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Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed

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Abstract

Fumonisins, mycotoxins primarily produced by *Fusarium verticillioides* and *Fusarium proliferatum*, occur predominantly in cereal grains, especially in maize. The European Commission asked EFSA for a scientific opinion on the risk to animal health related to fumonisins and their modified and hidden forms in feed. Fumonisin B_1 (FB_1), FB_2 and FB_3 are the most common forms of fumonisins in feedstuffs and thus were included in the assessment. FB_1, FB_2 and FB_3 have the same mode of action and were considered as having similar toxicological profile and potencies. For fumonisins, the EFSA Panel on Contaminants in the Food Chain (CONTAM) identified no-observed-adverse-effect levels (NOAELs) for cattle, pig, poultry (chicken, ducks and turkeys), horse, and lowest-observed-adverse-effect levels (LOAELs) for fish (extrapolated from carp) and rabbits. No reference points could be identified for sheep, goats, dogs, cats and mink. The dietary exposure was estimated on 18,140 feed samples on $FB_{1–3}$ representing most of the feed commodities with potential presence of fumonisins. Samples were collected between 2003 and 2016 from 19 different European countries, but most of them from four Member States. To take into account the possible occurrence of hidden forms, an additional factor of 1.6, derived from the literature, was applied to the occurrence data. Modified forms of fumonisins, for which no data were identified concerning both the occurrence and the toxicity, were not included in the assessment. Based on mean exposure estimates, the risk of adverse health effects of feeds containing $FB_{1–3}$ was considered very low for ruminants, low for poultry, horse, rabbits, fish and of potential concern for pigs. The same conclusions apply to the sum of $FB_{1–3}$ and their hidden forms, except for pigs for which the risk of adverse health effect was considered of concern.

Keywords: fumonisins, modified forms, hidden forms, feed, exposure, toxicity, animal health risk assessment

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Summary

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM) assessed the risk to animal health related to the presence of Fumonisins and their modified and hidden forms in feed. The CONTAM Panel was asked to consider all relevant adverse health effects, and in particular to address the co-occurrence of fumonisins and their modified and hidden forms, and to estimate the dietary exposure of different animal species.

Previous risk assessments from the European Food Safety Authority (EFSA) on fumonisins in feed (2005), modified forms of certain mycotoxins in food and feed (2014) and on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms (2018) have been used as a starting point for the present assessment.

Fumonisins are mycotoxins produced predominantly by *Fusarium verticillioides* and *Fusarium proliferatum*. In terms of chemical structure, fumonisins are long-chain aminopolyols with two tricarballylic acid side chains. The most relevant compounds are the B-type fumonisins (FBs), FB_1, FB_2 and FB_3 which differ in the number and position of hydroxy groups at the backbone. The most relevant modified forms are hydrolysed fumonisins B (HFBs) and partially hydrolysed fumonisins B (pHFBs). FBs may react during food processing, giving rise to the formation of Maillard-type modified forms, such as NCM-FBs and NDF-FBs.

Due to the chemical structure, FBs may strongly interact through non-covalent binding with the matrix macroconstituents, giving rise to the so-called hidden FBs. Hidden forms may be disrupted upon digestion, leading to the release of the unchanged parent forms of FBs in the gastrointestinal tract.

Analytical methods for $FB_{1–3}$ are well established and are mainly based on mass spectrometry (MS). Modified forms of FB_1 are commonly analysed under the same conditions as their parent compound. However, the strong physical interaction of fumonisins with the food matrix, which is well documented in the literature, may significantly affect the analytical performance in a matrix-related way. For the determination of hidden fumonisins, the food/feed matrix is usually treated under alkaline conditions prior to the analysis. Only $FB_{1–3}$ are available on the market as calibrant solutions. Except for HFB_1, analytical standards for modified forms are not commercially available.

There is poor information on the absorption, distribution, metabolism and excretion (ADME) of fumonisins in farm animal species, and the available studies are almost limited to FB_1. In orally exposed animals, fumonisins are in general poorly bioavailable, rapidly distributed mainly to liver and kidney, extensively biotransformed and rapidly excreted mostly via the faecal route. Hydrolytic biotransformations largely prevail; the main metabolites are $pHFB_1$ and HFB_1; both may be found in limited amounts in tissues. Unlike in rats, no further metabolites (e.g. *N*-acyl derivatives of FB_1 and its hydrolysed forms) have been detected in farm and companion animals. A very limited excretion of fumonisins in milk and negligible excretion in eggs have been documented. No information on $FB_{1–3}$ kinetics could be identified for farmed rabbits, fish, horses, farmed mink, dogs and cats.

In ruminants, the scant information available data indicate a very limited oral bioavailability and a remarkable biotransformation to the hydrolysed $pHFB_1$ and HFB_1. Hydrolytic biotransformation appear not occur in rumen or liver. Excretion in milk has been investigated and only been documented in cows.

In pigs, $FB_{1–3}$ are poorly bioavailable but extensively hydrolysed to $pHFB_1$ and HFB_1 in the enteric tract. Measurable amounts of the toxin and of both hydrolysed metabolites are detectable in livers and kidneys up to several days after treatment cessation. The faecal excretion largely outweighs the urinary one; the extent of biliary excretion might vary according to the dose and the duration of the exposure. The bioavailability of FB_2 is likely to be much lower than that of FB_1.

There is very limited knowledge on $FB_{1–3}$ kinetics in avian species, with no information of FB_1 biotransformations. Oral bioavailability is poor and in the order turkey>duck>chicken. Kinetic studies point to a more rapid elimination in ducks and chickens than in turkeys. In birds fed with feed at, or approaching the European Union (EU) recommended guidance, residues were detected only in the liver. The kinetics of FB_2 in ducks and turkeys is similar to that of FB_1, with evidence of a lower bioavailability.

Fumonisins are structural analogues of sphingoid bases and they inhibit ceramide synthase. This induces a disruption of sphingolipid metabolism and pathological changes. Even if the disruption of the sphingolipid metabolism at an early stage is closely related with fumonisin toxicity, there is no evidence that fumonisin-induced ceramide synthase inhibition is in itself an adverse effect. Therefore, reference points for fumonisins have been derived using endpoints other than the sole alteration of sphingolipid ratio in serum or organs. The implication of the disruption of sphingolipid metabolism in some of the observed critical adverse effects still remains to be established. At the cellular level, FB_1, FB_2 and FB_3 have the same mode of action and are considered as having similar toxicological profiles and potencies.

Ruminants are considered less sensitive than horses and pigs. Gross and histopathological lesions, as well as changes in serum enzymes and biochemistry indicate an impairment of liver and possibly kidney function. Taking as endpoints the increase in serum enzymes, cholesterol and bilirubin as well as the decrease in lymphocyte blastogenesis a no-observed-adverse-effect level (NOAEL) of (31 mg $FB_{1–3}$/kg feed) could be set only for cattle. However, a very limited data set indicates that sheep and goats would not seem to be more susceptible to fumonisins than cattle.

Porcine pulmonary oedema syndrome is the specific effect produced by FB_1 in pigs and cardiovascular toxic 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. A NOAEL of 1 mg FBs/kg feed and a lowest-observed-adverse-effect level (LOAEL) of 5 mg/kg feed could be identified for pigs based on lung lesions.

Fumonisins affect the liver and the immune system in investigated poultry species. In addition, decreases in feed intake and body weight gain were reported from feeding studies with ducks and Japanese quail, but not from studies with chickens and turkeys. Increased Sa and Sa/So levels have also been reported from low feed concentrations (2 mg FB_1/kg feed) in investigated poultry species. A NOAEL of 8 mg/kg feed based on alterations of liver enzymes indicative of liver toxicity was identified for ducks. A NOAEL of 20 mg/kg feed, corresponding to 2 mg/kg body weight (bw) per day was identified for chickens. This NOAEL was identified based on an increase in liver lipids which was considered as an adverse effect taking into consideration the observed liver toxicity in all investigated species. A NOAEL of 20 mg/kg feed per day was also identified for turkeys. This was the highest dose used in the studies published since the last EFSA opinion and no adverse effects were observed in these studies.

A NOAEL of 0.2 mg FB_1/kg bw per day, recalculated from an intravenous (i.v.) study (corresponding to 8.8 mg FB_1/kg feed) was identified for horses, based on neurological and cardiovascular effects.

Decreased performance, biochemical alterations in serum and blood formula, liver and kidney congestion, impaired spermatogenesis and delay of the onset of puberty as well as increased Sa level and the Sa/So ratio in urine, serum and liver were associated with exposure of rabbits to FBs. A LOAEL of 5 mg FBs/kg feed was identified based on alterations in liver.

There is limited information available from feeding studies with fish, and no information is available on the effects of FBs on salmonids. Observed effects of FBs in fish species include pathological damages in several organs, reduced body weight gain and haematological and immunological alterations. A NOAEL of 10 mg/kg feed has been identified for Nile tilapia based on reduced weight gain. This corresponds to 0.4 mg/kg bw per day. Similarly, a LOAEL of 10 mg/kg feed was identified for carp, corresponding to 0.5 mg/kg bw per day. This LOAEL was based on pathological alterations, changes in haematological parameters and reduced body weight gain. A NOAEL of 20 mg/kg feed was identified for catfish. This was based on reduced body weight gain and microscopic liver lesions.

No data could be identified concerning the effects of FBs in cats, dogs or farmed mink.

No data were available to establish a reference point for any modified form of fumonisin, for any of the animal species considered.

The dietary exposure was estimated using a final data set of 18,140 feed samples on FBs (i.e. FB_1, FB_2 and FB_3) representing most of the feed commodities with potential presence of fumonisin. Samples were collected between 2003 and 2016 in 19 different European countries, but most of them came from four Member States. The total concentration of FBs was estimated by summing available analytical concentrations for each sample. For samples for which no concentration was available, the levels were estimated by using the mean concentration of available data.

The percentage of left-censored data reported (results below limit of detection and/or limit of quantification) was high (~ 80%). The highest number of reported analytical results were in the feed group 'Cereal grains' (~ 47%) and in particular for maize, wheat and barley. Other feed groups included forages, land animal products, legume seeds, minerals, oil seeds and tubers. High quantified values were reported for maize wheat and compound feed. The compound feeds with highest levels were for unspecified species and were therefore not used for the exposure assessment. The animal exposure was presented as dietary concentrations because the animal risk characterisation was carried out on a feed concentration basis. Exposure to FBs and the hidden forms is primarily from the consumption of maize (corn) and its by-products. Except for forage maize, and maize silage produced from it, levels on forages are generally low.

The highest estimated dietary concentrations to FBs by cattle was for lactating dairy cows on a maize silage-based diet (mean lower bound (LB) = 368 and 95th percentile upper bound (UB) = 1,894 μg/kg feed), reflecting both the high levels of FBs in forage maize and the inclusion of cereal grains in the complementary compound feeds. For other cattle, the lowest overall dietary concentration was for beef cattle on a straw-based ration (LB mean = 14 UB P95 = 270 μg/kg feed). For sheep and goats, the calculated lowest LB to highest UB mean dietary concentrations of FBs were 25 and 187 μg/kg feed, respectively, while at the 95th percentile the range was from 42 (LB) to 716 (UB) μg/kg feed. For horses, the calculated mean LB and UB diet concentrations of FBs were 22 and 203 μg/kg feed, respectively, while for the 95th percentile the range (LB–UB) was 22–223 μg/kg feed. The calculated mean LB and UB exposures to FBs by pigs, derived from data for species-specific compound feeds, ranged from 23 to 413 μg/kg feed, respectively, while the 95th percentile exposures ranged from 568 (LB) to 943 (UB) μg/kg feed. For poultry, the calculated mean exposure ranged from 58 (LB) to 575 (UB) μg/kg feed, based on levels in individual feeds and their inclusion in diets. The equivalent range for the 95th percentile estimates of exposure was 72 and 1,749 μg/kg feed, respectively. For farmed salmonids and carp, the calculated mean LB and UB for dietary concentrations ranged from 121 to 370 μg/kg feed, respectively. At the 95th percentile, LB and UB estimates dietary concentrations ranged from 421 (LB) to 1,110 (UB) μg/kg feed. The calculated mean diet concentration for farmed rabbits ranged from 7.0 (LB) to 233 (UB) μg/kg dry matter (DM), while the equivalent range for the 95th percentile was from 20 to 296 μg/kg DM. The mean calculated diet concentration for farmed mink ranged from 58 (LB) to 84 (UB) μg/kg DM, while the equivalent range for the 95th percentile was 241 and 260 μg/kg DM. For companion animals (cats and dogs), the calculated LB and UB mean diet concentrations of FBs were 365 and 465 μg/kg DM, respectively, while at the 95th percentile the range was from 1,501 (LB) to 1,765 (UB) μg/kg DM.

Fumonisins hidden forms are assumed to be 60% of the dietary concentrations for FBs. The sum of FBs plus the hidden forms may be calculated by multiplying the values given above (for FBs) by 1.6.

The risk of exposure to fumonisins was evaluated taking into consideration the comparison between the exposure of the sum of FB_1, FB_2 and FB_3, and the identified NOAELs/LOAELs for chronic adverse effects. The risk characterisation of exposure to FBs and their hidden forms was evaluated based on the comparison between the exposure of FBs and their hidden forms (exposure to FBs multiplied by a factor of 1.6), and the identified NOAELs/LOAELs for chronic adverse effects of FBs. For dogs, cats and mink, the health risk from the exposure to FBs and to FBs and their hidden forms could not be assessed as no NOAEL or LOAEL have been identified. For cattle, the risk of an adverse health effects from feed containing FBs was considered very low. It is expected that sheep and goat have similar sensitivity to FBs as cattle and the risk was considered very low also for those species. For poultry, horses, rabbits and fish, the risk of adverse health effects of feed containing FBs was considered low. For pigs, the risk of adverse health effects of feed containing FBs was considered low for pigs exposed to mean levels but of potential concern for animals exposed to levels at the 95th percentile. The same conclusions apply to the sum of FBs and their hidden forms except for pigs for which the risk of adverse health effects from feeds containing FBs was considered low for exposure at the mean levels and of concern for animals exposed to levels at the 95th percentile.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

BACKGROUND

Following a request from the European Commission, the risks to human and animal health related to modified forms of the Fusarium toxins zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins were evaluated in the scientific opinion on the risks for human health related to the presence of modified forms of certain mycotoxins in food and feed, adopted by the EFSA Panel on Contaminants in the Food Chain (CONTAM) on 25 November 2014.

The CONTAM Panel indicated in the recommendations that the animal health effects of fumonisins needed to be re-assessed in order to possibly set NOAELs/LOAELs for fumonisins in order to be able to assess the risk for animal health related to the presence of fumonisins and their modified forms in feed.

TERMS OF REFERENCE

In accordance with Art. 29 (I) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on the risks for animal health related to the presence of fumonisins and their modified forms in feed.

1.2. Interpretation of the Terms of Reference

The CONTAM Panel assumed that the previous EFSA risk assessment of fumonisins in feed (EFSA, 2005) comprehensively covered all relevant aspects of fumonisins and therefore used it together with the recent opinion on modified mycotoxins (EFSA CONTAM Panel, 2014) and the opinion on appropriateness to set a group health based guidance value for Fumonisins and modified forms (EFSA CONTAM Panel, 2018) as a starting point for the present assessment.

The CONTAM Panel noted that, in addition to FB_1 and FB_2, FB_3 and FB_4 are among the most common forms of fumonisins, and therefore decided to also consider these in the assessment. The CONTAM Panel reviewed the new relevant data on $FB_{1–4}$ (i.e. published after 2004) to evaluate whether reference points for risk characterisation identified for FB_1 in some animal species need to be revised and to possibly set no-observed-adverse-effect levels (NOAELs)/lowest-observed-adverse-effect levels (LOAELs) for fumonisins to assess the risk for animal health related to the presence of fumonisins and their modified forms in feed.

The Panel decided to present the modified forms of $FB_{1–3}$ identified to date and reviewed the appropriateness of the methods currently available for their analysis as in the previous EFSA opinion (EFSA CONTAM Panel, 2018). FB_4 was not considered in this opinion as it occurs mainly in grapes, which are not a major feedstuff. In addition, data on the occurrence, toxicity and toxicokinetics could not be identified for FB_4.

In this opinion, the CONTAM Panel have considered the parent compound, the modified forms and 'physical entrapped' or 'hidden' forms' of fumonisins, as described in Section 1.3.1.

1.3. Additional information

1.3.1. Fumonisins, modified forms and hidden forms considered in this opinion

1.3.1.1. Fumonisins

Based on their different substituent groups, fumonisins are classified as A-, B-, C- and P-series (EFSA CONTAM Panel, 2018). Those belonging to group B such as fumonisin B_1 (FB_1), B_2 (FB_2), B_3 (FB_3), B_4 (FB_4) occur mainly in feed commodities (Gelderblom et al., 1988; Cawood et al., 1991). Other fumonisins belonging to group B, or those classified as A-, C- and P-series, usually account for less than 5% of the total fumonisin (Rheeder et al., 2002).

In view of their occurrence in grains (see Section 3.2 Feed occurrence data), the CONTAM Panel decided to include FB_1, FB_2 and FB_3 as parent compounds, since these are the most abundant forms of fumonisins of the B-type. However, the CONTAM Panel decided not to include other fumonisins of the B-type, or fumonisins of the A, C and P series, since these usually represent less than 5% of total fumonisins.

— **Modified forms**

Fumonisins, as with other mycotoxins, may undergo modification according to two different routes:

1) Biotransformation in the fungus, infested plant and animal organism. This includes phase I metabolism through hydrolysis of the parent toxin, and phase II metabolism involving conjugation with endogenous molecules.
2) Processing of food and feed by thermal or chemical treatment. This causes degradation reactions during processing, as well as covalent binding to food and feed matrices.

However, few data about the occurrence of modified forms are available in the literature.

1.3.1.2. Hidden forms

Due to their chemical structure, fumonisins may form non-covalent binding products with food or feed matrices as modified forms, although there is no change of the chemical structure involved. Such non-covalent interactions may be mediated by hydrogen-bonding or ionic bonding and are therefore of particular importance for fumonisins as they can seriously affect the analytical determination of the parent fumonisins in food and feed, leading in some cases to underestimation of their content (see Section 1.3.4 Methods of analysis). The complete disruption of such non-covalent interactions in the gastrointestinal tract of animals may lead to the release of parent forms, thus contributing to the total load of fumonisins. Therefore, the CONTAM Panel has decided to include hidden forms of fumonisins in this exposure assessment.

1.3.2. Previous animal health risk assessments

The Scientific Opinion related to fumonisins as undesirable substances in animal feed (EFSA, 2005) evaluated the toxicity of fumonisins in feed for different animal species. The CONTAM Panel concluded that FB_1 was the most prevalent and toxic derivative and derived NOAELs and LOAELs for a number of livestock species and farmed animals based on FB_1. Pigs and horses were identified as the most sensitive species to FB_1. LOAELs of 200 μg/kg body weight (bw) per day for FB_1 were derived for pigs and horses based on increased sphinganine/sphingosine (Sa/So) ratio levels detected at that dose in serum of both species. In ruminants, a NOAEL of 600 μg/kg bw per day for FB_1 was derived based on liver changes and impaired lymphocyte blastogenesis. A LOAEL of 10 mg FB_1/kg feed was identified for fish (carp) based on pathological alterations in liver, pancreas, kidney, heart and brain. At the time of the evaluation, experimental data available for catfish and Nile tilapia suggested a NOAEL corresponding to 20 mg FB_1/kg feed. A LOAEL of 2,000 μg/kg bw per day for FB_1 was identified for poultry based on increased Sa 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). The toxicity for animals and humans of metabolites and masked or bound forms of mycotoxins, including fumonisins, was evaluated. The EFSA occurrence database contained no data on modified fumonisins, and therefore occurrence was based on limited information reported in the literature.

An estimation of the human dietary exposure and animal feed exposure compared with the exposure to the parent mycotoxins and assessments of the human and animal health risks was performed. Based on occurrence data collected at the time of the evaluation (EFSA CONTAM Panel, 2014), modified forms[1] of fumonisins, which included physically entrapped forms, occurred – together with their precursor – occurred predominantly in corn and maize-based products. The exposure assessment was performed, and included an additional 60% to account for modified mycotoxins to the parent compound. 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 re-assessing the animal health effects of zearalenone and fumonisins in order to set NOAELs/LOAELs for these compounds.

[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, 2018) 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.

Recently, the CONTAM Panel assessed the appropriateness to set a group health-based guidance value (HBGV) for fumonisins and modified forms (EFSA CONTAM Panel, 2018). The CONTAM Panel considered modified forms of fumonisins phase I and phase II metabolites formed in fungi or infested plants or food or feed products of animal origin. In addition, the Panel considered forms arising from food or feed processing, including covalent adducts with matrix constituents. 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, and found it appropriate to include FB_2, FB_3 and FB_4 in a group TDI with FB_1 and exclude the modified fumonisins in the group TDI for $FB_{1–4}$ (EFSA CONTAM Panel, 2018).

1.3.3. Chemistry

1.3.3.1. Fumonisins

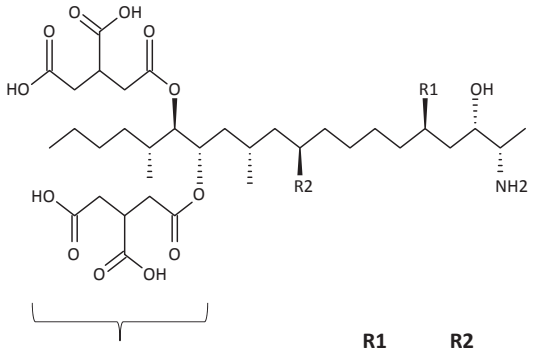
The chemical structure of fumonisins, and their classification into groups based on different chemical features, has been described in the EFSA CONTAM Opinion on the appropriateness to set up a group HBGV for fumonisins and their modified forms (EFSA CONTAM Panel, 2018), see Figure 1.

