultry** Command words: TS
#5	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

Search language = English.
(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
- EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed. EFSA Journal 2010;8(1):1457, 54 pp. https://doi.org/10.2903/j.efsa.2011.1457
- EFSA (European Food Safety Authority), 2010b. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557
- EFSA (European Food Safety Authority), 2011. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal 2011;9(12):2490, 33 pp. https://doi.org/10.2903/j.efsa.2011.2490
- EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579
- EFSA Scientific Committee, 2012b. Scientific Opinion on Risk Assessment Terminology. EFSA Journal 2012;10(5):2664, 43 pp. https://doi.org/10.2903/j.efsa.2012.2664
- EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M,Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Martino L, Merten C, Mosbach-Schulz O and Hardy A, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123