Briefly, fumonisins are formed by a C20 (or C19) long-chain amino-polyol backbone carrying two methyl groups. On the backbone, two propane-1,2,3-tricarboxylic acid (also named tricarballylic acid, TCA) side chains are esterified to hydroxy groups at positions C14 and C15.

Structurally the B-type fumonisin backbone resembles the sphingoid bases sphinganine (Sa) and sphingosine (So) especially with the amino and hydroxy functions in positions C2 and C3 (Figure 1).

According to IUPAC, FB_1 is named (2*R*,2′*R*)-2,2′-((((5*R*,6*R*,7*S*,9*S*,11*R*,16*R*,18*S*,19*S*)-19-amino-11,16,18-trihydroxy-5,9-dimethyleicosane-6,7-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))disuccinic acid (CAS No. 116355-83-0, $C_{34}H_{59}NO_{15}$, MW 721).

Fumonisins are highly polar compounds, soluble in water and in polar solvents, carrying various reactive groups, i.e. four carboxylic groups, two esterified tricarballylic side chains, one primary amine and several hydroxy groups. Therefore, they can react under thermal processing conditions giving rise to a number of modified forms.



Tricarballylic acid (TCA)

	R1	R2	Polar surface	Log P
FB1	OH	OH	288.51	-0.044
FB2	H	OH	268.27	1.3169
FB3	OH	H	268.27	1.3169
FB4	H	H	248.04	2.5538

Figure 1: Chemical structure of the main parent fumonisins FB_1, FB_2, FB_3 and FB_4

1.3.3.2. Modified forms of fumonisins

Based on the presence of several reactive groups on the fumonisin backbone, several modified forms have been elucidated, especially generated by thermal processes applied during food or feed production (Figure 2). However, phase I and phase II metabolites formed in plants, fungi, and animals have also been described.

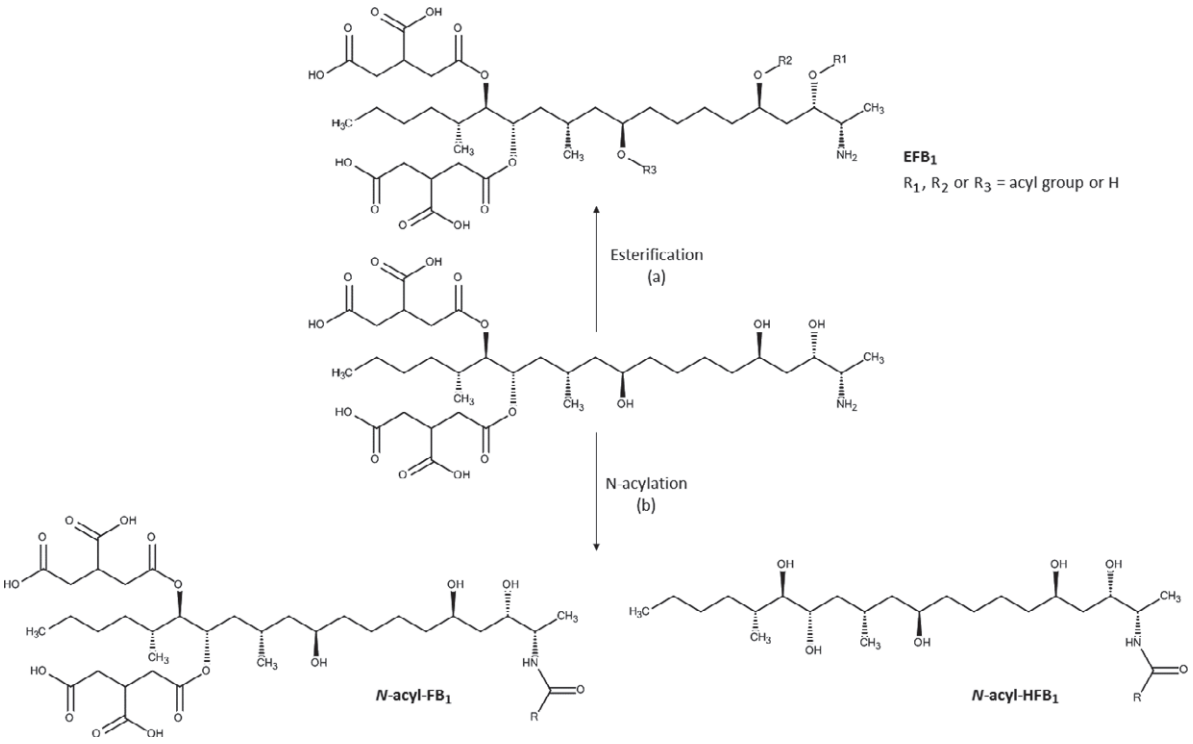
Phase I modification

Little is known about the phase I metabolism of fumonisins in living organisms. Due to their high polarity, $FB_{1–3}$ show a lower absorption, compared to other mycotoxins, and are often excreted as parent forms. The hydrolysis of the tricarballylic moieties, leading to the release of $HFB_{1–3}$, is the only phase I modification described in the literature. Hydrolysed and partially hydrolysed fumonisins may be formed by microbial and animal metabolism (Hahn et al., 2015), while the low occurrence of these forms in grains may be related to fungal/plant metabolisms as well as to chemical reactions occurring at harvest. It must be underlined that the hydrolysed form of FB_1 is often referred to as aminopentol in animal studies. Hydrolysed fumonisins can be formed through use of enzyme-based feed additive (EFSA FEEDAP Panel, 2014; EFSA FEEDAP Panel, 2016).

Phase II modification

Minor modified forms of fumonisins are *O*-fatty acyl fumonisin B_1 (EFB_1). These compounds are formed by the esterification of a long-chain fatty acid on the fumonisin backbone (3-*O*-, 5-*O*- or 10-*O*-acyl-fumonisins) (Figure 2) (Bartók et al., 2010a,b, 2013a; Falavigna et al., 2016). Besides *O*-fatty acyl-fumonisins, the corresponding *N*-fatty acyl-fumonisins were also detectable in low amounts in *Fusarium* (Bartók et al., 2013b). These phase II metabolites have been found in maize in the field, but it is still unclear if their formation is due to fungal or plant metabolism.

N-fatty acyl-fumonisins and *N*-fatty acyl-hydrolysed fumonisins with fatty acid chain length ranging from C16:0 to C24:1 are also described as *in vitro* and *in vivo* metabolites of fumonisins (Seiferlein et al., 2007; Harrer et al., 2013, 2015).



(a) formation of fatty acid esters of fumonisins (EFB_1); (b) formation of *N*-acyl-fumonisin B_1 and *N*-acyl-hydrolysed fumonisin B_1.

Figure 2: Formation of Phase I and Phase II metabolites of fumonisins

Process-derived forms

Fumonisins bear four carboxylic moieties, a primary amino group and several hydroxyl groups, which are prone to react with other molecules under thermal processing conditions commonly applied in food and feed production, leading to process-derived modified forms of fumonisins.

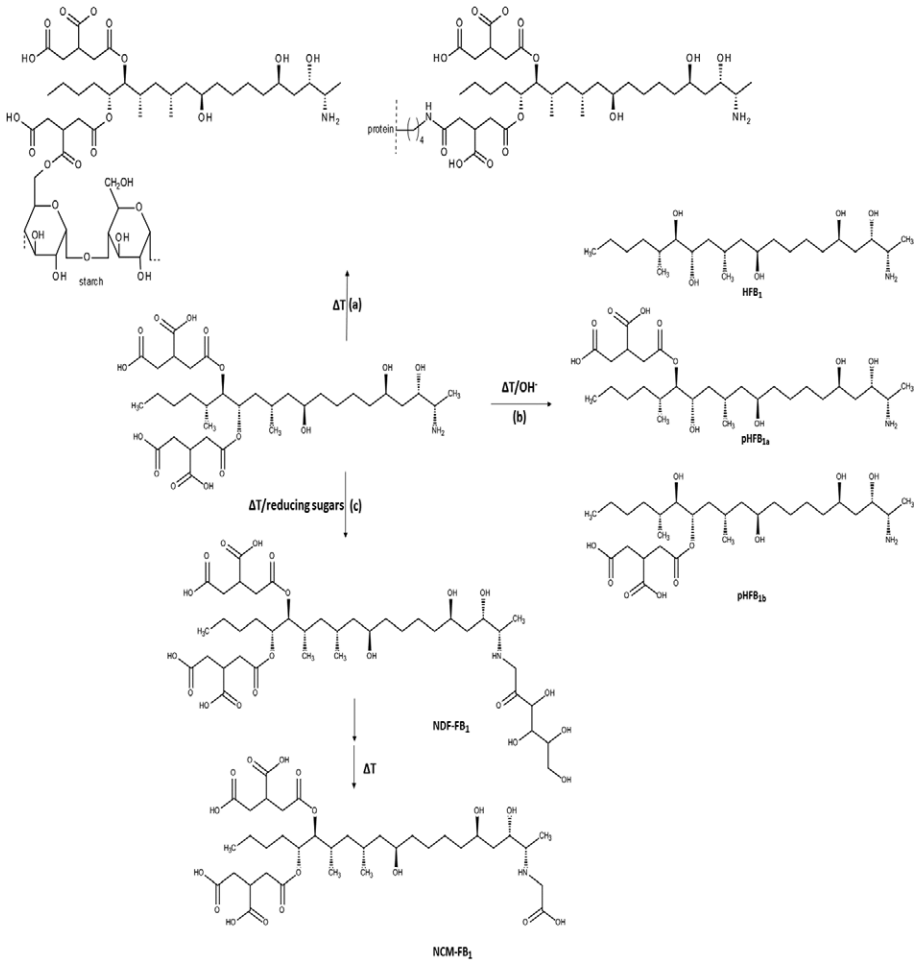
TCA side chains can be cleaved under alkaline conditions giving rise to hydrolysed fumonisins HFB_x (Humpf and Voss, 2004). When the hydrolysis is incomplete, partially hydrolysed fumonisins ($pHFB_{1–3}$)

are produced as isomeric forms from the cleavage of one of the two tricarballylic side chains on the fumonisin backbone. Their structure has been described in the EFSA opinion on Fumonisins HBGVs (EFSA CONTAM Panel, 2018 section on chemistry). $pHFB_{1–3}$, (Figure 3) are formed by cleavage of only one of the two TCA side chains. Hydrolysed fumonisin B_1 (HFB_1) occurs in nixtamalised corn products and canned yellow corn, but usually at lower concentrations than FB_1.

The primary amine group of fumonisins may easily react with reducing sugar upon heating, originating from Maillard-type products. Among possible degradation products, only *N*-(carboxymethyl)-fumonisin B_1 (NCM-FB_1) and *N*-(1-deoxy-D-fructos-1-yl)-fumonisin B_1 (NDF-FB_1) have been detected in food and feed so far (Figure 3) (Humpf and Voss, 2004). These reactions have been primarily shown for FB_1 and HFB_1 but all other fumonisins with a free primary amino group can react in the same way. Recently, NDF derivatives of FB_2 and FB_3 have been identified in corn samples (Matsuo et al., 2015).

Fumonisins can also covalently bind to macromolecules such as starch and proteins via their two reactive TCA side chains. These matrix-bound forms of fumonisins were first described and partially characterised by Shier et al. (2000a,b) in model experiments with radiolabelled FB_1 (Shier, 2000; Resch and Shier, 2000; Shier et al., 2000a,b), and were further characterised by Seefelder et al. (2003).

Such covalent binding has been described so far only for FB_1, which is the most abundant fumonisin in crops. However, due to the chemical similarity of FB_1 with other B-type fumonisins, the formation of modified forms of FB_2 and FB_3 is very likely. Although these compounds have been isolated and characterised in model systems, their direct determination in food as such is not possible, as the covalently bound fumonisins have to be released first by chemical hydrolysis. Therefore, these matrix-bound forms of fumonisins can be determined indirectly by quantifying free $FB_{1–3}$ and $HFB_{1–3}$ before and after chemical hydrolysis or after digestion of the macromolecules (Dall'Asta et al., 2010).



(a) Formation of matrix-bound forms; (b) formation of hydrolysed (HFB_1) and partially hydrolysed fumonisins B_1 ($pHFB_1$); (c) N-alkylation with sugars (*N*-(carboxymethyl)-fumonisin B_1 ($NCM\text{-}FB_1$), *N*-(1-deoxy-D-fructos-1-yl) fumonisin B_1 ($NDF\text{-}FB_1$).

Figure 3: Process-derived modified fumonisins

1.3.3.3. Hidden forms/Non-covalently bound fumonisins

While modified fumonisins have been isolated and structurally characterised, the presence of other non-covalent forms of fumonisins have been assumed based on experimental observation, such as poor recovery rates from different food matrices in interlaboratory studies (Dall'Asta et al., 2009b; Bryła et al., 2015). These forms have been already discussed by EFSA CONTAM Panel (2018).

Due to their chemical structure, which is highly prone to form hydrogen bonds as well as apolar interactions, fumonisins may undergo non-covalent binding with macromolecules occurring in food (e.g. starch, proteins, lipids, etc.). This gives rise to the formation of non-extractable, non-covalent forms, often described as 'hidden' or 'physically entrapped' fumonisins. In the same context, the extractable fraction is commonly referred to as 'free fumonisins'. Within this opinion, 'hidden fumonisins' will be the term used for defining such non-covalent forms.

Due to the non-covalent nature of these non-specific interactions and the structural diversity of such complexation, which can range from quite weak to very strong, such forms cannot be isolated and chemically characterised.

Although the physicochemical nature of such interaction has not been fully described, data collected so far indicate that biopolymers – preferentially amylose and amylopectine, but also proteins – may form inclusion complexes with fumonisins. These complexes are stable under the routine extraction conditions, but can be easily destroyed under *in vitro* digestion conditions, when biopolymers are enzymatically degraded (Dall'Asta et al., 2010).

Such interactions have been indicated as responsible for the difficulties in obtaining comparable and reproducible results using different analytical methods. Complexation may be disrupted during the extraction process as a consequence of different experimental parameters (i.e. pH, solvents, temperature, etc.). This will lead to the release of parent forms, and thus to changes in the final recovery of analytes (Dall'Asta et al., 2009b). Moreover, it has been demonstrated that the instability of fumonisins in stored analytical samples, and in particular spiked samples used in collaborative method studies (Kim et al., 2002), may involve the formation of hidden fumonisins.

Unfortunately, current protocols for matrix macrocompounds disruption are based on alkaline treatment, and cannot avoid the simultaneous hydrolysis of fumonisins. Therefore, as a result, hidden fumonisins are determined indirectly as hydrolysed fumonisins, and not as parent compounds.

Data reported in the literature indicated that such forms can be related to the chemical composition of maize hybrids, as well as to other environmental factors (Dall'Asta et al., 2012). In addition, technological processes may affect the distribution ratio between extractable and non-extractable fumonisins, mainly in consideration of starch-related phenomena (Bryła et al., 2015).

It has been demonstrated that matrix-fumonisin complexes can be destroyed by human digestive enzymes in an artificial system, thus releasing the corresponding parent forms (Oomen et al., 2003; Versantvoort et al., 2005; Dall'Asta et al., 2010). Indeed, enzymatic activity may induce the formation of hidden forms which may significantly contribute to the overall fumonisins exposure. Therefore, these should be considered to avoid underestimation of the exposure in risk assessment.

1.3.4. Methods of analysis

1.3.4.1. Fumonisins

The methods of analysis for fumonisins have been largely described by the EFSA CONTAM Panel (2018).

Group B fumonisins are soluble in water and polar solvents, and therefore, they can be extracted from raw and processed materials with water/methanol or water/acetonitrile mixtures. As for other mycotoxins, sample clean-up strategies may involve the use of SPE cartridges, as well as immunoaffinity columns (Hubner et al., 2012; Berthiller et al., 2014).

The analytical determination of fumonisins is usually carried out by reverse phase liquid chromatography separation, using water/methanol or water/acetonitrile as elution solvents (Möller and Gustavsson, 2000; Bartók et al., 2010b). Due to the lack of UV-absorbing or fluorescent chromophores, measurement of fumonisins involves a derivatisation step with fluorescent labels, such as *o*-phthaldialdehyde (OPA) (Wilkes and Sutherland, 1998; Arranz et al., 2004). Such derivatisation is not needed when liquid chromatography-mass spectrometry (LC–MS) methods are implemented.

These high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) methods are still in use for routine purposes, but LC coupled to tandem mass spectrometry (LC–MS/MS) has over the last decade become the method of choice for fumonisin determination. Common procedures are based on electrospray ionisation (ESI) in positive mode. The sensitivity is often very good, reaching

the limit of quantification (LOQ) in the range 50–100 μg/kg for FB_1 and FB_2. However, the inclusion of fumonisins in multitoxin methods is still difficult, due to the different polarity and the increased matrix effect, compared to other mycotoxins, i.e. trichothecenes. Therefore, such approaches often suffer from poor recovery (≤ 60%) and lower accuracy for fumonisins, when compared to other analytes. Such effects can be counteracted by using stable isotopic standards or matrix-matched calibration (Rychlik and Asam, 2008; Varga et al., 2012).

Several tests, based on immunochemical detection, are available on the market for $FB_{1–3}$ determination. The limit of detection (LOD) for enzyme-linked immunosorbent assay (ELISA) kits is usually in the range 25–50 μg FBs/kg, with specificity of 100% for FB_1 and FB_3 and of 40% for FB_2. Lateral flow devices have been developed for semiquantification in maize and show a limit of detection in the range 0.3–3.0 mg FBs/kg feed.

1.3.4.2. Modified forms of fumonisins

Methods for analysing modified fumonisins are commonly based on two different approaches, i.e. direct analysis, or indirect analysis obtained by alkaline hydrolysis or enzymatic digestion of the sample. According to the selected strategies, the monitored final analyte may be different, and the result may require a correction based on stoichiometric factors for the evaluation of the contamination in terms of FBs. Since the calculation step may introduce an additional factor of uncertainty, this should be considered in the exposure assessment procedure.

Direct methods

Phase I metabolites

Extraction and analysis methods for modified fumonisins are very similar to the parent compounds, and therefore $FB_{1–3}$, as well as $HFB_{1–3}$ and other modified forms, are often determined within the same chromatographic run. Historically, many protocols were based on HPLC-FLD with OPA derivatisation, as already used for FBs. However, recent methods mainly involve mass spectrometry (MS) (De Girolamo et al., 2014), and $pHFB_{1–3}$ are less frequently measured because of their lower stability, although the protocols in use are the same proposed for $FB_{1–3}$ and $HFB_{1–3}$.

Phase II metabolites

Phase II metabolites of fumonisins are often characterised by the conjugation with long-chain fatty acids. These forms are, therefore, less polar than the parent compounds, and their co-extraction with parent compounds can be challenging in terms of recovery and chromatographic separation. For this reason, few studies are reported in the literature and the incidence of these forms compared to parent compounds could be under- or over-estimated.

Fatty acid esters of FB_1 have been recently reported in rice and maize (Bartók et al., 2010a; Falavigna et al., 2013). These rather apolar compounds are commonly extracted from the matrix using water: methanol (25/75, v/v), then the sample is directly analysed by LC–MS/MS. Similar conditions have been applied to the determination of *N*-acyl forms of fumonisins (Bartók et al., 2013b).

Process-derived forms

Process derived forms of fumonisins are mainly Maillard-type compounds that can be easily extracted from the matrix under the same conditions applied for parent compounds.

The main N-alkyl-conjugates of fumonisins, NDF-FB_1 and NCM-FB_1, are extracted with the same methods used for FB_1, mainly based on the use of water/methanol or water/acetonitrile mixture. The clean-up step is usually avoided (Castelo et al., 2001; Seefelder et al., 2001, 2003; Voss et al., 2001).

Following the extraction, the analysis of modified fumonisins is almost exclusively based on LC–MS/MS. The separation is obtained on a C18 column, using 0.1% aqueous formic acid or acetic acid and methanol/water or acetonitrile/water as mobile phase, under positive ESI as an ionisation mode. As with the parent compounds, modified fumonisins determination suffers from matrix effect. Therefore, the use of matrix-matched calibration or of isotopic standards (when available), is strongly required.

Indirect methods

Starting from the 1990s, it has been observed that performing alkaline hydrolysis of contaminated corn products often leads to a higher amount of released hydrolysed fumonisins than that stoichiometrically derived by the conversion of the fumonisins detectable by routine analytical methods. This additional amount of FBs may be due to both non-covalently and covalently bound fumonisins, although it is not possible to distinguish between the two.

Under alkaline conditions, $FB_{1–3}$ lose their side chains (TCAs) and, if the reaction is complete, they can be fully recovered as $HFB_{1–3}$. As sugar, starch, peptide or protein conjugates are also attached to the side chains, fumonisins can be liberated by this treatment and measured (Dall'Asta et al., 2009a, 2010; Bryła et al., 2014, 2015). However, although often used for total fumonisin determination, the protocol may be easily affected by bias, especially when calculation is applied for obtaining free and bound FB amounts (Dall'Asta et al., 2009b; Bryła et al., 2014, 2015).

The main drawback of this approach is the lack of information about the single modified forms occurring in the samples, since all forms are detected as $HFB_{1–3}$ and results are given as $FB_{1–3}$ equivalents. Besides modified forms, under this approach non-covalently bound fumonisins are also detected as $HFB_{1–3}$, thus leading to additional difficulties in the estimation of exposure.

1.3.4.3. Hidden forms/non-covalently bound fumonisins

The term 'hidden forms' refers to the fraction of fumonisins associated with the matrix via strong non-covalent interaction, and thus non-extractable. Such non-covalent interactions may be weakened when matrix macrocompounds are disrupted, i.e. following protein denaturation, starch hydrolysis, etc. Therefore, changes in extraction parameters such as pH, salts, temperature, particle size, etc., may strongly affect the extractability of fumonisins.

To address this analytical issue, several approaches have been proposed, mainly based on the use of strong chemical and/or enzymatic hydrolysis of the matrix. Alkaline hydrolysis, already discussed as an indirect determination of modified forms, is actually the most widely used approach, in spite of possible bias due to analytical difficulties (Dall'Asta et al., 2009b; Bryła et al., 2013, 2014, 2015). In addition, the enzymatic digestion of the matrix has been proposed by several authors (Dall'Asta et al., 2010; Bertuzzi et al., 2016).

1.3.5. Legislation

Directive 2002/32/EC on undesirable substances in animal feed stipulates that rules on feedingstuffs are needed to ensure agricultural productivity and sustainability and to ensure public and animal health and animal welfare. Annex I of this Directive contains maximum levels of a number of undesirable substances (chemical contaminants) that may be tolerated in products intended for use as animal feed. Fumonisins are not regulated under this Directive.

Guidance values for fumonisins (fumonisins $B_1 + B_2$) have been recommended under Commission Recommendation 2016/1319/EC.[2] The guidance values are shown in Table 1. Currently, modified forms of fumonisins are not considered in the legislation.

Table 1: Guidance values for fumonisins $B_1 + B_2$ in products intended for animal feed in the EU (Commission Recommendation 2016/1319/EC)

Products intended for animal feed	Guidance value in mg/kg relative to a feedingstuff with a moisture content of 12%
Feed materials[(a)]	
• Maize by-products[(b)]	60
Compound feed for	
• pigs, horses (Equidae), rabbits and pet animals	5
• fish	10
• poultry, calves (< 4 months), lambs and kids	20
• adult ruminants (> 4 months) and mink	50

(a): Particular attention has to be paid to cereals and cereals products fed directly to the animals that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feedingstuffs are used in a daily ration.

(b): The term 'Maize and maize products' includes not only the feed materials listed under heading 1 'Cereal grains and products derived thereof' of the list of feed materials referred to in part C of the Annex to Regulation (EU) No 68/2013 but also other feed materials derived from maize in particular maize forages and roughages.

[2] 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.

2. Data and methodologies

2.1. Data

2.1.1. Feed occurrence data

Following an European Commission mandate to EFSA, a call for an annual collection of chemical contaminant occurrence data in food and feed, including fumonisins, was issued by the former EFSA Dietary and Chemical Monitoring Unit (now DATA Unit)[3] in December 2010 with a closing date of 1 October of each year. The data submissions to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a); occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and 'Data analysis and reporting'. By the end of July 2017, a total of 18,273 analytical results from 8,057 samples on fumonisins in feed were available in the EFSA database. Data received after that date were not included in the data set used to estimate dietary exposure. No data on the modified forms of fumonisins were available in the EFSA Chemical Occurrence database.

Following the EFSA SOP on 'Data analysis and reporting' to guarantee an appropriate quality of the data used in the exposure assessment, the initial data set was carefully evaluated applying several data cleaning and validation steps. Special attention was paid to different parameters such as 'Sampling strategy', 'Sampling year', 'Sampling country', 'Analytical methods' and the 'Reporting unit'. Feeds were classified based on the catalogue of feed materials specified in the Commission Regulation (EU) No 68/2013[4].

Analytical results were reported either on a whole weight basis or with a dry matter (DM) content of 88%. Before estimating dietary exposure, all results were converted into 88% DM mg/kg. For those samples expressed on whole weight basis, the moisture content was used to convert the analytical result into 88% DM; when the moisture content was missing, whenever possible, the moisture content was estimated from reported values (see Section 3.2.2).

In analysing the occurrence data of fumonisins, the left-censored data (results below LOD or below LOQ[5]) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009) and in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b). The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for naturally occurring contaminants, nutrients and mycotoxins. The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD)/LOQ (< LOQ). The UB is obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

According to the previous studies reported in the literature, hidden fumonisins contribute to the overall fumonisins occurrence for an additional amount ranging from 40% to 70% of the parent compounds, and in a few cases may reach an additional 100% (See Appendix D). In maize, the presence of hidden fumonisins is influenced by the growing season, the genotype, and on the processing (Dall'Asta and Battilani, 2016). As a general observation, the ratio of modified fumonisins is higher when the overall contamination is low, while it is lower in highly contaminated samples (Dall'Asta and Battilani, 2016). Although this percentage can vary depending on the processing, different factors cannot be derived for single products, due to the lack of sufficient data from the literature.

Therefore, the CONTAM Panel agreed that the exposure assessment would be performed assuming an additional contribution of 60% with respect to the parent compound.

2.1.2. Feed consumption data

Fumonisins and their modified forms are predominantly found in cereal crops, cereal grains and by-products of cereal processing and the highest levels are generally reported in maize grains and maize

[3] From 1 January 2014 onwards, Evidence Management Unit (DATA).
[4] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials.OJ L 29, 16.1.2013, p. 1–64.
[5] The LOD can be defined as the lowest concentration level that can be determined to be statistically different from a blank. Similarly, the LOQ is the minimum concentration or mass of the analyte that can be quantified with acceptable accuracy and precision (Keith et al., 1983. Principles of environmental analysis, Analytical Chemistry 55 (14), 2210–2218).

by-products. Cereals and their by-products are widely used as feed for livestock, almost all of which (> 95%) are grown or produced in the EU.[6]

Forages are also important constituents of livestock diets (principally for ruminants and horses), and frequently are the sole feed. Since fumonisins and modified forms have been identified in certain forages – and particularly maize silage – estimates of intake of forages are also required to assess likely exposure.

In this opinion, two approaches have been adopted to estimate exposure to fumonisins and its modified forms. For many livestock in the EU, part or all of the daily ration is provided in the form of manufactured compound feeds, and where data on levels of fumonisins in species-specific compound feeds[7] are available these have been used to estimate exposure. Since compound feeds represent the complete diet for many livestock, this is the preferred method of calculating exposure. However, for some livestock categories, information on levels in compound feeds has not been given, or insufficient data have been provided to allow reliable estimates of exposure to be made, and for these, the occurrence data on individual feed materials have been used, together with example diets (Appendix C) to estimate exposure. It should be stressed that these do not represent 'average' diets, nor are the feeding systems 'typical' for all of Europe. Instead, they are used to estimate levels of exposure to fumonisins and their modified forms that might be indicative. They are based on published guidelines on nutrition and feeding (AFRC, 1993; Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2008; McDonald et al., 2011; EFSA FEEDAP Panel, 2012; OECD, 2013) and expert knowledge of production systems in Europe. Details of the rations used and live weights assumed are given in Appendix C.

2.1.3. Toxicokinetic and toxicological data

Data were obtained from the scientific literature as described in 2.2.2.

2.2. Methodologies

2.2.1. Use of default value for Fumonisins, modified forms and hidden forms included in the assessment

2.2.1.1. Modified forms

As described in Section 1.3.1 (Fumonisins, modified forms and hidden forms considered in this opinion) $FB_{1–3}$ as parent forms, modified forms of fumonisins and hidden forms of fumonisins have been included in the assessment, according to the available occurrence data.

Due to the lack of information on their toxicity, the CONTAM Panel was unable to derive any relative potency factor (RPF) for modified fumonisins (EFSA CONTAM Panel, 2018).

In consideration of the lack of occurrence data for modified forms of fumonisins in the EFSA database, and since studies from the literature indicate a low occurrence (less than 10%) of these forms compared to the parent compounds, modified forms of $FB_{1–3}$ were not included in the exposure assessment.

FB_4 was not considered in this opinion since it occurs mainly in grapes, which is not a major feedstuff. In addition, data on the occurrence, toxicity and toxicokinetics (TK) could not be identified for FB_4.

2.2.1.2. Hidden forms

As discussed in Section 1.3.3.3, hidden fumonisins may be available after digestion along with the parent compounds, thus increasing the total fumonisin exposure.

Although the proportion of hidden fumonisins may vary depending on the food process, different factors cannot be derived for different matrices due to the lack of appropriate information.

Based on the data from the literature and in agreement with the previous assessment (EFSA CONTAM Panel, 2014), an additional factor of 60% was applied for hidden fumonisins to the occurrence of parent compounds in feed. Therefore, two exposure scenarios were calculated, one for the parent fumonisins ($FB_1 + FB_2 + FB_3$) and one increased by a factor of 60% to take into account the contribution of hidden fumonisins.

[6] Source: FEFAC Feed and Food Statistical Yearbook 2014. Available online: www.fefac.eu
[7] Complete and complementary feedingstuffs.

2.2.2. Methodology for data collection and study appraisal

In 2015, the CONTAM Panel received from European Commission the mandate for an assessment of the risk to animal health of fumonisins and their modified forms. In addition, a mandate was received to assess whether it is appropriate and feasible to set a group HBGV for fumonisin B_1 and B_2 and their modified forms identified in the CONTAM opinion on the risks for human health related to the presence of modified forms of certain mycotoxins in food and feed (EFSA CONTAM Panel, 2018), and to consider, if relevant, the appropriateness to use the parent compounds as a marker for presence and toxicity of fumonisin B_1 and B_2 and their modified forms.

A call for a literature search and review was launched in March 2016 within the Framework Contract (FWC) No OC/EFSA/AMU/2014/01 Lot 2 Chemical/toxicological – FWC 6 with the aim of identifying and collecting relevant literature related to fumonisins and their modified forms to support preparatory work for the present opinion and that on HBGVs (EFSA CONTAM Panel, 2018). A final project report was delivered in November 2016 and published on 23 February 2018, together with the opinion on HBGVs for fumonisins (EFSA CONTAM Panel, 2018; NFI-DTU, 2018). Briefly, nine search strings were designed to identify potentially relevant studies and after removal of duplicates and applying inclusion/exclusion criteria (as described in NFI-DTU, 2018) potentially relevant references were identified. Papers published in the period from 1/1/2000 (the year of publication of the SCF opinion) until 21/7/2016 were considered (except for adverse effects in farm and companion animals where the starting date was 1/1/1980). The total number of publications identified, and the number of publications identified as potentially relevant for each of the scientific areas, were: Chemistry and analysis (4,456/532), toxicokinetics (2,262/114), mode of action (1,649/273), *in vivo* toxicity (3,555/87), *in vitro* toxicity (1,632/138), observations in humans (2,424/38), adverse effects in farm and companion animals (5,087/270), occurrence in food (3,284/709) and occurrence in feed and animal exposure (3,283/270). The report contains as an annex all abstracts screened together with an evaluation of their relevance and the corner points of the individual publications.

The abstracts proposed as potentially relevant in the report were then screened by the working group (WG) members and, by applying expert judgement, were used in the assessment if considered relevant for animal risk assessment.

Since a series of previous assessments of either EFSA or other scientific bodies were available (IARC, 1993, 2002; SCF, 2000, 2003; FAO/WHO 2001, 2012; EFSA, 2005; EFSA CONTAM Panel, 2014, 2018), these were also considered for the present assessment. Whenever necessary, original publications referenced in these previous assessments were retrieved.

In addition to the systematic search and the use of previous evaluations for retrieval of relevant literature, a 'forward snowballing' approach[8] was applied by all WG members in order to obtain any relevant information published up to 1 October 2017.

2.2.3. Methodologies for dietary exposure assessment in animals

Exposure to fumonisin by livestock is a function of its concentration in their diets and the amount of the diet consumed. In the absence of a comprehensive database on the amounts or types of feed consumed by livestock in the EU, estimates of feed consumed for each of the main categories of farmed livestock and companion animals are based on published guidelines on nutrition (e.g. Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2008; McDonald et al., 2011; EFSA FEEDAP Panel, 2012; OECD, 2013), together with expert knowledge of production systems in Europe.

For many farmed livestock and companion animals, their nutritional requirements are provided in commercially manufactured complete (compound) feeds. Where sufficient (reliable) data on the concentrations of fumonisins in compound feeds have been provided, these have been used to estimate exposure. However, where insufficient compound feed data were available, the CONTAM Panel identified example diets and feed inclusion rates, and used concentrations of fumonisin in individual feed materials to estimate P95 and mean exposure both LB and UB. Details of the intakes and composition of diets used in estimating animal exposure to fumonisins are given in Appendix C.

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

2.2.4. Methodology applied for risk assessment

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. The principles described by WHO/IPCS (2009) and EFSA guidances pertaining to risk assessment have been applied for the present assessment. For details on the specific EFSA guidances applied, see Appendix A.

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics

3.1.1.1. Fumonisins

The absorption, distribution, metabolism and excretion, (ADME) of fumonisins was reviewed by EFSA in 2005 (EFSA, 2005) and, more recently in 2018 (EFSA CONTAM Panel, 2018), in an opinion addressing the appropriateness to set an HBGV for fumonisins and their modified forms in humans.

Based on a limited data set in laboratory species, farm animals and humans, it was concluded that, upon oral exposure, fumonisins display a limited bioavailability (3–6%) and exhibit peak plasma levels a few hours after the exposure. The poor bioavailability is mainly due a very limited absorption rate, as confirmed by *in vivo* investigations with the labelled toxin and *in vitro* studies using differentiated Caco-2 cells, an established model of human enteric absorption.

Once absorbed, fumonisins are rapidly cleared from the systemic circulation with half-lives of few hours. Although relatively higher concentrations are usually detected in the liver and kidney, no specific target tissues for fumonisins accumulation have been found.

Overall, fumonisins are known to be biotransformed to a limited extent in mammalian species. The first step entails the hydrolysis of the ester groups yielding two metabolites of $pHFB_1$ (also referred to as aminopolyols) and HFB_1. The generation of HFB_1 is of note due to the higher lipid solubility (and hence potential bioavailability) of this metabolite compared to FB_1 (Humpf et al., 1998). Accordingly, an *in vitro* study performed with differentiated Caco-2 cells, HFB_1, but not FB_1, was able to cross the epithelial cell barrier and its absorption appeared to be regulated by the drug transporter P-gp (De Angelis et al., 2005).

Most of the hydrolytic reactions appear to be carried out by microorganisms occurring in the lower enteric tract. Unlike studies with chyme suspensions, a number of *in vitro* experiments conducted with primary cell cultures and/or tissue subfractions failed to detect any hydrolysed derivatives or other metabolites following the incubation of the parent compounds. This notwithstanding, the incubation of clofibrate-induced[9] pig liver microsomes with 2–100 μM FB_1 has been reported to generate a type I spectrum upon ultraviolet-visible (UV–vis) absorption spectroscopy, indicating that the toxin may be a substrate of CYP4A with an affinity of around 5 μM; a putative hydroxylated metabolite distinct from the hydrolysed ones was tentatively identified (Marvasi et al., 2006).

Despite the scant information concerning the role of drug transporters and tissue biotransformation enzymes in fumonisins kinetics, it has been reported that both may be modulated by fumonisins. The modulation of biotransformation enzymes has been recently reviewed by Wang et al. (2016) and Wen et al. (2016). For example, the intraperitoneal (i.p.) administration of FB_1 (0.125, 0.25, 2.5 mg/kg bw per day for 6 days) was documented to upregulate CYP1A and CYP4A in rat liver (Martinez-Larrañaga et al., 1996). In addition, the oral administration of 0, 5, 15 and 45 mg FB_1/day to ducks over 12 days resulted in the increase in a number of hepatic CYP-mediated biotransformations (mainly CYP3A) even at the lowest dose, while phase II enzymes were less affected (Raynal et al., 2001). More recently (Antonissen et al., 2017), a trial was conducted on broiler chickens which were offered for 15 days a diet containing FBs at levels approaching the EU guidance ones (20 mg/kg). Treated animals showed an almost 25-fold increase in jejunum CYP1A4, an isoform which is orthologous to mammalian CYP1A1; at the same time, a threefold increase in MDR1/ABCB1 (P-gp) expression was also noticed. Interestingly, birds exposed to same dosages revealed minor but detectable changes in enrofloxacin kinetic parameters following an oral bolus administration of the drug. Although the effects of FBs on

[9] Clofibrate is a typical CYP4A inducer and peroxisome proliferator in mammalian species; CYP4A metabolizes mainly fatty acids at their omega carbon.

biotransformation enzymes and drug transporters have not been thoroughly investigated, there is the potential for the alteration of the kinetics of xenobiotics that are substrates of the affected enzymes/ drug transporters.

A further metabolic pathway, i.e. the N-acylation of the hydrolysed forms at the primary amino group with fatty acids of various chain length, has been documented in cell lines and in rodents, but not in livestock or companion species; the *in vivo* formation of *N*-acyl-FB_1 has been also demonstrated in rats. It is generally accepted that the N-acylation reactions are carried out by tissue ceramide synthase. The main metabolic pathways of fumonisins are depicted in Figure 4.

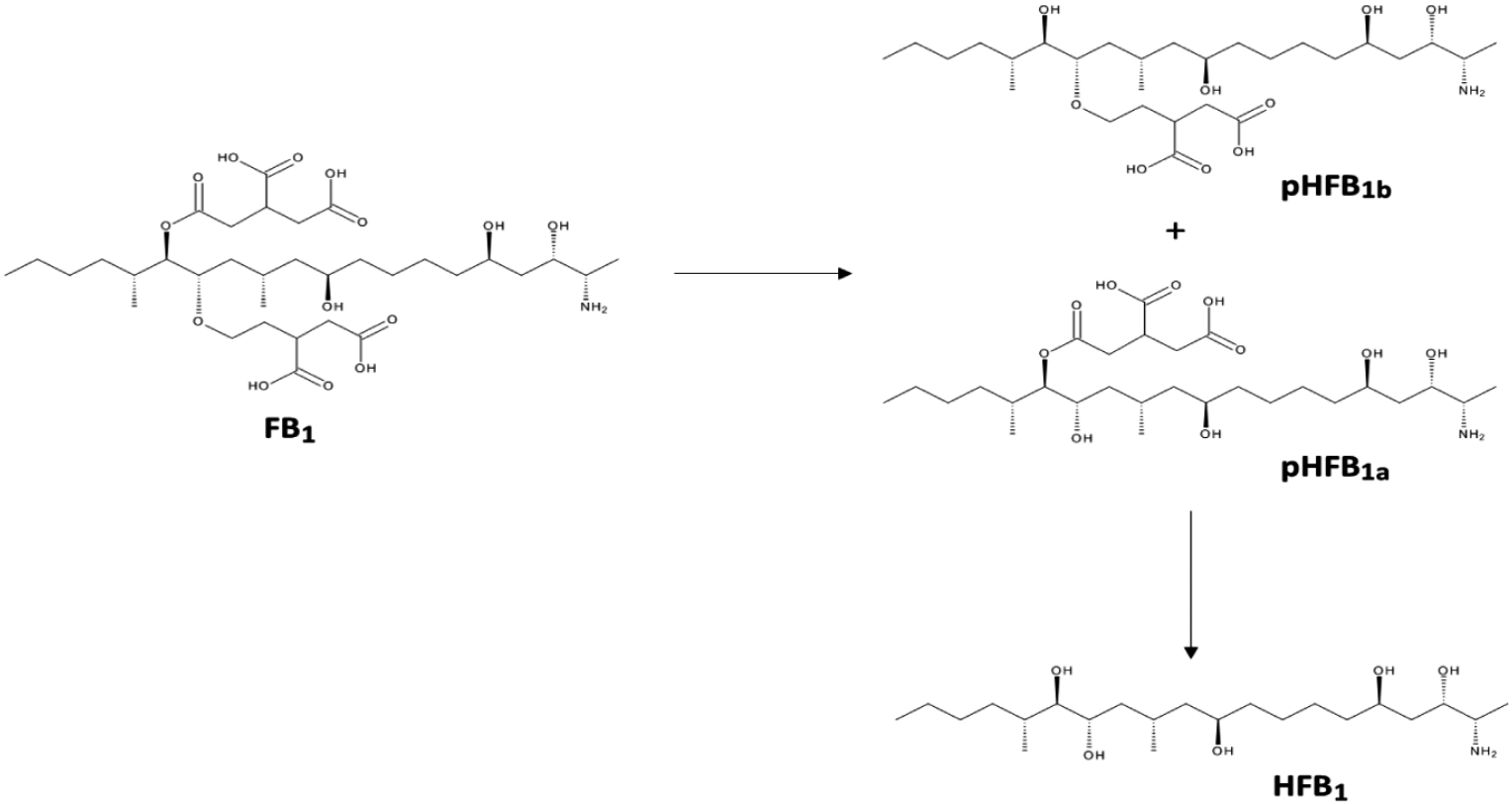


Figure 4: Metabolic pathways of fumonisins

Biliary excretion of FBs has been documented in a number of species, followed by enterohepatic circulation. Urinary excretion has been reported as a minor route, fumonisins being primarily excreted via the faecal route.

No data on fumonisin biotransformations are available for avian species and no information on fumonisin kinetics could be identified for companion animals, horses, rabbits, farmed mink and fish.

Appreciable interspecies differences in fumonisin TK have been reported (see Section 3.1.1.2). However, due to a limited data set, a link between such differences, the various peculiar syndromes occurring in farm animals and species sensitivity has not yet been established. Although contrasting results have been reported in rats (reviewed in Wang et al., 2016), the majority of the available *in vivo* studies carried out in laboratory species point to a lower toxicological significance of FB metabolites (mainly HFB_1) vs the unmodified toxins. There is a limited knowledge concerning food producing species. Based on plasma and liver Sa/So ratios, liver and enteric morphology, and cytokine expression, a much lower effect of HFB_1 compared to FB_1 was documented in piglets fed a diet contaminated compound feed at a concentration of approximately 37–44 mg/kg for 14 days (Grenier et al., 2012). More recently, the toxic effects of FB_1 or HFB_1 were compared in turkeys and piglets (Masching et al., 2016). Animals were offered a contaminated diet in the presence or absence of a commercial carboxylesterase, which was intended to cleave FB_1 into its hydrolysed metabolites. As expected, marked reductions in FB_1 content and a parallel rise in HFB_1 concentration were detected in the excreta of animals receiving the carboxylesterase fortified diet; this finding was matched by a significant reduction in the Sa/So ratio which was taken as a biomarker of FB_1 toxicity. Although the study was not performed with the purified metabolite, the results reinforce the view that FBs hydrolysis should be considered as a detoxification mechanism.

3.1.1.2. Species-related kinetics

Ruminants

Cattle

According to Smith and Thakur (1996) and Caloni et al. (2000), using an artificial model of a cow's rumen, a very limited decline (9–12%) in the amount of measurable fumonisins was observed after up to 72 h incubation, and it was not possible to detect any hydrolysed metabolic derivative. A limited degradation (8–10%) of FB_1 was also reported by Gurung et al. (1999) following incubation of 50 or 100 mg FB_1/kg in ruminal fluid.

Cattle hepatic microsomes were incubated with FB_1 (7, 14 or 28 μM) for up to 60 min in the presence of an NADPH-generating system and the incubates were analysed for the presence of FB_1, $pHFB_1$ and HFB_1 by HPLC. Neither an appreciable decrease in the parent molecule concentration nor the appearance of measurable amounts of the examined metabolites could be detected (Spotti et al., 2001).

To gain insight into the excretion of FB_1 in milk, *in vitro* experiments were carried out with the isolated and perfused udder (Spotti et al., 2001). For each udder (n = 3), 2 mg of FB_1 were injected in the perfusion blood of a pair of quarters to reach a concentration of 400 ng/mL, while the other two were left untreated. The concentration of FB_1 was measured in both serum and milk samples at 0, 30, 60, 120 and 150 min after dosing. At the end of the monitoring period, serum FB_1 concentrations were about the half of those measured after 30 min, with no appreciable binding to erythrocytes. Measurable levels of FB_1 (up to around 20 ng/mL) were found in milk samples. The authors concluded that FB_1 is able to cross the mammary barrier but did not provide evidence of the mycotoxin fate in the udder tissue.

In a study specifically designed to set up analytical methods to measure FB_1 and metabolites in feeding stuffs and animal excreta (Rice and Ross, 1994), cattle (gender, breed and trial duration not reported) were administered with a diet containing 200 or 400 mg FB_1/kg (n = 5/dose). Faecal and urine samples (sampling time not specified) were collected and analysed by HPLC for the presence of FB_1 and the sum of $pHFB_1$ and HFB_1 (the latter only in faeces). Faeces were found to contain FB_1 (1–6 mg/kg) and a higher amount of $pHFB_1$ + HFB_1 (14 mg/kg), whereas a lower concentration of FB_1 (0.1–0.7 mg/kg) was measured in urine. For comparison, the dietary exposure of rats to a higher FB_1 concentration (1,000 mg/kg) resulted instead in a prevalent faecal excretion of the parent compound with respect to $pHFB_1$ + HFB_1 (530 vs 282 mg/kg) and in urine FB_1 concentrations of the same order of magnitude as those reported for cattle. The study suggests that, upon oral exposure of cattle, FB_1 is largely excreted via the faecal route and to a lesser extent via urine; faeces also contain a measurable amounts of hydrolysed metabolites.

Prelusky and collaborators (1995) investigated FB_1 kinetics in four dairy cows (452–630 kg bw, unspecified breed) following either i.v. dosing (50 or 200 μg/kg bw) or oral gavage (1 or 5 mg/kg bw). Both FB_1 (LOD = 4 ng/mL) and HFB_1 (aminopentol) (LOD = 8 ng/mL) were assayed in plasma using an HPLC technique with fluorescence detection. Data from the i.v. administration best fitted a two-compartment model, with similar values irrespective of the dose. There was a very rapid distribution phase ($t_{½}$ α ~ 2 min) and a slower but still rapid elimination phase ($t_{½}$ β 15–18 min) with the parent compound and the metabolite being no longer detectable 120 min after dosing. Similar and relatively low values also occurred for the volume of distribution (V_d ~ 0.25 L/kg) pointing to a prevalent presence of the toxin in the extracellular compartments before being excreted. Whatever the dosage, no measurable amounts of either compound were recovered in plasma from orally exposed animals. The authors concluded that a low absorption and/or a very efficient pre-systemic metabolism might explain the observed results.

Sheep

The temporarily isolated rumen model is an experimental technique performed in living animals to assess both the ruminal metabolism and the systemic absorption across ruminal walls of a given molecule. Applying this technique to Texel wethers (N = 3, average weight 65 kg), no ruminal degradation of FB_1 (1 μg/mL) or systemic absorption could be demonstrated (Pantaya et al., 2014).

The only paper identified dealing with the *in vivo* TK of FB_1 in sheep is the study of Rice and Ross (1994). Sheep (gender, breed, sampling time and trial duration not reported) were exposed to a diet containing 50 mg FB_1/kg (n = 5/dose). The proportion of FB_1/$pHFB_1$ + HFB_1 recovered in faeces (6/10 μg/g) and the urinary levels (0.1–3.8 μg/g) were of the same orders of magnitude as those reported for cattle.

Goats

Eight weanling female Angora goats (15 ± 2.1 kg bw) were randomly allotted to a control group (< 1 mg/g FB_1) and a treated group receiving a contaminated diet (95 mg of FB_1/kg diet) for 112 days, with four goats per diet (Gurung et al., 1998). Using an HPLC method with a low sensitivity (LOD 1 mg/kg), an average daily consumption of 45 ± 4 mg FB_1 could be estimated for the whole trial. Only 21 ± 4 mg FB_1 (47%) of the daily ingested toxin was excreted as such in faeces during the last 7 days trial; in addition, no FB_1 residues > LOD could be detected in the liver, kidneys or hearts of the treated animals (metabolites not determined). Taken together, these results point to an extensive biotransformation of the toxin, but no indication about FB_1 bioavailability could be derived.

In conclusion, there is scant information on the kinetics of fumonisins in ruminants, and all what is known refers to FB_1. The available data indicate a very limited bioavailability of the toxin per se, along with an extensive biotransformation to HFB_1 and $pHFB_1$. The *in vitro* data would exclude the substantial involvement of either the ruminal microbiota or microsomal liver drug metabolising enzymes in the generation of the hydrolysed derivatives. Both the parent compound and the hydrolysed metabolites are mainly eliminated via the faeces, the urinary route representing only a minor excretion pathway. Excretion in milk has been investigated and documented in cows only.

Pigs

To study the *in vitro* metabolism of FB_1 in pigs, cecal chyme suspensions were incubated anaerobically with 5 μM FB_1 up to 72 h. Samples were collected at 12 h intervals and analysed for the presence of FB_1, $pHFB_1$ and HFB_1 with LC–MS. A very low amount of HFB_1 was detected at each time point, overall accounting for less than 1% conversion of the parent molecule. By contrast, a negative correlation was found between FB_1 and $pHFB_1$ concentrations at the different sampling times; overall, the conversion of FB_1 into the measured metabolites amounted to about 50%. It was concluded that under *in vitro* conditions, a significant portion of FB_1 is biotransformed into its hydrolysed derivatives (Fodor et al., 2007).

A previous evaluation (EFSA, 2005) reported a study in which the kinetics of ^{14}C-FB_1 was investigated in pigs after i.v. (0.40 mg/kg bw) or oral (intragastric, 0.50 mg/kg bw) single administration. After i.v. dosing, a tri-exponential concentration–time profile was observed, with apparent plasma half-lives of 2.2 min ($t_{½}$ α), 10.5 min ($t_{½}$ β), and 192 min ($t_{½}$ γ), respectively. The latter was assumed to reflect a significant enterohepatic re-circulation. Biliary recovery was 70.8% of the administered dose, while 3 days after treatment 21.2% and 58.3% of the administered FB_1 were found in urine and faeces, respectively. Based on plasma and excretion data, FB_1 systemic bioavailability in orally exposed pigs was estimated to be very limited (3–4%). No FB_1 residues

(LOD = 1 mg/kg) were found in milk from sows exposed to a diet containing 100 or 200 mg FB_1/kg for 14 days (Becker et al., 1995).

Meyer et al. (2003) investigated the tissue distribution of FB_1 in 13 weaned castrated pigs (12–14 kg bw, breed and age not mentioned) exposed to a diet contaminated by *Fusarium verticillioides* fungal culture to ensure a daily intake of 100 mg FB_1/head. Five individuals died during the treatment. Six of the remaining animals were sacrificed after 5 days, while the two remaining (living) animals were euthanised after 10 days of treatment. The amount of FB_1 was determined by a LC–MS analysis on plasma, bile and samples of lungs, liver, bile, kidney, brain, spleen, pancreas, heart, eye, muscle (m. longissimus dorsi, m. biceps femoris and m. psoas major), subcutaneous and abdominal fat. On average, FB_1 content was highest in kidneys (1,530 μg/kg) followed by spleen (1,020 μg/kg), liver (379 μg/kg) and lungs (204 μg/kg). Taken together, muscles were found to contain 43 μg/kg and fat 6 μg/kg. Relatively high levels (384 μg/kg) were recovered in the bile, likely indicating the occurrence of an important enterohepatic cycling.

Distribution and elimination of fumonisins in tissues was investigated in weaned barrows (breed not specified, 12–14 kg bw) (Fodor et al., 2006). Piglets (N = 10) received a diet containing *F. verticillioides* fungal culture to provide a daily intake of 50 mg FB_1, 20 mg FB_2, and 5 mg FB_3 per animal for 22 days, corresponding to 2.2, 0.88 and 0.22 mg FB_1, FB_2 or FB_3/kg bw, respectively. Total collection of quantity of faeces and urine was undertaken for 5 days, i.e. between days 13 and 17 of the treatment period. At the end of the trial, animals were necropsied and samples of liver, lungs, kidney, brain, spleen, heart, muscle longissimus dorsi and psoas, abdominal and subcutaneous fat, as well as bile, were collected. All samples were analysed for FB_1 and FB_2 by a LC–MS method. Tissue levels of FB_1 were in the order liver (99 ± 37 μg/kg) > kidney (31 ± 10 μg/kg) > myocardium ~ spleen (7–9 μg/kg) > lung (about 3 μg/kg). No appreciable levels were detected in brain and muscles or in fat. Measurable levels of FB_2 could only be found in livers, lungs and fat from some animals in very low concentrations, with an estimated ratio of 1:19 with FB_1. As regards excretion, only bile samples from 1 out of 10 individuals were found to contain measurable FB_1 levels. During the 5-day test collection, faecal excretion of FB_1 largely outweighed that in urine, being on average 28.2 mg vs 4.5 mg. In the same period, it could be calculated that only 13% of the ingested FB_1 was eliminated, faecal and urinary excretion amounting to 86% and 14%, respectively. By contrast, the extent of the excretion of FB_2 appeared to be much less pronounced since concentrations of 1/9 and 1/14 with respect to those of FB_1 were measured in urine and faces, respectively. Overall, due to the large discrepancy between the amount of the ingested toxin and that recovered in the excreta, the results point to an extensive biotransformation of FB_1 and FB_2.

To address this issue, a further study was designed involving sixteen weaned barrows (Hungarian Large White, 12–14 kg bw) (Fodor et al., 2008). For the assessment of FB_1 absorption, as calculated from the Cr-FB_1 ratio in feed, piglets were offered a Cr_2O_3-fortified diet containing *F. verticillioides* fungal culture to provide a concentration of 45 mg FB_1/kg (36.6 ± 6.5 mg/day), 8.6 mg FB_2/kg and 4.6 mg FB_3/animal for 10 days, respectively. Half of the experimental animals (five treated and three controls) were sacrificed at the end of the trial, while the remaining were killed 10 days after treatment cessation. A special T-cannula was implanted into the distal part of the ileum to allow for the determination of FB_1 absorption from the Cr-fortified feed. During the whole 10-day treatment faeces and urines were quantitatively collected and samples of chymus and of the same tissues as described in the previous paper (Fodor et al., 2006) were taken. The amounts of FB_1, FB_2 and the hydrolysed metabolites $pHFB_1$ and HFB_1 were determined by a GC–MS method. On average, it could be calculated that the amount of the absorbed FB_1 over the treatment was of 4%. It could also be estimated that in the colonic chymus the conversion rate of FB_1 into $pHFB_1$ and HFB_1 amounted to 3.9% and 1%, respectively. At the end of the treatment, all examined organs contained measurable amounts of FB_1 and FB_2, the latter being present at much lower concentrations in all tissues but muscles, where FB_2 levels were of the same order of magnitude. As regards FB_1, liver (17.4 ± 1.7 μg/kg) and kidney (9.9 ± 0.3 μg/kg) exhibited the highest values, but remarkable levels could also be found in m. longissimus dorsi (11.2 ± 1.2 μg/kg) and m. psoas major (4.75 ± 1.5 μg/kg). Besides FB_1, both metabolites were consistently recorded, with HFB_1 levels being similar or lower than those of $pHFB_1$ in most tissues but the kidney. Overall, taking into account the levels of FB_1 and its hydrolysed metabolites recovered in the examined organs after 10-day of exposure, 50% was made by the parent compound while HFB_1 and $pHFB_1$ accounted for 30% and 20%, respectively. After comparing these results with those from the colonic chymus, the authors concluded that the hydrolysed metabolites are also likely to be generated in the proximal enteric tracts, where a significant absorption may occur. Of note, measurable levels (μg/kg) of both FB_1 and HFB_1 were still detected in most of the organs 10 days after treatment.

In the same study, during the 10-day feeding period, about 360 mg FB_1 was calculated to be ingested by piglets; of this, during the toxin exposure and the 10-day recovery period, 69% (247 mg) appeared in the excreta as the sum of the parent compound and its hydrolysed metabolites. The faecal route accounted for the majority of the eliminated toxins (98.5%), with 41% as FB_1, 47% as $pHFB_1$, and 12% as HFB_1. Conversely, only a very limited amount (1.5%) of the ingested toxins appeared in urine during the entire trial, and in this case about one-third was represented by the parent compound, the remaining being $pHFB_1$ (~ 20%), and HFB_1 (~ 15%). As regards FB_2, 23% of the ingested toxin was eliminated via the faeces and only 0.6% via the urine. On the whole, results from this study are consistent with a low absorption and an extensive biotransformation of FB_1 to $pHFB_1$ and to a lesser extent HFB_1, both of which may be detected in tissues even after treatment cessation.

The kinetics of FB_1 in blood and excreta was investigated with an HPLC method in four 8-week-old weaned pigs (Landrace × Large White × Duroc, average weight 25 kg) exposed to a single oral dose (gavage) of culture material of *F. verticillioides* containing 5 mg FB_1/kg bw[10] (Dilkin et al., 2010). Samples of blood were taken at 1 h interval up to 6 h and at 12 h intervals up to 60 h. Urine and faeces were collected up to 72 and 96 h from dosing, respectively. Bile samples were not collected. The toxin was rapidly absorbed, as reflected by the occurrence of measurable plasma levels as early as 1 h post-dosing (average 125 ± 13[11] ng/mL). FB_1 concentrations plateaued at 2 h (average 282 ± 38 ng/mL) and rapidly declined so that detectable levels could be measured in 2/4 animals and in 0/4 animals 36 and 48 h after treatment, respectively. A significant amount of the toxin (average 551 ± 117 μg[12]) was excreted in urines within 8 h of FB_1 administration, and a similar amount (average 561 ± 102 μg) occurred within 24 h. On the whole, a very limited amount of the administered toxin was detected in urine (0.93%) while approximately 76.5% of FB_1 was measured in faeces. According to the authors, the unaccounted fraction in faeces could be due to a limited absorption rate, an intense enterohepatic circulation and biotransformation to FB_1 hydrolysed derivatives.

In summary, the studies published since the previous EFSA evaluations (EFSA CONTAM Panel, 2014) do not modify the earlier conclusions on FB_1 kinetics in pigs, and indicate a very limited oral bioavailability followed by a rapid tissue distribution and an extensive biotransformation into $pHFB_1$ and HFB_1. Both metabolites are also detectable in tissues. This suggests that the generation of $pHFB_1$ and HFB_1 could not only occur in the distal enteric tract but might also take place in the proximal tract, where a higher absorption rate may be expected. Both the parent compound and its hydrolysed metabolites tend to accumulate in liver and kidney, while conflicting results are reported for muscles. Measurable levels of FB_1 and HFB_1 (μg/kg) may be detected several days after treatment cessation. The faecal excretion largely outweighs the urinary one, while the extent of biliary excretion might vary according to the dose and the duration of the exposure.

Very little is known about FB_2 kinetics. No evidence has been identified of a higher bioavailability compared to FB_1. Both the urinary and faecal excretion, as well as tissue deposition, appears to be much lower than that displayed by FB_1, pointing to a high rate of biotransformation of FB_2 into hydrolysed and possibly other metabolites.

Poultry

The TK of FB_1 in avian species has been recently reviewed by Guerre (2015).

Little is known concerning fumonisin ADME in **chickens**. In the only report found (Vudathala et al., 1994), the kinetics of ^{14}C-FB_1 (2 mg/kg bw) was investigated in 30-week-old White Leghorn laying hens (1.3–1.7 kg bw) following i.v. or oral administration. After 24 h, animals were sacrificed and in the i.v. study, the kinetics was described as bi-exponential with a very rapid equilibrium ($t_{1/2}$ α = 2.5 min) and a short $t_{1/2}$ β (40–69 min), which is consistent with a very low V_d (0.063–0.125 L/kg) and a rapid clearance of the toxin, which was present in the systemic circulation as largely unbound. Following the oral exposure, C_{max} was reached at 1.5–2.5 h in different birds with plasma levels in the range 28–103 ng/FB_1 equivalents; no radioactivity was detected in the 24 h plasma sample. The estimated bioavailability was 0.71 ± 0.5%, indicating a very limited systemic absorption. The largest fraction of the administered dose (80%) appeared in the excreta collected between 2 and 6 h post-dosing; excretion was virtually completed after 24 h from toxin administration. Besides crop and intestine, liver and kidney were the only organs with measurable levels of radioactivity; no radioactivity could be measured in eggs.

[10] According to the authors, this dose corresponded to 83 mg FB_1/kg feed.
[11] Mean ± SD.
[12] As such in the paper.

It was concluded that, in laying hens exposed to a single oral dose, FB_1 is poorly absorbed and quickly eliminated, giving rise to negligible residues in edible tissues and eggs.

In a more recent paper (Antonissen et al., 2015a), six 24-day-old Ross broiler chickens were administered 1.91 mg FB_1/kg bw and 0.59 mg FB_2/kg bw as a single intracrop administration. Blood was collected at 10 min intervals up to 60 min and at 240 min and plasma FB_1 levels were quantified by a LC–MS/MS method. The dose was calculated according to the EU guidance levels for fumonisins in poultry feed (20 mg/kg for the sum of $FB_1 + FB_2$) and a feed consumption of 125 g/kg bw. Relatively low peak levels (about 35 μg/L) were reached after 20 min, indicating a rapid but limited absorption rate. In addition, chicks exhibited elimination half-life ($t_{1/2el}$ 106 min) and mean residence time (MRT 165 min) values consistent with a rapid elimination.

Turkeys

Very little is known about fumonisin TK in turkeys. In the only paper that could be identified, Tardieu et al. (2008) investigated the comparative (i.v. vs oral) **FB_1** TK in 1-week-old BUT9 male turkeys. For i.v. studies, eight individuals were dosed with 10 mg FB_1/kg bw and blood samples were taken at different intervals up to 2,000 min after treatment. For studies using the oral route, further eight animals received a single dose of 100 mg FB_1/kg bw and blood sampling was performed at 30–60 min intervals up to 600 min after dosing. Plasma and tissue levels of FB_1 were measured by an HPLC method (fluorescence detector, LOD 13 μg/kg). Data after i.v. dosing were best fitted to a three-compartment open model and were consistent with a rapid ($t_{1/2}$ α 3.5 min) and notable distribution within the body ($V_{d\ area}$ around 1 L/kg) along with a rapid clearance ($t_{1/2}$ β 85 min, MRT 52 min, clearance around 8 mL/min per h). Following the oral administration, a C_{max} of nearly 1,000 μg/mL was reached after 180 min, while a bioavailability of 3.2% was estimated. A considerable $V_{d\ area}$ (more than 2 L/kg) and both relatively long MRT (around 400 min) and $t_{1/2\ \beta}$ (214 min) indicate the potential for tissue accumulation of FB_1 (and possibly its derivatives) in turkeys exposed to contaminated feed. To test this hypothesis, the same animals used in the oral study were sacrificed 20 h after dosing (100 mg FB_1/kg bw); measurable values of FB_1 were detected in serum (279 ± 30 μg/L), liver (5,458 ± 509 μg/kg), kidney (5,785 ± 1,002 μg/kg), and muscle (113 ± 15 μg/kg).

The **FB_2** TK was examined by Benlashehr et al. (2011) in BUT9 turkeys (6- to 7-week-old, 2 kg bw) using the purified toxin. In the i.v. study, five individuals were dosed with 1 mg FB_2/mg bw and blood samples were taken at different intervals up to 240 min after treatment. For the study by the oral route, eight animals received a single dose of 1 mg FB_2/mg bw; blood samples were collected up to 600 min after treatment. In i.v. dosed turkeys, the toxin was cleared very rapidly, with extremely short values of both MRT (around 5 min) and $t_{1/2}$ β (about 12 min) along with a very limited extent of tissue distribution ($V_{d\ area}$ around 0.15 L/kg). Accordingly, plasma levels declined very quickly, reaching values below the LOQ (25 ng FB_2/mL) already 60 min after toxin administration. As to the study involving the oral route, measurable (> LOQ) FB_2 plasma levels were found in only two out of eight animals and data could not be fitted to any TK model. Data are therefore consistent with a very limited oral bioavailability of FB_2 in turkeys.

Ducks

There is scant information about fumonisin ADME in ducks and only one report could be identified in the open literature (Tardieu et al., 2009). Kinetic parameters were first investigated in 42-day-old ducks treated by either the i.v. or the oral route using the purified toxin (96%). For the i.v. study, six animals received 10 mg FB_1/kg bw in the jugular vein and blood samples were taken at different intervals up to 1,200 min after dosing. The TK via the oral route was investigated in further six animals which were administered a single dose of 100 mg FB_1/kg bw and subjected to blood sampling up to 1,200 min after treatment. A second study (oral route only) was carried out on 96-day-old ducks after a force feeding period of 12 days with an uncontaminated diet, using the same protocol as above. After the last blood sampling, all animals were sacrificed and liver, kidney and muscle samples were taken. Plasma and tissue levels of FB_1 were measured by an HPLC method (fluorescence detector, LOD 13 μg/kg).

A two-compartment open model was demonstrated in i.v. dosed animals, showing a very rapid distribution phase (2.6 ± 0.3 min) which was followed by a relatively slower elimination phase (26 ± 2 min); the V_d was about 800 mL/kg, while the MRT and the clearance were 24 ± 1 min and 19 ± 2 mL/min per kg, respectively. A three-compartment open model best described the kinetic data in orally dosed ducks. The toxin was rapidly absorbed, with maximum serum levels of the toxin (628 μg/mL) being reached 60 min after dosing, extensively distributed ($V_{d\ area}$ = 1.7 L/kg bw) but

also rapid cleared (MRT 200 min, $t_{1/2}$ β around 70 min). A very limited bioavailability (2.3%) could be calculated. Measurable levels of FB_1 (see Section 3.1.1.5) could be detected only in liver.

The **FB_2** TK in ducks (male mule ducks, 10 weeks old, 2 kg bw) was examined in the study of Benlashehr et al. (2011) cited above. In the i.v. study, five individuals received 1 mg FB_2/kg bw and blood samples were taken at different intervals up to 240 min after treatment. For the study by the oral route, eight subjects were treated with a single dose of 1 mg FB_2/kg bw; blood samples were collected up 600 min after dosing. In i.v. dosed animals, there was a rapid decline in plasma levels and values below the LOQ (25 ng FB_2/mL) were reached already 120 min after toxin administration. A rapid clearance of the toxin was observed, with very short values of both MRT (around 13 min) and $t_{1/2}$ β (about 32 min) along with a limited extent of tissue distribution ($V_{d\ area}$ around 0.40 L/kg). Measurable (> LOQ) FB_2 plasma levels were not detected in any of the orally treated animals. Data point to a negligible oral bioavailability of FB_2 in ducks.

In conclusion, sparse information is available concerning **FB_1** kinetics in avian species. Bioavailability is very low and in the order turkey>ducks>chickens. In general, the toxin is rapidly absorbed and distributed, but also rapidly cleared. Kinetic parameters (MRT and $t_{1/2el}$) suggest a lower FB_1 clearance in turkeys compared to ducks and chickens, with the potential for tissue accumulation in turkeys (see Section 3.1.1.5). Currently, there is no information on FB_1 metabolism in avian species.

Only one study could be identified on **FB_2** kinetics for turkeys and ducks, indicating that the oral bioavailability of the toxin seems to be even lower than that of FB_1. No data on chickens could be retrieved.

No information on fumonisin kinetics could be identified for companion animals, horses, rabbits, farmed mink, and fish.

The main TK parameters measured in cows, pigs, laying hens, boilers, turkeys and ducks are reported in Table 2.

Table 2: Parameters of toxicokinetics of fumonisins in various species

Species/category	Dose (mg/kg bw) (N)	Route of admin.	C_{max} (ng/mL)	T_{max} (min)	$t_{1/2\ \alpha}$ (min)	$t_{1/2\ \beta}$ (min)	$t_{1/2\ \gamma}$ (min)	V_d L/kg	Bioavailability (%)	Reference
Cows	0.050 (1)	i.v.	–	–	1.7	15.1		0.251[(a)]	–	Prelusky et al. (1995)
	0.200 (1)	i.v.	–	–	1.7	18.7		0.278[(a)]	–	
	1	Oral	< LOD	–	–	–	–	–	–	
	5	Oral	< LOD	–	–	–	–	–	–	
Pigs	0.40[(b)] (5)	i.v.	–	–	3[(c)]	10.5[(c)]	183[(c)]	2.4 ± 0.6	–	Prelusky et al. (1994)
	0.50[(b)] (5)	Oral	–	70[(d)]	–	96[(d)]		–	4.1 ± 1.1[(d)]	
	5 (4)	Oral	282 ± 38	120	–	–	–	–	–	Dilkin et al. (2010)
Laying hen	2[(b)] (6)	i.v.	–		2.5 ± 0.3	49 ± 11		0.08 ± 0.01	–	Vudathala et al. (1994)
	2[(b)] (6)	Oral	–	130[3]	–	86[(c)]	–	–	0.7 ± 0.5	
Broiler	2.5 (6)	Oral	33 ± 21	20 ± 5	–	106 ± 8		0.23 ± 0.02	–	Antonissen et al. (2015a,b)
Turkey	10 (8)	i.v.	–	–	3.5 ± 0.8	85 ± 4		0.39 ± 0.02	–	Tardieu et al. (2008)
	100 (8)	Oral	991 ± 61	180	29 ± 3	214 ± 36		2.3 ± 0.4	3.2 ± 0.2	
Duck	10 (6)	i.v.	–	–	2.6 ± 0.3	26 ± 2		0.79 ± 0.11	–	Tardieu et al. (2009)
	100 (6)	Oral	559 ± 95	60	80 ± 13	70 ± 10		1.7 ± 0.23	2.3 ± 0.3	

C_{max}: maximum concentration achieved in the plasma following dose administration; t_{max}: time at maximum plasma/serum concentration, $t_{1/2el}$: plasma/serum elimination half life; bw: body weight; i.v.: intravenous; LOD: limit of detection.
(a): Based on area under the curve (AUC) method.
(b): ^{14}C FB_1.
(c): Average values.
(d): Average values of 4/5 individuals.

3.1.1.3. Modified forms and hidden forms

Modified forms

No specific studies on the metabolic fate of modified forms of FBs in farm and companion animals have been identified. As regards HFB_1, only indirect evidence is available from studies in pigs and turkeys. Lower intestinal and hepatic toxicity was recorded in pigs orally exposed to HFB_1 (2 μM/kg bw per day for 14 days) as compared to pigs receiving equimolar doses of the parent compound (Grenier et al., 2012). Accordingly, the alteration of sphingolipid metabolism (serum Sa/So ratio) was much less pronounced in pigs or turkeys receiving FB_1 contaminated rations supplemented with carboxylesterase (able to extensively hydrolyse FB_1 to HFB_1) in comparison with animals administered with the unsupplemented diets (Masching et al., 2016). In keeping with the conclusions of a previous EFSA opinion on the risks for animal and human health related to the presence of modified forms of certain mycotoxins in food and feed (EFSA CONTAM Panel, 2014), the reduced toxicity of FB_1 hydrolysed derivatives might be due to poor absorption. However, based on studies performed in rats (Hahn et al., 2015), other hypotheses (e.g. presystemic metabolism) cannot be ruled out.

A different behaviour has been shown by covalently bound FBs, such as NDF- and NCM-FB_1 conjugates, which are rather stable in the *in vitro* model system and not further biotransformed *in vitro* by a suspension culture of human gut microbiome (Falavigna et al., 2012; Cirlini et al., 2015).

Nothing is known so far about the stability *in vitro* of *O*- and *N*-acyl conjugates of fumonisins.

Hidden forms

Studies performed *in vitro* on the bio availability of modified FBs in maize showed that their release is strongly affected by the nature of the feed matrix modification. Non-covalent associations leading to hidden FBs can be easily disrupted *in vitro* using a digestion assay that simulates human gastrointestinal conditions (Dall'Asta et al., 2010; Falavigna et al., 2012). In these studies, the amount of fumonisin detected in the sample before the digestive assay was lower than that found in the chyme after the treatment. The release of hidden FBs from the matrix is likely due to the enzymatic degradation of starch and proteins (Dall'Asta et al., 2010). After hydrolysis in the gut the fate would be the same of parent FBs. However, specific studies on the TK of hidden forms have not been identified.

3.1.1.4. Conclusions on toxicokinetics

Little is known on fumonisin TK in food-producing animals and in companion species, and the available information is almost entirely related to FB_1 fate in ruminants, pigs and avian species. In general, the toxin is poorly bioavailable (1–6%). The absorbed fraction is rapidly distributed, mainly to the liver and kidneys, and rapidly excreted through the faeces, with the urinary route playing an ancillary role. Biliary excretion has so far been documented only in the porcine species. Likely at the enteric level, FB_1 undergoes hydrolysis to both $pHFB_1$ and HFB_1, which may be detected in tissues and excreta. However, data are lacking concerning the species-related extent, as well as the site of generation and the further metabolism (e.g. formation of *N*-acyl derivatives) of both hydrolysed derivatives. Based on a very limited data set, FB_2 shows a metabolic fate similar to FB_1 with poor bioavailability. However, both the urinary and faecal excretion, as well as tissue deposition, appear to be much lower than that displayed by FB_1.

3.1.1.5. Contribution of products of animal origin to the presence of FBs and modified forms in feed

The carry-over of FB_1 in milk, eggs and edible tissues was addressed in a previous EFSA evaluation (EFSA, 2005). Based on *in vitro* and *in vivo* studies, a limited to negligible carry over (namely 0.11–0.001%) of the toxin in cows' milk and in sows milk, respectively, was identified. A low transfer with levels in the ng/g range also occurred in eggs. Although the transfer rates were not mentioned, different studies performed in pigs, with various dosages, duration and withdrawal times, showed that livers and kidneys could be considered the target tissues of FB_1 deposition, while much lower residual levels were detected in muscles. No measurable amounts of FB_1 (LOD 1 mg/kg) were found in the liver or kidneys from goats exposed to a diet containing 95 mg FB_1/kg (Gurung et al., 1998; see Section 3.1.1.2). It was concluded that the low residue levels found in animal products from experimentally exposed farm animals 'do not contribute substantially to human exposure'. These conclusions are in line with those drawn by the SCF (2000) and have been substantially confirmed by JECFA (2011, 2017). Interestingly, as mentioned in the opinion addressing the appropriateness to set an HBGV for fumonisins and their modified forms (EFSA CONTAM Panel, 2018), a survey performed on

a few dairy milk samples (N = 10) purchased in Italian retail shops revealed the presence of trace levels of FB_1 in eight samples (mean 0.33 μg/kg, range 0.26–0.43 μg/kg, LOQ 0.33 μg/kg) (Gazzotti et al., 2009).

Since the 2005 EFSA evaluation, a limited number of reports have been published dealing with tissue residues of FB_1 and occasionally its metabolites, particularly in animals fed with fumonisin concentrations corresponding or approaching those recommended in feedingstuffs by the EU legislation.

In a preliminary study, Gazzotti et al. (2011) fed seven **piglets** (unspecified age and breed) with a diet containing the EU recommended limits for fumonisins (5 mg/kg as the sum $FB_1 + FB_2$) for 7 weeks, providing an average daily intake of about 1.66 mg/head. At the end of the experiment, the animals were sacrificed and liver samples were analysed for the presence of FB_1, HFB_1, FB_2 and HFB_2 by a LC–MS/MS method, with a LOD of 0.05 ng/g and a LOQ of 10 ng/g for each analyte. FB_1 was detected in 5/7 samples (range 15.8–42.5 μg/kg) and HFB_1 in 1/7 (17.4 μg/kg), while traces of FB_2 (between LOD and LOQ) were detected in 5/7 samples. No measurable amounts of HFB_2 were found. The authors concluded that detectable amounts of FB_1 and its metabolites may be detected in liver of piglets fed diets compliant with the EU recommended limits for fumonisins in feedingstuffs. Of note, in a previously published review, Prelusky et al. (1996) concluded that, despite a poor bioavailability, pigs are characterised by an extensive enterohepatic circulation resulting in a long elimination phase and a rapid accumulation of FB_1 in liver and kidney even in animals orally exposed to relatively low toxin concentrations (2–3 mg/kg).

Twenty-four male Ross **broiler chicks** were fed a diet containing 10 mg FB_1/kg from 21 to 42 days of age. At the end of trial, the average FB_1 content of pooled liver samples amounted to 24 μg/kg (Del Bianchi et al., 2005).

A complementary study (Tardieu et al., 2008) on the tissue accumulation of FB_1 was carried out in 1-week-old BUT9 **turkeys** which were offered a diet containing 0, 5, 10 or 20 mg $FB_1 + FB_2$/kg for 9 weeks. In accordance with the TK data, the highest levels were found in livers amounting to 33, 44 and 117 μg/kg in animals receiving 5, 10 or 20 mg $FB_1 + FB_2$/kg feed, respectively. Measurable kidney levels (22 μg/kg) were observed only at the highest dietary concentration, while muscles did not exhibit FB_1 levels > LOD (13 μg/kg).

The same dosages (5, 10 or 20 mg $FB_1 + FB_2$/kg feed) were administered to 12-week-old **ducks** for 12 days (Tardieu et al., 2009). Tissue levels > LOD (13 μg/kg) could be detected in livers from animals exposed to 10 or 20 mg $FB_1 + FB_2$/kg feed only, while in all other cases liver, kidney and muscle sample were free from measurable FB_1 concentrations.

Conclusions

Overall, based on a limited data set, the experimental data on the transfer of FBs from contaminated feedstuffs into animal tissues or products indicate that animal derived feedstuffs are unlikely to contribute quantitatively to the exposure of animals to fumonisins and its modified forms where foodstuffs of animal origin are included in their diets.

In evaluating the risk, target animal species fed with higher proportions of feedstuffs of animal origin, such as dogs and cats, fish and farmed mink might need to be considered.

3.1.2. Mode of action

Recent evaluations, including FAO/WHO (2017) and EFSA CONTAM Panel (2018), have described in detail the mode of action of fumonisins. Due to a structural resemblance with ceramide, fumonisins competitively inhibit ceramide synthases (CerS), a group of key enzymes in the biosynthesis of ceramide and more complex sphingolipids. Inhibition of these enzymes results in the disruption of the *de novo* synthesis of ceramide as well as sphingolipid metabolism and, as a consequence, alterations in other lipid pathways. Of note, six mammalian isoforms of CerS have been described which differ in their tissue distribution as well as in their specificity of the fatty acid chain length used for N-acylation (Loiseau et al., 2015).

Most of the data concern the mode of action of FB_1; however, early studies indicated that $FB_{1–3}$ are inhibitors of CerS in rat liver slices at equimolar concentrations (Norred et al., 1997). As the inhibition of CerS is the initial step of fumonisin toxicity, the previous opinion assumed that at the cellular level FB_1, FB_2 and FB_3 have the same mode of action (EFSA CONTAM Panel, 2018). Thus, even if toxicity studies deal mainly with effects of FB_1; the other forms, FB_2 and FB_3 are considered as having similar toxicological profiles and potencies (EFSA CONTAM Panel, 2018).

The inhibition of CerS by fumonisins leads to an increase of So in blood and tissues as well as a greater increase of Sa. The change in Sa/So is observed upon exposure to fumonisin and considered as a potential biomarker of FBs exposure in several animal species (Masching et al., 2016). However, this biomarker varies according to the animal species, the dosage and the duration of the exposure (Zomborszky-Kovács et al., 2002a; Tran et al., 2006; Masching et al., 2016).

Sphingolipids are both highly bioactive compounds and important structural components in cell membranes. The inhibition of CerS by fumonisins leads to broad impairment of cellular signalling mechanisms (EFSA CONTAM Panel, 2018) with multiple cellular consequences such as apoptosis, inhibition of cell proliferation, altered S1P receptor function, impairment of lipid raft formation, altered cell–cell and cell matrix interaction. The disruption of the sphingolipid metabolism is closely related at an early stage with fumonisin toxicity (EFSA CONTAM Panel, 2018); however, there is no evidence of fumonisin-induced CerS inhibition in any human/animal disease, nor is there evidence that fumonisin - induced CerS inhibition is in itself an adverse effect (FAO/WHO, 2017).

Of note the effect on FBs on sphingolipid metabolism has not yet been related to some of the critical adverse effects observed in some target species such as impairment of the immune system in cattle, brain alteration in and cardiovascular effects in horses, lung alterations in pigs and reduced weight gain in most of the species.

The mode of action of modified form of fumonisins is well described (EFSA CONTAM Panel, 2018). However it has been shown that *N*-acyl-FB_1 derivatives are more cytotoxic *in vitro* compared with FB_1 but no *in vivo* data are available. Similarly HFB_1 has been shown repeatedly to be much less toxic compared to FB_1 in feeding studies (Grenier et al., 2012; Voss et al., 2013; Masching et al., 2016).

3.1.3. Adverse effects in livestock, fish, horses and companion animals

Toxicity studies deal mainly with effects of FB_1, but $FB_{2–3}$ are considered as having similar toxicological profiles and potencies (EFSA CONTAM Panel, 2018).

In the previous EFSA evaluations (EFSA, 2005; EFSA CONTAM Panel, 2014), the increase in the Sa/So ratio in serum and/or organs was taken as an endpoint for deriving reference points for certain species. 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.

3.1.3.1. Fumonisins

Ruminants

Despite the limited number of suitable studies, ruminants are considered less sensitive to fumonisins than other livestock species, notably pigs or horses (Mostrom and Jacobsen, 2011; Smith, 2012), In addition, ruminants tend to avoid mouldy feed (Voss et al., 2007).

No new studies on fumonisin adverse effects in ruminants could be retrieved since the last EFSA evaluations.

For **cattle**, the previous EFSA evaluations (EFSA, 2005, EFSA CONTAM Panel, 2014) covered several studies. The pivotal study used in the previous EFSA opinions (EFSA, 2005) is summarised in Table 3.

Studies that could not be used for identifying NOAELs or LOAELs are summarised in the text below.

Table 3: Adverse effects in ruminants

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentrations	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 18 Crossbred Limousine × Angus Hereford steers 230 kg bw 3 groups 31 days	1) Control (N = 6) 2) Low FB (26 mg/kg diet FB_1, 5 mg/kg diet FB_2, < 5 mg/kg diet FB_3) → 31 mg/kg diet (N = 6) 3) High FB (105 mg/kg diet FB_1, 32 mg/kg diet FB_2, 11 mg/kg diet FB_3) → 148 mg/kg diet (N = 6)	No effects on feed consumption and weight gain; in the highest dosed animals only: ↑↑ Serum AST, GGT, LDH, cholesterol ↓Mitogen-induced lymphocyte blastogenesis	Necropsy performed only on two calves from High FB group and control Mild hydropic liver degeneration and cloudy swelling	NOAEL 31 mg/kg feed, corresponding to 600 μg/kg bw (sum of FB_1–FB_2–FB_3) Endpoint: serum enzymes and cholesterol, suggesting alteration of liver function, and reduced immune function	Pivotal study used in EFSA (2005) FB_1 and FB_2 naturally contaminated corn; levels of the most common mycotoxins below LOD FB_3 content not taken into account	Osweiler et al. (1993)

AST: aspartate aminotransferase; bw: body weight; FB: fumonisin B; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; LOD: limit of detection; N: number of animals; NOAEL: no-observed-adverse-effect level.

$FB_{1–3}$ in cattle are endowed primarily with hepatic toxicity, as reflected by the increase in serum enzymes and bilirubin, hepatocellular injury and biliary duct hyperplasia. Kidney involvement (increase in BUN and in urinary GGT along with tubular nephrosis) has been demonstrated only in i.v. dosed neonatal calves (Mathur et al., 2001).

In the study used by EFSA in 2005 to derive a reference point (Osweiler et al., 1993), 18 crossbred feeder calves (around 230 kg bw) were allotted to one of the following experimental groups: control (N = 6), low FB (26 mg/kg diet FB_1, 5 mg/kg diet FB_2, < 5 mg/kg diet FB_3) amounting to 31 mg FBs/kg diet (N = 6) and high FB (105 mg/kg diet FB_1, 32 mg/kg diet FB_2, 11 mg/kg diet FB_3) amounting to 148 mg FBs/kg diet (N = 6) for 31 days. Weight gain and feed consumption were not affected by the treatment. In contrast, animals exposed to the higher FB dosage exhibited an increase in AST, LDH and GGT as well as in serum cholesterol and bilirubin suggesting an impairment of liver function. There was also a decrease in the mitogen-induced lymphocyte blastogenesis. No such changes were noticed in low FB_1-dosed animals. According to the available data, a NOAEL of 31 mg/kg feed corresponding to 600 μg/kg bw for the sum of FB_1-FB_2-FB_3 could be calculated, based on the lack of the increase in serum enzymes, cholesterol and bilirubin as well as the lack of decrease in lymphocyte blastogenesis observed in this group compared to animals exposed to the high fumonisin dose (148 mg FB_1-FB_2-FB_3/kg feed).

Scant information is available concerning the adverse effects of $FB_{1–3}$ in **sheep**. Two sheep died after the oral administration of 5 g of a *F. verticillioides* isolate (fumonisin content unknown)/head for 8 or 10 days, respectively; at necropsy, 'acute nephrosis and hepatosis' were recorded (Kriek et al., 1981). The previous EFSA evaluation (EFSA, 2005) reported a study (Edrington et al., 1995) without deriving a reference point. Fifteen crossbred whether lambs (average weight 32 kg) were allotted to four experimental groups and dosed intraruminally with fumonisin-containing culture material at doses of 0 (N = 3), 11.1 (N = 4), 22.2 (N = 4) or 45.5 (N = 4) mg total fumonisins (FB_1 + FB_2 + FB_3) for four consecutive days, respectively, equivalent to approximately 0, 0.35, 0.7 or 1.4 mg total fumonisins/kg bw. In all treated animals, there was a statistically significant decrease in feed intake together with an increase in serum ALT, GGT AST, BUN, creatinine, cholesterol and triglycerides. All the animals from the high dose level and one from the intermediate dose level died. All dosed animal showed diarrhoea and lethargy as well kidney and liver degeneration. Due to the very short exposure period in the only available study, the CONTAM Panel concluded that no NOAEL could be derived for sheep.

Only one report (already examined in the EFSA previous opinion from 2005) addressed the adverse effects in **goats** (Gurung et al., 1998, see Section 3.1.1.2). No overt signs of toxicity or effects on weight gain were exhibited by weanling Angora goats (N = 4) receiving a FB_1 contaminated diet (95 mg FB_1/kg) for 112 days. However, with respect to pretreatment values (T = 0), dosed lambs exhibited a progressive, statistically significant increase ($p < 0.1$) in serum cholesterol, triglycerides, creatinine, LDH and GGT along with a tendency toward the increase in the Sa/So ratio in liver and kidney. Due to the poor experimental design, no NOAEL could be derived from this study, in line with the previous EFSA assessment.

In summary, there is scant information available concerning the adverse effects of FBs in ruminants. The reported changes in organ macro- and microscopic appearance (cattle and sheep) as well as in serum enzymes and biochemistry (cattle, sheep, and goats) are consistent with an impairment of liver and possibly kidney function. Reference points (NOAEL) of 31 mg $FB_{1–3}$/kg feed could only be set for cattle based on the increase in serum enzymes, cholesterol and bilirubin as well as the decrease in lymphocyte blastogenesis. However, a very limited data set indicate that sheep and goats would not seem to be more susceptible to fumonisins than cattle.

Pigs

Pigs are considered one of the most sensitive farm animal species to $FB_{1–3}$. For pigs a LOAEL of 200 μg/kg bw per day of fumonisins (based on FB_1) was derived by EFSA in 2005 based on one study of Riley et al. (1993) which reported accumulation in sphingoid bases in serum and tissue organs. Since the publication of this opinion, several new studies, mainly on piglets around weaning, have reported adverse effect produced by FBs exposure (see Table 4). The majority of these studies indicated that changes in sphinganine: sphingosine ratio (Sa/So) is a sensitive biomarker in the assessment of adverse effect exerted by FBs but other effects have been reported. These studies confirmed that FBs affect mainly the lungs and liver, producing a specific syndrome, pulmonary oedema. Histological changes in the pancreas, intestines, spleen and lymph nodes were also observed (Fodor et al., 2005; Piva et al., 2005; Stoev et al., 2012). Moreover, Gbore et al. (2010) described alterations in brain neurochemistry: decrease in acetylcholinesterase (AChE) and specific

acetylcholinesterase (SAChE) release and activity in different brain regions in pigs fed ≥ 5.0 mg FB_1/kg feed for 6 months.

Pulmonary oedema is observed in animals exposed to low (3–10 mg FB_1/kg feed) and high (20–100 mg FB_1/kg feed) concentrations of fumonisins although with different degrees of severity. Histological lesions were observed in the lungs from all piglets fed diets containing low concentrations as for example 3, 6 and 9 mg FB_1/kg feed (Grenier et al., 2013; Souto et al., 2015) for 35 and 28 days respectively, whereas the exposure to 12 mg FB_1/kg feed of FB_1 for 18 days produced slight interstitial pneumonia and only one pig showed severe haemorrhagic congestion and some oedema (Moreno Ramos et al., 2010). In two studies performed by Zomborszky-Kovács et al. (2002a,b) weaned pigs were exposed to 0, 1, 5 and 10 mg FB_1/kg feed for 8 and 20 weeks. Slightly changes in lung in one animal was observed at 1 mg FB_1/kg feed while changes in lungs and in liver in more than two animals was found at 5 and 10 mg of FB_1/kg feed after 8 weeks of exposure. An increase in permeability of blood vessels, which was responsible for perivascular and especially pericapillary oedema in the lungs after three months oral administration of 10 mg FB_1/kg feed was also observed by Stoev et al. (2012). Increases in lung weight, irreversible fibrosis and histopathological changes in lungs and liver were also reported after prolonged exposure to FB_1 (20 weeks). Administration of higher doses (20–100 FB_1/kg feed) of FB_1 caused more severe alterations in lungs. Strong oedematous changes, accumulation of serofibrinous exudate or fibrin in the interlobular and interalveolar tissue as well as thickening of interalveolar septa due to epithelial hyperplasia were observed at 20 mg FB_1/kg FB (42 days) by Pósa et al. (2013, 2016); distinct lesions, yellowish fluid with clotting characteristics in the lungs, pleural cavity and marked pulmonary oedema in all animals were reported at 30 mg FB_1/kg (42 days), 10–40 mg FB_1/kg feed (28 days) and 45 mg FB_1/kg feed (10 days) (Zomborszky-Kovács et al., 2002a; Piva et al., 2005; Fodor et al., 2008). Similar effects such as severe dyspnoea, the presence of fluid in thoracic cavity and pulmonary oedema were reported in all piglets, and lead to death within 12–24 h at 50 and 100 mg FB_1/kg with the difference that these effects occurred in a much shorter time (5, 10 and 22 days) (Fodor et al., 2005) (Table 4).

As in the case of pulmonary oedema, hepatic injuries were observed, with various concentrations of FB_1 concentrations examined (Fodor et al., 2005). Hepatotoxicity was noticed in piglets exposed to doses ranging from 1.5 to 100 mg FB_1/kg feed. For instance, pigs fed diets containing 6 mg FB_1/kg feed (35 days) presented disorganisation of hepatic cords, cytoplasmatic and nuclear vacuolisation of hepatocytes, and megalocytosis (Grenier et al., 2013). Pigs fed for 42 days with 30 mg FB_1/kg feed, and with 50 and 100 mg FB_1/kg feed for 22, 5 and 10 days, respectively, had enlarged, friable, pale, yellowish liver, visible discoloration (fibrosis), vacuolation and necrosis (including occasional single cell necrosis) of the liver (Fodor et al., 2005; Piva et al., 2005). Other studies showed increase in liver weight at 1.5 and 30 mg FB_1/kg feed (Piva et al., 2005; Lessard et al., 2009; Lalles et al., 2010), polyploidy and fatty change in the liver at 12 mg FB_1/kg feed (Moreno Ramos et al., 2010) but no macroscopic or histological lesions in the liver and other organs (spleen, kidneys and heart) at 3.0, 6.0 or 9.0 mg FB_1/kg diet and 28 days of exposure (Souto et al., 2015).

Liver alterations also led to changes in the level of serum biochemical analytes. Increases in concentrations of albumin, total protein, cholesterol, triglycerides, creatinine and GGT were found in pigs exposed for 28–42 days to 6, 8, 30 and 44 mg FB_1/kg feed (Piva et al., 2005; Marin et al., 2006; Grenier et al., 2012, 2013), while a lower level of hepatic enzymes (GGT, AST, ALT, LDH) was observed by Marin et al. (2006) in the serum of male pigs receiving feed contaminated with *F. verticillioides* culture material (8 mg FB_1/kg feed) for 28 days.

Nephrotoxicity induced by FBs has been reported in several studies. Pigs fed with *F. verticillioides* culture material showed slight to moderate degenerative histopathological changes in the kidneys (Moreno Ramos et al., 2010; Stoev et al., 2012; Pósa et al., 2016) in addition to increase in permeability of vessels in the lungs, brain, cerebellum and kidney (Stoev et al., 2012). Alterations in the brain were also reported by Gbore et al. (2010). This study demonstrated that feed contaminated with FB_1 ≥ 5 mg/kg feed for a 6-month period decreased in a dose dependent manner the release AChE and SAChE activity from some brain regions (Gbore, 2010).

Several studies showed that ingestion of feed contaminated with fumonisins results in various intestinal disorders. Thus, impaired morphology of the different segments of the small intestine, reduced villi height and cell proliferation, reduced number of goblet cells and modified intestinal cytokine expression were found by Grenier et al. (2012) and Bracarense et al. (2012) in pigs exposed by gavage with 200 μg FB_1/kg bw per day for 14 days or fed with 5.9 mg FB_{1-2}/kg feed for 35 days. Intestinal inflammation by the upregulation of proinflammatory cytokines, IL-1β, IL-6, TNF-α and IFN-γ was observed (Grenier et al., 2013). Also, consumption of 1.5 mg FB_1/bw per day during 9 days increased

eightfold alphaB crystallin and 12-fold COX-1 in the colon and various stress proteins along the GIT (COX-1 and nNOS in the stomach, HSP 70 in the jejunum and HO-2 in the colon) (Lalles et al., 2010).

Changes in Sa/So ratio are considered as the most sensitive parameter in the assessment of adverse effect exerted by fumonisins (EFSA, 2005). Increase in Sa/So ratio was found when pigs were exposed from 2 mg FB/kg feed to 20 mg/kg FB_1 (Pósa et al., 2011, 2013; Grenier et al., 2013; Masching et al., 2016). Sa/So alterations appear to be time dependent. Indeed, Masching et al. (2016) reported a significant increase in Sa/So ratio in serum of pig exposed to 2 mg FB/kg feed for 42 days starting with day 28 of exposure in pigs fed 2 mg FB/kg feed for 42 days. Also, fumonisins at a level of 11.8 mg FB_1/kg feed were responsible for a statistically significant increase in the Sa/So ratio in serum, kidney and liver, 9 days after the beginning of toxin exposure of 63 days (Burel et al., 2013).

Several studies showed that FBs are reproductive toxicants in pigs. Indeed, the exposure of male pigs to dietary $FB_1 \geq 5$ mg/kg feed produced delayed in sexual maturity by reducing testicular and epididymal sperm reserves and daily sperm production (Gbore and Egbunike, 2008; Gbore, 2009), as well as semen quality and motility (Gbore, 2009).

In pigs, FBs also impair both local and systemic immune responses. Ingestion of 8 mg FB_1/kg feed decreased in blood of pigs the gene expression of Th2 cytokines IL-4, IL-6 and IL-10 (Taranu et al., 2005; Marin et al., 2006). These authors found also that short time exposure of piglets to 1.5 mg FB_1/kg feed altered the cytokine balance (IL-4 and IFN-γ) in mesenteric lymph nodes and spleen. A reduced expression of cytokines (IL-6, IL-1β, IL-12p40 and IL-8) in spleen was also reported by Grenier et al. (2013). Following ingestion of 2.8 μM FB_1/kg bw (37–44 mg FB_1/kg feed), a decreased expression of most of the cytokines was found in the different part of the intestine segments after 14 days of exposure (Grenier et al., 2012).

An important number of studies investigated the situation when pigs given diet contaminated with fumonisins were subjected to microbial or viral infection. Some studies analysed whether combined treatment with fumonisin predisposed animals to lung inflammation by pathogenic bacteria like *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica,* generating respiratory disorders (Halloy et al., 2005; Pósa et al., 2011, 2013). In all cases, the interaction between fumonisins and pathogens aggravated the progression of infection, exacerbating the severity of lung pathology. For instance, in a recent study, Pósa et al. (2016) found that pigs fed with 20 mg FB_1/kg for 23 days and infected with *M. hyopneumoniae* presented a catarrhal bronchointerstitial pneumonia with development of prominent peribronchial and peribronchiolar lymphocytes infiltration in the lungs (due to *M. hyopneumoniae* infection); animals also showed accumulation of serous exudates in the pleura and in the interstitium, mostly due to FB_1 action (not characteristic for *M. hyopneumoniae* infection) and in addition an increased permeability of vessels, responsible for the prominent perivascular and especially pericapillary oedema mainly in the lungs. In another study of Halloy et al. (2005), induced cough, and increased bronchoalveolar lavage fluid (BALF) total cells, macrophages and lymphocytes were also found in pigs exposed to 5–8 mg FB_1/kg feed for 7 days and infected with *P. multocida*. TNF-α, IFN-γ and IL-18 mRNA expression was also increased in lung tissue for 7 days.

Similar results were obtained in the case of intestinal disorders caused by *Escherichia coli* or *Salmonella* in pigs fed fumonisin contaminated diet. Using an infectious model with *E. coli* F4+, Devriendt et al. (2009) showed that intoxication with a low dose of FB_1 (1 mg/kg bw for 10 days) led to a lower numbers of antigen-specific IgM antibody-secreting cells in the jejunal Peyer's patches, a significantly reduced mucosal IgA immune response in FB_1 exposed piglets and a prolonged shedding of F4(+) enterotoxigenic *E. coli* (ETEC) following infection. Exposure to naturally contaminated feed containing 11.8 mg fumonisins/kg over 63 days inhibited the ability of *Salmonella*–specific lymphocytes to proliferate in the presence of a selective mitotic agent, result which remains to be confirmed. Similar concentration of FB_1 (8 and 10 mg/kg) received by feed administration to piglets after weaning altered the vaccinal antibody response by decreasing the antibody titre against Aujeszky's disease at days 21 and 35 after vaccination (Stoev et al., 2012) and IgG-specific antibody against *Mycoplasma agalactiae* at 28 days (Taranu et al., 2005). Consumption of fumonisins contaminated feed had no effect on pig health but affected the microbiota profiles and this phenomena was amplified by the presence of *Salmonella* (Burel et al., 2013). Little or no effect of fumonisins on pig performance has been reported (Burel et al., 2013; Pósa et al., 2013). However, some studies showed a decreased of average daily gain at 8, 10, 15 and 100 mg FB_1/kg feed (Marin et al., 2006; Gbore, 2009; Fodor et al., 2005). The effects of FBs on feed intake and feed efficiency are also variable. No differences in feed intake was observed by Piva et al. (2005), but Moreno Ramos et al. (2010) showed moderate anorexia and Gbore (2009) and Fodor et al. (2005) reported a decreased in feed intake in pigs fed contaminated diet.

In summary, *in vivo* pig experiments indicate that exposure to FBs disturb the Sa/So ratio in blood and tissues, and induces specific syndromes for $FB_{1–3}$ toxicity such as pulmonary oedema, lung and hepatic lesions. Alteration of intestinal physiology, villous architecture and enzyme activities, hypofunctions of brain regions with decrease of the activity and secretion of neurotransmitter (AChE) were recently reported. A NOAEL of 1 mg FB_1/kg feed (corresponding to 40 μg/kg bw per day) which did not cause clinical signs and significant performance impairment for short (8 weeks, Zomborszky-Kovács et al., 2002a) as well as for long (20 weeks, Zomborszky-Kovács et al., 2002b) term exposure could be considered for pig based on the studies of Zomborszky-Kovács et al., 2002a,b). Also, a LOAEL of 5 mg FB_1/kg feed (corresponding to 200 μg/kg bw per day) could be identified for pigs based on increased biochemical parameters in blood, serum Sa/So ratio as well as lungs and liver histological changes (Zomborszky-Kovács et al., 2002a,b). This LOAEL was supported recently by studies showing alteration in brain neurochemistry by the decrease in AChE and SAChE activity and delayed sexual maturity in pigs at this concentration (Gbore et al., 2010).

Table 4: Adverse effects in pigs

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 136 male SPF pigs Average weight 13 kg bw 14 days of exposure	1) Control, 0 mg FB_{1-2}/kg feed (N = 5) 2) 5 mg FB_{1-2}/kg feed (N = 5) 3) 23 mg FB_{1-2}/kg feed (N = 5) 4) 39 mg FB_{1-2}/kg feed (N = 5) 5) 101 mg FB_{1-2}/kg feed (N = 5) 6) 175 ppm mg FB_{1-2}/kg feed (N = 5)	↑ Sa/So ratio in serum starting at 5 mg FB_{1-2}/kg feed ↑ serum liver enzymes at 101 mg FB_1/kg feed ↑biochemical parameters at 101 and 175 mg FB_{1-2}/kg feed ↑sign of respiratory distress	Pulmonary oedema at 175 mg FB_{1-2}/kg feed ↑Sa/So ratio in liver starting with 5 mg FB_{1-2}/kg feed ↑Sa/So ratio in liver, lungs, kidney and histological liver damage at ≥ 23 mg/kg	LOAEL 200 µg/kg bw per day corresponding to 5 mg FB_{1-2}/kg feed Endpoint: accumulation in sphingoid bases in serum and tissue organs	Pivotal study used in the EFSA (2005) opinion to calculate LOAEL based on Sa/So ratio Feed-containing corn or corn screenings naturally contaminated with fumonisins (166 mg FB_1/kg feed FB_1 and 48 mg/kg FB_2 feed)	Riley et al. (1993)
N = 20 pigs Average weight 10 kg bw 8 weeks of exposure	1) Control, 0 mg FB_1/kg feed (N = 5) 2) 1 mg FB_1/kg feed (N = 5) 3) 5 mg FB_1/kg feed (N = 5) 4) 10 mg FB_1/kg feed (N = 5)	No effects on productive parameters ↑ some serum parameters (ALP, ALT and AST activities) at 1, 5 and 10 mg FB_1/kg feed	Slightly changes in lung in only one animal at 1 mg FB_1/kg feed 5 and 10 mg FB_1/kg feed caused dose-dependent increase in the weight of the lungs, pathological and histopathological chronic pulmonary changes in the lungs and liver	NOAEL 1 mg FB_1/kg feed LOAEL 5 mg FB_1/kg feed Endpoint: increase in the weight of the lungs, pathological and histopathological chronic pulmonary changes in the lung and liver	Study mentioned in the EFSA (2005) opinion LOAEL based on lung lesions Feed contaminated with fungal (*Fusarium moniliforme*) culture	Zomborszky-Kovács et al. (2002a)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 20 pigs Average weight 10 kg bw 4 weeks of exposure 1st experiment 8 weeks of exposure 2nd experiment 20 weeks of exposure 3rd experiment	4 weeks: 1) control, 0 mg FB_1/kg feed (N = 5) 2) 10 mg FB_1/kg feed (N = 5) 3) 20 mg FB_1/kg feed (N = 5) 4) 40 mg FB_1/kg feed (N = 5) 8 weeks and 20 weeks: 1) control, 0 mg FB_1/kg feed (N = 5) 2) 1 mg FB_1/kg feed (N = 5) 3) 5 mg FB_1/kg feed (N = 5) 4) 10 mg FB_1/kg feed (N = 5)	No effects on productive parameters ↑ some serum parameters (AKLP, ALT and AST activities) at 1, 5 and 10 mg FB_1/kg feed ↑ time- and dose-dependent increase in the AST activities at 20 and 40 mg FB_1/kg feed ↑ Sa/So ratio at 10–40 mg FB_1/kg feed	10–40 mg FB_1/kg feed caused mild or severe pulmonary oedema since the 2nd weeks In chronic toxicosis (2–20 weeks, the pathological changes like pulmonary oedema turned to irreversible fibrosis at lower doses (10 mg FB_1/kg feed)	NOAEL 1 mg FB_1/kg feed Endpoint: no clinical signs and no effect on feed consumption, body weight gain and feed conversion; no increase in serum Sa/So ratio LOAEL 5 mg FB_1/kg feed Endpoint: increase in serum Sa/So ratio; macroscopic alteration in lung	Study mentioned in the EFSA (2005) opinion LOAEL based on lung lesions Feed contaminated with fungal (*F. moniliforme*) culture	Zomborszky-Kovács et al. (2002b)
N = 15 conventional piglets Average weight 9.6 kg bw 7 days of exposure	1) Control, 0 mg FB_1/bw per day (N = 5) 2) 0.5 mg FB_1/kg bw per day (5–8 mg FB_1/kg feed) (N = 5) Administrated by gavage	No clinical sign	↑ expression of IL-8, IL-18 and IFN-γ mRNA in the lung tissue minimal enlargement of the alveolar septa	LOAEL 500 μg/kg bw per day corresponding to 5–8 mg/kg feed Endpoint: Immunological (increased expression of cytokines IL-8, IL-18 and IFN-γ) and histological effects (lung lesions and minimal enlargement of the alveolar septa due to an increase in the macrophage and lymphocyte number)	Soluble crude extract of fungal *F. verticillioides,* 54% FB_1, 8% FB_2 and 9% FB_3)	Halloy et al. (2005)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 28 castrated male weanling piglets (Landrace × Large White) Average weight 6.9 kg bw 42 days of exposure	1) Control, < 2 mg FB_1/kg feed (N = 16) 2) 30 mg FB_1/kg feed as fed basis (N = 12)	No clinical signs (e.g. difficulty in breathing) ↑ concentrations of cholesterol, GGT, GOT, free sphinganine, sphingosine-1-phosphate and sphinganine 1-phosphate	↓ performance Marked pulmonary oedema; Lesions in the lungs, heart and liver of pigs changes in the pancreas, intestines, spleen and lymph nodes	LOAEL 2,250 μg/bw per day corresponding to 30 mg/kg feed Endpoint: increase in sphingolipid profile biochemical changes, organ lesions and pulmonary oedema	Feed contaminated with fungal (*F. proliferatum*) culture corn Addition of activated carbon Control feed contaminated with < 2 mg FB_1/kg Only one dose	Piva et al. (2005)
N = 12 male and female weaned piglets Average weight 7.3 kg bw 7 days of exposure	1) Control, 0 mg FB_1/kg bw (N = 6) 2) 1.5 mg FB_1/kg bw per day (N = 6)	–	Altered the cytokine balance (↓ IL-4 and ↑ IFN-γ) in mesenteric lymph nodes and spleen	LOEL 1,500 μg FB_1/kg bw per day Endpoint: alteration of Th1/Th2 cytokines production	Purified FB_1 Only one dose Gavage administration	Taranu et al. (2005)
N = 20 male and female weaned piglets Average weight 12.3 kg bw 28 days of exposure	1) Control, 0 mg FB_1/kg bw (N = 10) 2) 8 mg FB_1/kg feed (N = 10)	–	↓ IL-4 mRNA expression by porcine WBC	LOEL 500 μg FB_1/kg bw per day corresponding to 8 mg FB_1/kg feed Endpoint: decrease in cytokine production (IL-4, IFN-γ)	Feed contaminated with fungal (*F. verticillioides)* purified culture material Only one dose	Taranu et al. (2005)
N = 12 castrated pigs, same genotype Average weight 13.0 kg bw 22 days of exposure	1) Control, 0 mg FB_1/animal per day (N = 4) 2) 50 mg FB_1/animal per day (2.5 mg FB_1/kg bw per day) (N = 8)	No clinical signs	Pulmonary oedema developed	LOEL 2,500 μg FB_1/bw per day corresponding to 50 mg FB_1/kg feed Endpoint: pulmonary oedema	Feed supplemented with fungal (*F. verticillioides*) culture material Only one dose	Fodor et al. (2005)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 12 castrated pigs, same genotype Average weight 13.0 kg bw 5 days of exposure	1) Control, 0 mg FB_1/animal per day (N = 4) 2) 100 mg FB_1/animal per day (6.6 mg FB_1/kg bw per day) (N = 8)	Lost appetite, ↓ feed intake on the 5th–6th day	Pulmonary oedema; High significant FB_1 concentration in the liver, kidney, lung and spleen	LOAEL 6,600 µg FB_1/kg bw per day corresponding to 100 mg FB_1/kg feed Endpoint: pulmonary oedema and increased FB_1 content in organs, lower feed intake	Feed supplemented with fungal (*F. verticillioides*) culture material Only one dose	Fodor et al. (2005)
N = 12 castrated pigs, same genotype Average weight 13.0 kg bw 10 days of exposure	1) Control, 0 mg FB_1/animal per day (N = 4) 2) 100 mg FB_1/animal per day (N = 8)	Lost appetite, ↓ feed intake on the 5th–6th day	Pulmonary oedema developed ↑FB_1 content in organs	LOAEL 6,600 µg FB_1/kg bw per day corresponding to 10 mg FB_1/kg feed Endpoint: pulmonary oedema and increased FB_1 content in organs, lower feed intake	Feed supplemented with fungal (*F. verticillioides*) culture material Only one dose	Fodor et al. (2005)
N = 20, 4 weeks old males and females weaned pigs Average weight, 12.3 kg bw 28 days of exposure	1) Control, 0 mg FB_1/kg feed (N = 10) 2) 8 mg FB_1/kg feed (0.99 and 1.49 mg/bw per day) (N = 10)	↓ weight gain (males only) ↑ creatinine level in serum	↓ sex-dependent decrease in the expression of Th2 cytokines; ↓ IL-4, IL-6, IL-10 mRNA expression in male	LOEL 500 µg/kg bw per day corresponding to 8 mg/kg feed Endpoint: decrease in cytokine production, serum biochemistry (creatinine)	Feed contaminated with *F. verticillioides* purified crude extract Only one dose	Marin et al. (2006)
N = 16, weaned barrows, 8 weeks of age Average weight, 12–14 kg bw 10 days of exposure	1) Control, 0 mg FB_1/kg feed (N = 8) 2) 45 mg FB_1, 8.6 mg FB_2, 4.6 mg FB_3/kg feed (N = 8)	No clinical signs	Pulmonary oedema in all animals ↓ decrease the reduced glutathione content in blood plasma and R haemolysate, pathological change in organs	LOAEL 3,500 µg FB_1/kg bw per day corresponding to 58 mg FB/kg feed Endpoint: pulmonary oedema and reduction in the second line of the antioxidant system	Feed contaminated with fungal *(F. verticillioides)* no purified culture material containing FB_1, FB_2, FB_3 FB_2, FB_3 content not taken into account Only one dose	Fodor et al. (2008)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 24, Large White male weanling piglets, 8–9 weeks old Average weight, 6.94 kg bw 6 months of exposure (3 physiological phases: weaning, prepubertal and pubertal)	1) Control, 0.2 mg FB_1/kg feed (N = 6) 2) 5.0 mg FB_1/kg feed (N = 6) 3) 10.0 mg FB_1/kg feed (N = 6) 4) 15.0 mg FB_1/kg feed (N = 6)	–	Reduced testicular and epididymal sperm reserves and daily sperm production	LOAEL 300 μg FB_1/kg bw corresponding to ≥ 10.0 mg FB_1/kg feed Endpoint: reduction of daily sperm production and reproductive performance	Feed contaminated with fungal *(F. verticillioides)* no purified culture maize grains Control feed contaminated with 0.2 mg FB_1/kg	Gbore and Egbunike (2008)
N = 24, Large White male weanling piglets, 8–9 weeks old Average weight, 6.94 kg bw 24 weeks of exposure (measurements in pubertal phase at 36 weeks old)	1) Control, 0.2 mg FB_1/kg feed (N = 6) 2) 5.0 mg FB_1/kg feed (N = 6) 3) 10.0 mg FB_1/kg feed (N = 6) 4) 15.0 mg FB_1/kg feed (N = 6)	No effect on performance	No effect on relative weights of the testis (and volume) and epididymides, reduced sperm concentration, total sperm and motile sperm per ejaculate	LOAEL 300 μg FB_1/kg bw corresponding to ≥ 10.0 mg FB_1/kg feed Endpoint: reduced semen quality, motility and concentration	Feed contaminated with fungal *(F. verticillioides)* no purified culture maize grains Control feed contaminated with 0.2 mg FB_1/kg	Gbore (2009)
N = 24, Large White male weanling piglets, 8–9 weeks old Average weight, 6.94 kg bw 24 weeks of exposure	1) Control, 0.2 (N = 6) 2) 5.0 mg/kg feed (N = 6) 3) 10.0 mg/kg feed (N = 6) 4) 15.0 mg FB_1/kg feed (N = 6)	↓ feed intake during 0–4 months and a FB_1 concentration-dependent decrease in body weight and DWGs at 10 and 15 mg FB_1/kg feed in pubertal phase	Delayed sexual maturity	LOAEL 200 μg FB_1/kg bw, corresponding to ≥ 5.0 mg FB_1/kg feed Endpoint: reduced semen quality and capacity of fertility, lower performance in growing pigs	Feed contaminated with fungal *(F. verticillioides)* no purified culture maize grains Control feed contaminated with 0.2 mg FB_1/kg	Gbore (2009)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 36 [Pietrain X (Landrace X Large-White)] castrated male weaned pigs (intralitter paired), 35 days of age Average weight, 10.87 kg bw (control) and 10.94 kg bw (FB_1 group) 9 days of exposure	1) Control, 0 mg FB_1/kg bw (N = 18) 2) 1.5 mg FB_1/kg bw per day (25–30 mg FB_1/kg feed) (N = 18)	↓ the gain: feed ratio	↑ liver weight Alteration of intestinal physiology, villous architecture, and enzyme activities	LOAEL 1,500 µg FB_1/kg bw per day corresponding to 25–30 mg FB_1/kg feed Endpoint: modulation of intestinal structure and physiology, reduced performance	Purified extract (2.3 g/L FB_1, 0.34 g/L FB_2, 0.38 g/L FB_3) Only one dose	Lessard et al. (2009)
N = 14, 16-day-old weaned piglets 42 days of exposure	1) Control, 0 mg FB_1/kg feed (N = 7) 2) 20 mg FB_1/kg feed (N = 7)	No clinical signs	No significant differences in body weight gain and no macroscopic and CT lung lesions	NOAEL 1,000 µg FB_1/kg bw per day corresponding to 20 mg FB_1/kg feed	Feed contaminated with fungal *(F. verticillioides)* no purified culture material Only one dose	Pósa et al. (2009)
N = 10, weaned piglets, 34 days of age, both sexes Average weight, 5.8 kg bw 18 days of exposure	1) Control, 0 mg FB_1/kg/ feed (N = 5) 2) 12 mg FB_1/kg feed (N = 5)	Moderate anorexia, depression, prostration and fluid stools	Pathologic and histopathologic changes in the lungs, liver and kidney	LOAEL 800 µg FB_1/kg bw per day Endpoint: lesions in lungs, liver and kidney	Feed contaminated with FB_1 standard pure toxin Only one dose	Moreno Ramos et al. (2010)
N = 36 [Pietrain X (Landrace X Large-White)] castrated male weaned pigs (intralitter paired), 35 days of age Average weight, 10.87 kg bw (control) and 10.94 kg bw (FB_1 group) 9 days of exposure	1) Control, 0 mg FB_1/kg bw (N = 18) 2) 1.5 mg FB_1/kg bw per day (N = 18)	Little effects on growth rate	↑ liver weight ↑ increased alphaB crystallin, COX-1 and HO-2 in the colon, nNOS in the stomach, HSP70 in the jejunum	LOAEL 1,500 µg FB_1/kg bw per day corresponding 25–30 mg FB_1/kg feed Endpoint: induces stress protein responses along the GIT, especially in the colon	Purified FB_1 extract (2.3 g/L FB_1, 0.34 g/L FB_2, 0.38 g/L FB_3) Only one dose	Lalles et al. (2010)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 24, Large White male weanling piglets, 8–9 weeks old Average weight, 6.94 kg bw 6 months of exposure (3 physiological phases: weaning, prepubertal and pubertal)	1) Control, 0.2 (N = 6) 2) 5.0 mg/kg feed (N = 6) 3) 10.0 mg/kg feed (N = 6) 4) 15.0 mg FB_1/kg feed (N = 6)	–	Altered brain neurochemistry; Significant influence of dietary FB_1 on regional brain and ↓ dose-dependent release of AChE (corresponding to 2.0 mg FB1/kg bw per day) from some brain regions ↓ acetylcholinesterase (AChE) activities	LOAEL 200 μg FB_1/kg bw corresponding ≥ 5.0 mg FB_1/kg feed Endpoint: hypofunctions of brain regions, ↓ of AChE activities and secretion	Feed contaminated with fungal (*F. verticillioides)* no purified culture maize grains Control feed contaminated with 0.2 mg FB_1/kg	Gbore et al. (2010)
N = 14 female piglets, 16 days old Average weight, 3.0 kg bw 23 days of exposure	1) Control 0 mg FB_1/kg feed (N = 7) 2) 10 mg FB_1/kg feed (N = 7)	No clinical signs, only a pronounced heterogeneity of body weight on day 39 ↑ Sa/So ratio in blood at 39 days	No lung lesions	LOAEL 800 μg FB_1/kg bw per day corresponding to 10 mg FB_1/kg feed Endpoint: increase in serum Sa/So ratio	Receiving diets included fungal (*F. verticillioides*) no purified culture material Only one dose	Pósa et al. (2011)
N = 24 castrated male piglets, 5 weeks old Average weight, 9.54 kg bw (control) and 9.52 (FB group) 35 days of exposure	1) Control, 0 mg $FB_{1\text{-}2}$/kg feed (N = 12) 2) 5.9 mg $FB_{1\text{-}2}$/kg feed (4.1 mg FB_1 + 1.8 mg FB_2) (N = 12)	No clinical signs	Atrophy and fusion of villi ↓ villi height and cell proliferation in the jejunum; reduced number of goblet cells and lymphocytes ↑ TNF-α, IL-1β, IFN-γ, IL-6 and IL-10 in the ileum or the jejunum ↓ expression of E-cadherin and occluding in the intestine	LOAEL 400 μg FB/kg bw per day corresponding to 5.9 mg FB/kg feed Endpoint: intestinal and immunological changes	Feed artificially contaminated with fungal culture material Only one dose	Bracarense et al. (2012)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 12, Pietrain/Duroc/ Large-White, female piglets Average weight 10.98 kg bw (control) and 10.92 kg bw (FB_1) 14 days of exposure	1) Control, 0 mg FB_1/kg bw per day (N = 6) 2) 2.8 μmol FB_1/kg bw per day; corresponding to 2.0 mg FB_1/kg bw per day (N = 6)	↑ biochemical analytes	FB_1 induced hepatotoxicity, impaired morphology of the different segments of the small intestine, ↓villi height and modified intestinal cytokine expression	LOAEL 2,000 μg FB/kg bw per day corresponding to 37–44 mg FB_1/kg Endpoint: increase in biochemical analytes, morphological and immunological effect in intestine	Fumonisins extract containing 530.85 mg/ L FB_1, 133.30 mg/L FB_2, and 35.60 mg/L FB_3 Only one dose gavage administration	Grenier et al. (2012)
N = 6 (3 males and 3 females) piglets Average weight 12–14 kg bw 3 months of exposure	1) Control, 0 mg FB_1/kg fed (N = 6) 2) 10 mg FB_1/kg feed (N = 6)	Scarce clinical signs: transient cases of diarrhoea ↑ of serum creatinine, urea and enzyme activity of AST/ALT ↓ of serum cholesterol, total protein, albumin and glucose	↑ in permeability of vessels mainly in lung, brain, cerebellum or kidneys; slight to moderate degenerative changes in kidneys	LOAEL 500 μg FB_1/kg bw per day corresponding to 10 mg FB_1/kg feed Endpoint: increase in biochemical parameters, changes in organs	Feed contaminated with fungal (*F. verticillioides*) no purified culture material Only one dose	Stoev et al. (2012)
N = 12 castrated males Pietrain/Duroc/Large-White piglets 4 weeks old 35 days of exposure	1) Control 0 mg $FB_{1\text{-}2}$/kg feed (N = 6) 2) 5.9 mg/kg feed $FB_{1\text{-}2}$ (N = 6)	↑ Sa/So ratio in plasma ↑ creatinine concentration	↑ lesions in lung, liver and intestine ↓ lymphocytes proliferation ↑ inflammatory cytokines in spleen and jejunum ↓ anti-OVA IgG antibodies	LOAEL 400 μg FB_1/kg bw per day corresponding to 5.9 mg FB_1/kg feed Endpoint: increase in plasma parameters (Sa/So ratio, creatinine), histological and immunological effects	Feed contaminated with fungal *(F. verticillioides)* not purified culture material Only one dose	Grenier et al. (2013)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 24 Large-White, SPF growing pigs (1/3 females and 2/3 males), 4 weeks old Average weight 41.6 kg bw 63 days of exposure	1) Control, 0 mg FB/kg feed (N = 12) 2) 11.8 mg $FB_{1\text{-}2}$/kg (8.6 mg FB_1 + 3.2 mg FB_2) (N = 12)	No effect on performance, mortality or disease ↑ Sa/So ratio in serum	Imbalance in digestive microbiota, with *Salmonella* exposure amplifying this phenomenon	LOAEL 500 μg FB_1/kg bw per day corresponding to 11.8 mg FB_1/kg feed Endpoint: imbalance in digestive microbiota	Feed contaminated with maize naturally contaminated with FB Only one dose	Burel et al. (2013)
N = 14 weaned piglets, 16 days old Average weight, 3.0 kg bw 42 days of exposure	1) Control, 0 mg FB_1/kg feed (N = 7) 2) 20 mg FB_1 (+3.5 mg/FB_2 and 1.9 mg FB_3)/kg feed (N = 7)	No significant differences in the body weights ↑ Sa/So ratio	Lesions extending to the cranial and middle or in the cranial third of the caudal lobe of the lungs; pulmonary oedema; aggravated progression of catarrhal bronchointerstitial pneumonia	LOAEL 1,000 μg FB_1/kg bw per day corresponding to 20 mg FB_1/kg feed Endpoint: increase serum Sa/So ratio and pulmonary lesions	Feed contaminated with fungal *(F. verticillioides)* no purified culture material Only one dose	Pósa et al. (2013)
N = 24 castrated males pigs, 4 weeks old Average weight, 10.8 kg bw 28 days of exposure	1) Control, 0 mg FB_1/kg feed (N = 6) 2) 3.0 mg FB_1/kg feed (N = 6) 3) 6.0 mg FB_1/kg feed (N = 6) 4) 9.0 mg FB_1/kg feed (N = 6)	No clinical signs	No significant differences in the body weights of the pigs; no macroscopic or histological lesions in the spleen, liver, kidneys and heart Histological lesions in lungs but not quantified	LOAEL 400 μg FB_1/kg bw per day corresponding to 6–9 mg FB_1/kg feed Endpoint: Histological lesions in lungs	Feed contaminated with fungal (*F. verticillioides)* no purified culture material	Souto et al. (2015)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 70 PIC 337 male and female 28 days old, weaned piglets 42 days of exposure	1) Control, 0 mg FB/kg feed (N = 35) 2) 2 mg FB_{1-2}/kg feed (N = 35)	↑ Sa/So ratio starting with day 28	No other pathological findings	LOEL 100 μg FB/kg bw per day corresponding to 2 mg FB/kg feed Endpoint: increase serum Sa/So ratio	Feed contaminated with fungal (*F. verticillioides*) no purified culture material Only one dose	Masching et al. (2016)
N = 14 female piglets, 16 day old Average weight, 3.0 kg bw 42 days of exposure	1) Control, 0 mg FB_1/kg (N = 7) 2) 20 mg FB_1/kg feed (N = 7)	No clinical signs throughout the experiment No significant differences in the body weights	Strong oedema in the lung and slight oedema in the other internal organs and mild degenerative changes in the kidneys	LOAEL 1,000 μg FB_1/kg bw per day corresponding to 20 mg FB_1/kg feed Endpoint: pulmonary alterations	Feed contaminated with fungal (*F. verticillioides*) no purified culture material Only one dose	Pósa et al. (2016)

AChE: acetylcholinesterase; AKLP or ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; bw: body weight; DWG: daily weight gain; FB: fumonisin B; GGT: gamma-glutamyl transferase; GIT: gastrointestinal tract; GOT: glutamic-oxaloacetic transaminase; LDH: lactate dehydrogenase; IFN: interferon; IL: interleukin; LOAEL: lowest-observed-adverse-effect level; LOEL: lowest-observed-effect level; LOD: limit of detection; mRNA: Messenger Ribonucleic Acid; N: number of animals; NOAEL: no-observed-adverse-effect level; Sa/So: sphinganine-to-sphingosine ratio; TNF: tumour necrosis factor; WBC: white blood cells.

Poultry

EFSA derived a LOAEL of 2 mg/kg bw per day for poultry (EFSA, 2005). This was based on a 21-day feeding study where broiler chickens were given 0, 20, 40 or 80 mg pure FB_1/kg feed for 21 days from day 1 (Henry et al., 2000). FB_1 did not affect body weight or growth in this study. FB_1 induced a dose-dependent increase in liver sphinganine and 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. These birds also had an increased serum GOT/ASP ratio. Cholesterol, ALP and LDH were not affected by any treatment. EFSA calculated that a LOAEL of 20 mg/kg feed would correspond to 2 mg/kg bw per day. EFSA also concluded that the LOAELs for other poultry species were higher, 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 (EFSA, 2005). The more recent papers identified are summarised below.

Chickens

Ninety-six-day-old chicks (breed not specified) were given 0 (control), 5, 10 or 15 mg FB_1/kg feed for 21 days in two experiments Cheng et al., 2006). FB_1 was prepared by inoculation of grains with *F. moniliforme*. The cultured material was analysed with HPLC and contained deoxynivalenol (DON) (0.5 mg/kg, zearalenone (< 1.0 μg/kg) aflatoxins (3.3 μg/kg) and FB_1 (5,250 mg/kg feed). The mycotoxin concentrations were diluted to approximately 1/1,000 of this in the lowest dose group. The relative weight of the bursa was reduced in chicks given 10 or 15 mg FB_1/kg feed. Increased serum AST was observed in chicks exposed to FB_1 levels from 5 mg/kg feed and serum albumin and cholesterol in chicks given 15 mg FB_1/kg feed. In the first experiments, chickens were vaccinated against Newcastle disease at 4 days of age with a booster injection 10 days later. Chickens from the groups given 10 or 15 mg FB_1/kg feed had significantly lower antibody titres against Newcastle disease than controls. Finally, peritoneal macrophages were collected, counted and the macrophages phagocytic activity towards *Candida albicans* was tested *ex vivo*. A dose-dependent decrease in number of macrophages and % of phagocytic macrophages was observed with the high dose group being statistically significant lower than controls. The number of *Candida* per phagocytic macrophage was significantly lower in treated chickens compared to controls. In addition, decreased gene transcription of proinflammatory cytokines in spleen after challenge with LPS was observed in all treated birds. There were some unclarities in the reporting of the studies related to performance parameters and the CONTAM panel could not derive a reference point based on the study.

Ross broiler chickens (6 replicate cages, 6 chickens/cage) were fed 0 (control), 5.6, 11.3, 17.5, 47.8 or 104.8 mg of sum of FB_1 and FB_2 from fungal cultures mixed into the diet for 20 days from day 1 of age (Grenier et al., 2015). FBs in the diet had no effect on body weight or feed intake. The levels of Sa and the Sa/So ratio was increased ratio in liver, kidney, jejunum, ileum and caecum from chickens given from 11.3 mg $FB_1 + FB_2$ in the diet, but not in chickens given 5.6 mg $FB_1 + FB_2$ in the diet. Furthermore, FB increased the gene expression of proinflammatory regulatory genes in the small intestines. The upregulation was not dose-dependent and the largest increase was found in chickens given 11.3 mg $FB_1 + FB_2$ in the feed. The effects observed in this study are not considered as adverse.

A decrease in liver lipids was observed in chickens given from 40 mg FB/kg feed in the studies by Henry et al. (2000). Taking the known liver toxicity observed in most tested species into consideration, the WG considered the decreased liver lipids as an adverse effect and identified a NOAEL of 20 mg/kg feed, at. At this level, only the Sa/So ratio was altered and this is not considered as an adverse effect. A NOAEL of 20 mg/kg feed (corresponding to 2 mg/kg bw per day) could be identified based on the studies by Henry et al. (2000).

Laying hens

Only one feeding study with laying hens was available in which Hisex Brown layer hens (37 weeks of age) were fed either a control diet or a diet containing 25 mg $FB_1 + FB_2$/kg feed for 56 days (two cycles of 28 days). There were six replicates, with four birds/replicate for each treatment group. The feed was prepared by mixing cultures of *F. verticillioides* into the feed. Laying hens given $FB_1 + FB_2$ in the feed had shorter small intestines (1.37 vs 1.57 m) compared to controls. The treatment did not have any effect on performance, blood lipids or plasma cholesterol (Siloto et al., 2013). Only one dose of FBs was used in the study and no NOAEL could be derived. The feed concentration used in the trial corresponded to 1.6 mg/kg bw per day.

Ducks

EFSA concluded in 2005 that there was no evidence that ducks or ducklings were more sensitive than chickens. The statement was based on two published feeding experiments where LOAELs of 5 mg/kg bw per day for Mallard ducks and 17 mg/kg bw per day for Pecking ducklings were reported. These were, however, the lowest doses tested in the studies. The more recent papers are summarised below.

Benlashehr et al. (2011) gave mule ducks (25/diet) a diet where culture material of *F. verticillioides* was mixed into the diet. The final diet contained 10 mg $FB_1 + FB_2$/kg feed while aflatoxin B1, ochratoxin A, zearalenone, DON and T2 toxin were all below their respective limit of detection. Five birds from each group were examined on days 0, 3, 7, 14 and 21. The ducks given $FB_1 + FB_2$ in the feed had a decreased feed consumption and body weight gain compared to the control. Furthermore, the Sa and Sa/So ratio was increased compared to the control group. The relative organ weighs were not statistically different in exposed birds compared to controls, but the serum concentrations of cholesterol, LDH, ALT and AST were elevated in ducks given $FB_1 + FB_2$ in the feed.

Growing Mallard ducks (age and start weight not specified) were force-fed a diet containing 0, 10 or 20 mg FB_1 from naturally contaminated maize in the feed for 12 days (25 ducks/treated group, 30 controls). The feed contained traces of FB_2 and FB_3 while aflatoxins B_1, ochratoxin A, zearalenone, trichothecenes, fusarine C, fusaric acid and moniliformine could not be detected. The mortality increased in the high dose group (8% vs 0%). A dose-related increase in levels of Sa and the Sa/So ratio was observed in treated ducks. The liver of the high dose birds were slightly discoloured and microscopic examinations of the livers indicated steatosis in all exposed ducks (Tardieu et al., 2004).

Mule ducks from 1 week of age received daily oral doses corresponding to dietary concentrations of 0, 2, 8, 32 or 128 mg FB_1/kg feed from a purified culture material of *F. verticillioides* for 77 days (Tran et al., 2006). The purified extract contained 54% FB_1, 8% FB_2, 9% FB_3 and 29% maize pigments. The concentrations of aflatoxin B1, ochratoxin A, zearalenone and T-2 were below their respective LODs. The treatments had no effect on feed intake or body weight gain and did not give any macroscopic lesions. Serum Sa and Sa/So ratio were increased in ducks receiving more than 2 mg FB_1/kg feed. The increase in serum Sa and Sa/So ratio was highest during days 1–21 and decreased thereafter. No visible signs of toxicity or effects on body weight gain and feed intake was observed even at the highest dose, even though the Sa and Sa/So ratio was increased. Tardieu et al. (2006) examined the effects of FB_1 on Sa and Sa/So ratio in liver and kidneys and the serum biochemistry of the same birds. The Sa and Sa/So ratio were increased in liver and kidney from all ducks from 2 mg/kg feed, with the maximum concentrations reached on days 3–21. FB_1 also increased serum protein, cholesterol, ALP, LDH, ALT, AST in birds given doses corresponding to 32 mg/kg feed. Like for sphinganine, the increase was highest after 7–21 days for most parameters and decreased thereafter. In addition, a microglandular structure in both periportal and centrolobular areas was observed in the livers of exposed animals on treatment days 7, 14, 21 and 28 but not on treatment day 77. The structure was not characterised. Based on the high Sa concentrations found in birds without any visible toxic effects in this study, the authors suggested that ducks may be relatively resistant to increased Sa concentrations compared to other species. In this study, 8 mg FB_1/kg feed could be considered a NOAEL for effects other than increased Sa and Sa/So ratio. Using the EFSA conversion tables, a feed concentration of 8 mg FB_1/kg feed would correspond to 0.4 mg FB_1/kg bw per day.

As an overall evaluation of feeding studies with ducks, a NOAEL of 8 mg FB_1/g feed could be identified for ducks. This NOAEL was based on alterations of liver enzymes indicating liver damages of birds given 32 mg FB_1/kg feed, but not in birds given 8 mg/kg feed (Tardieu et al., 2006). In addition, the Sa and Sa/So ratio was increased in birds given from 2 mg/kg feed.

Turkeys

EFSA concluded in 2005 that there was no evidence that turkeys are more sensitive than chickens. The statement was based on two published feeding experiments where high feed concentrations were used and effects had been observed at the lowest doses used.

Since then, a few feeding studies have been published. Increased Sa and Sa/So ratio were observed in two feeding studies using 10 or 15 mg $FB_1 + FB_2$ in the diet (Benlasher et al., 2012; Masching et al., 2016). No other effects were reported from these studies.

Male turkey chicks of the BUT-9 strain (n = 36/group) were given fumonisins B_1 and B_2 in the diet for 9 weeks (Tardieu et al., 2007). The diet was prepared by replacing some of the non-infected maize in the feed with naturally infected maize. The final feed contained 0, 5, 10 or 20 mg sum of FB_1 and

FB_2 in the feed. Aflatoxin B_1, ochratoxin A, zearalenone, DON and T-2 toxin were not detected in the final feed. No macroscopic lesions were detected in any tissues and histopathological examinations of liver and kidneys did not reveal any alterations. There were no effects on body weight gain, relative organ weights or feed conversion but a slight but statistically significant increase in feed consumption (177.7 vs 189.3 g/day) was reported from chicks given 20 mg/kg feed. Furthermore, there were no significant changes in serum levels of total protein, cholesterol or enzymatic activities of LDH, AAT and AST. The Sa concentrations and Sa/So ratios were increased in liver and kidneys but not in plasma from turkeys receiving from 10 mg FB_1 + FB_2 in the feed during the experiment. No adverse effect was observed in turkeys even at the highest dose used.

In conclusion, the information available for oral feeding studies with dose–response relationships from relevant feed concentrations in turkeys is scarce, but no adverse effects have been reported from turkeys given up to 20 mg FB_1/kg feed, corresponding to 0.67 mg/kg bw per day, and this could be considered as a NOAEL.

Japanese quail

EFSA did not evaluate the toxicity of fumonisins in quails in 2005. Several studies with one high concentration of fumonisins in the feed have been published since then, demonstrating that fumonisins potentially may have toxic effects in quails. Increased mortality, ruffled feathers, reduced feed intake and body weight gain and increased pathological alterations after infection with *Salmonella* Gallinarum and effects on spleen and lymphoid cell depletion in tissues have been reported from feeding studies where quails were a given single dietary feed concentration from 150 mg FB_1/kg feed (see Table 5, Asrani et al., 2006; Deshmukh et al., 2005a,b, 2007; Sharma et al., 2008). Reduced feed consumption and bw gain and reduced egg weight were also reported from laying quails given a feed containing 10 mg FB_1/kg feed from *F. verticillioides* culture material for 140 days (5 egg laying circles of 28 days) (Ogido et al., 2004), but even in this study only one feed concentration was used and no reference points could be identified.

Young laying Japanese quails (4 replicate pens with 8 birds/treatment) were given 0 (control), 10, 50 or 250 mg FB_1/kg feed for 28 days (Butkeraitis et al., 2004). FB_1 was added as a fungal culture material of *F. verticillioides* containing 6,500 mg FB_1/kg, 2,100 mg FB_2/kg and 680 mg FB_3/kg. Aflatoxins, ochratoxin A. DON and zearalenone were not detected in the basal feed. Feed intake and body weight gain were lower in birds receiving 50 or 250 mg FB_1/kg feed compared to controls while no effects were found in birds given 10 mg FB_1/kg feed.

Feed conversion was reduced in quails receiving 250 mg FB_1/kg feed. Histopathological examinations did not reveal any changes in liver, kidney or heart from any group. The egg production was only reduced in quails given 250 mg FB_1/kg feed, but egg weight and the thickness of the egg shells were reduced in eggs from quails receiving from 50 mg/kg feed. No effects were reported from the group fed 10 mg FB_1/kg diet. This could be considered as a NOAEL.

Japanese quail were fed *F. verticillioides* culture material mixed into the feed to produce feed containing 10 mg FB_1/kg feed for 140 days, which constitutes five egg laying cycles of 28 days (Ogido et al., 2004). The treatment resulted in decreased feed intake in cycles 4 and 5, but not in the first 3 cycles. The body weight was reduced only in cycle 5. In addition, the egg weight was lower in eggs from the exposed birds compared to the controls.

In summary, only one feeding study with several doses of fumonisin in the feed to Japanese quails was available (Butkeraitis et al., 2004). In this study, 10 mg could be considered as a NOAEL. However, there are indications of adverse effects in Japanese quail given 10 mg/kg feed in a study where this was the only dose used (Ogido et al., 2004).

In summary, even though low feed concentrations have been shown to alter the Sa levels and Sa/So ratios in both tissues and serum of poultry, in chickens, adverse effects were observed at feed concentrations exceeding 20 mg/kg feed. For ducks, a NOAEL of 8 mg FB_1/kg feed and a LOAEL of 32 mg FB_1/kg feed were identified and for turkeys, no adverse effects have been reported from birds given up to 20 mg FB_1/kg feed, corresponding to 0.67 mg FB_1/kg bw per day. The overall LOAEL for Japanese quail was 10 mg FB_1/kg feed used (Ogido et al., 2004).

Table 5: Adverse effects in poultry

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Broiler chickens from day 1 21 days	1) 0 mg FB_1/kg feed (n = 5 × 6) 2) 20 mg FB_1/kg feed (n = 5 × 6) 3) 40 mg FB_1/kg feed (n = 5 × 6) 4) 80 mg FB_1/kg feed (n = 5 × 6)	↑ SA and Sa/So ratios (from 20 mg/kg feed) Increased liver lipids (from 40 mg/kg) Increased ratio GOT:ASP (from 80 mg/kg) No effect on body weight gain, serum cholesterol, ALP and LDH	–	NOAEL 20 mg/kg feed Corresponding to 2,000 μg/kg bw per day Endpoint: increased liver lipids	Pivotal study used in EFSA (2005) Pure Fumonisin B_1 added to the feed	Henry et al. (2000)
Day-old broilers (breed not specified) given contaminated feed for 21 days Grains inoculated with *F. moniliforme* mixed into the feed (culture material also contained 0.5 mg DON/kg, < 1.0 mg ZEN/kg, aflatoxins 3.3 μg/kg and fumonisins (B_1) 5250 mg/kg 20 days	1) 0 mg FB_1/kg feed (n = 24) 2) 5 mg FB_1/kg feed (n = 24) 3) 10 mg FB_1/kg feed (n = 24) 4) 15 mg FB_1/kg feed (n = 24)	No effect on bw gain Increased serum albumin and cholesterol (from 10 mg/kg Increased AST (from 5 mg/kg) Decreased antibody titre response towards vaccination (from 15 mg/kg feed) Altered macrophage function (from 15 mg/kg feed) Decreased gene expression of proinflammatory cytokines (from 5 mg/kg feed)	Decreased relative weight of bursa (from 10 mg/kg)	No reference points could be identified due to unclarities in the reporting	Contaminated feed used in the study. Other mycotoxins present in low concentrations Breed not specified Limited time (3 weeks) Limitations with data provided in Table 3	Cheng et al. (2006)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
1-day old male broilers (Ross) Toxins from *F. verticillioides* cultures. Feed contained DON (0.236–0.344 mg/kg) and ZEN (0.015–0.029 mg/kg) 21 days	1) 0 (control), (n = 6 × 6) 2) 5.6 mg FB_1 + FB_2/kg (n = 6 × 6) 3) 11.3 mg FB_1 + FB_2/kg (n = 6 × 6) 4) 17.5 mg FB_1 + FB_2/kg (n = 6 × 6) 5) 47.8 mg FB_1 + FB_2/kg (n = 6 × 6) 6) 104.8 mg FB_1 + FB_2/kg (n = 6 × 6)	No effect on performance Increased Sa/So ratio in liver, kidney, jejunum, ileum and caecum (from 11.3 mg/kg feed) — Upregulation of proinflammatory cytokine gene transcription in all groups. Response not dose-dependent		NOAEL > 105 mg/kg feed No adverse effects reported	Culture material used in the study Short-term study (21 days)	Grenier et al. (2015)
Ross 308 broiler chickens, 3 × 34 animals/treatment culture material (*F. verticillioides*) in the feed 15 (6/dose) or 21–23 days	1) 0 (control) 2) (16.2 mg/kg feed for days 1–8, 27.6 days 9–16, 18.0 from day 17. fed a mixture of B_1, B_2 and B_3 Average B_1 10.4 mg/kg feed, average total FB_{1-3}: 20.6 mg/kg feed	Increased plasma Sa and Sa/So ratio No effect on body weight gain	Reduced small intestine length villus height and crypt depth Increased relative liver weight Altered microbiota composition in ileum, but not in duodenum Increased susceptibility to *Clostridium perfringens*-induced necrotic enteritis	LOAEL 16.2 mg/kg feed Endpoint: Altered gut morphology, increased susceptibility to *C. perfringens*-induced necrotic enteritis	Culture material used Only one dose Short-term	Antonissen et al. (2015a)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Ross 308 broiler chickens Purified from culture material 15 days	1) 0 (control) 2) Average level of FB_1, FB_2, and FB_3 in the two batches were 8.4, 7.0 and 1.7 mg/kg	Increased plasma Sa and Sa/So ratio No effect on weight gain, feed conversion — Altered mucus layer composition in duodenum — Altered ileal gene expression of genes involved in antioxidative responses		LOAEL 17.1 mg/kg feed (dose could not be estimated as bw not given) Endpoint: altered mucus	Culture material used Only one dose Short-term	Antonissen et al. (2015b)
Broiler chickens (Ross) Pure FB_1 21 days (21–42 days of age)	1) 0 (control) (n = 4 × 6) 2) 10 mg/kg feed (n = 4 × 6)		Bile duct hyperplasia with fibrosis	NOAEL of 10 mg/kg feed No adverse effect	Only one dose Short-term No details given of the pathological alterations	Del Bianchi et al., 2005
Male broiler chicks *F. proliferatum* culture extracts mixed into the feed (trial 1–3) or pure FB_1 (Trial 4) 7–28 days in four different trials (trial 1: 1–28 days of age, trial 2: 8–28 days of age; trial 3: 21–28 days of age; Trial 4: 1–14 days of age)	Trial 1: 1) 0 (control) (n = 30) 2) 75, (n = 30) 3, 231 (n = 30) 3) 644 mg FB_1 + FB_2/kg feed (n = 30) Trial 2: 1) 0 (control, n = 6) 2) 75 (n = 6) 3, 231 (n = 6) 3) 644 mg FB_1 + FB_2/kg feed (n = 6)		Gross and histopathological lesions in all investigated organs (liver, lungs, kidneys, heart, intestine, gizzard, bursa, brain, pancreas pericardium, peritoneal cavity)	LOAEL 75 mg FB_1 + FB_2/kg feed Endpoint: Pathological lesions in several organs	Only high doses used in the experiments Culture material used in most trials High concentrations of moniliformin present in the contaminated feed (66–367 mg/kg feed) Several short-term trials	Javed et al. (2005)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
	Trial 3: 1) 0 (control, n = 6) 2) 75 (n = 6) 3, 231 (n = 6) 3) 644 mg FB_1 + FB_2/kg feed (n = 6) Trial 4: 1) 125 mg FB_1/kg feed (n = 10) 2) 274 mg FB_1/kg feed (n = 10)					
One-day-old chicks (Cobb 500) *F. verticillioides* culture material was mixed into the feed 21 days	1) 0 (control, n = 12) 2) 100 mg FB_1/kg feed (n = 12)	Increased Sa/So ratio	Increased liver weight, relative liver weight, feed conversion ratio Increased lipid peroxidation and ascorbic acid and CAT activity in the liver	LOAEL 100 mg/kg feed Feed conversion ratio, indications of oxidative damages	Only one dose Culture material High dose Indication of oxidative stress in the livers	Poersch et al. (2014)
One-day-old chicks (Cobb 500) given culture material from *F. verticillioides* in the diet 28 days (days 1–28)	1) 0 (control), (n = 6 × 11) 2) 100 mg FB_1/kg feed (n = 6 × 11) 3) 200 mg FB_1/kg feed (n = 6 × 11) The diet also contained 0, 20 or 40 mg FB_2/kg in addition to FB_1	— No mortality — Reduced feed intake and bw gain — Increased feed conversion rate — Increased Sa/So ratio — Increased plasma protein and albumin — Increased serum Ca — Decreased serum uric acid — Alterations in serum ALT, AST, GGT, Chol, Tri	Increased rel. liver weight	LOAEL 100 mg/kg feed Endpoint: Reduced reed intake and bw gain and increased feed conversion ratio, alteration in serum biochemistry	Only high doses used Culture material	Rauber et al. (2013)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
One-day-old chicks (Vencobb) (n = 25/ treatment) Culture material of *F. moniliforme* was mixed into the feed 8 weeks	1) 0 (control, n = 25) 2) 50 (n = 25) 3) 100 (n = 25) 4) 200 (n = 25) 5) 400 mg FB_1/kg (n = 25)		Histopathological alterations reported from liver, kidney, bursa of Fabricius, proventriculus heart and intestines	LOAEL 50 mg/kg feed Endpoint: Histopathological alterations in several organs	Culture material Only high doses Lack of details on findings from each treatment No statistics	Satheesh et al. (2005)
Male broiler chicks commercial Hybro-PG). Fumonisins prepared from cultures extracts of *F verticillioides* *34 days (*from 8 to 41 days of age)	1) 2.23 mg FB_1/kg feed (control, n = 12) 2) 50 mg FB_1/kg feed (n = 12) 3) 200 mg FB_1/kg feed (n = 12) Also contained FB_2 and FB_3	No visible clinical effects Reduced body weight gain (from 50 mg/kg feed) Increased rel. weight of heart (from 50 mg/kg), liver and bursa (from 200 mg/kg feed). No effect on rel. weight of spleen	Vacuolar degeneration in liver Cell proliferation in bile ducts near The liver portal space or between the hepatocytes (from 50 mg/kg feed) Reduced antibody titres against Newcastle disease (from 50 mg/kg feed)	LOAEL 50 mg/kg feed Endpoint: Reduced bw gain, pathological alterations in liver and reduced antibody titres	Culture material used Only high doses No pure control	Tessari et al. (2006)
Male broiler chicks commercial Hybro-PG). Fumonisins prepared from cultures extracts of *F verticillioides* *34 days (*from 8 to 41 days of age)	1) 2.23, mg FB_1/kg feed (control, n = 12) 2) 50 mg FB_1/kg feed (n = 12) 3) 200 mg FB_1/kg feed (n = 12) Also contained FB_2 and FB_3	Increased plasma AST (from 200 mg/kg feed) No effects on plasma total protein		No reference points could be identified	Culture material used Only high doses No pure control	Tessari et al. (2010)
Laying hens (Hisex Brown layer hens), 37 weeks of age, 56 days exposure	1) 0 (control) 2) 25 mg FB/kg feed	No effect on performance No effect on blood lipids or plasma cholesterol	— No effect on feed intake, bw gain or relative organ weights — Reduced small intestine length — Increased abdominal fat	LOAEL 25 mg/kg bw per day Endpoint: Reduced small intestinal length and increased abdominal fat	Only one dose	Siloto et al. (2013)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Male mule **ducks** 22 days old Force-fed Culture extracts containing B_1 and B_2, from 22 days of age 21 days	1) 0 (control) 2) oral administration of 10 mg FB_1 + FB_2/kg bw per day Other mycotoxins were not detected	Decreased body weight gain and feed consumption Increased Sa and Sa/So ratio in serum, liver and kidney Increased serum cholesterol, LDH, ALT, AST	No lesions, increased rel. liver weight	LOAEL 10 mg/kg bw per day Endpoint: Decreased bw gain and feed consumption, altered serum biochemistry	Only one dose tested Force feeding	Benlasher et al. (2012)
Mallard **ducks** (n = 25/ group) were given a feed where naturally contaminated maize was used in the feed 12 days	1) 0 (control) 2) 10 mg FB_1/kg feed 3) 20 mg FB_1/kg feed Other mycotoxins were not detected	Increased mortality in the high-dose group Increased ratio Sa/So in plasma No effect on standard plasma biochemical parameters		NOAEL 10 mg/kg feed (corresponding to 0.5 mg/kg bw per day) Endpoint: Increased mortality	Force feeding	Tardieu et al. (2004)
Mule **ducks** from 1 week of age Culture material from *F verticillioides* was mixed in the feed traces of FB_2 and FB_3, AFB_1, ochratoxin A, zearalenone, trichothecenes, fusarine C, fusaric acid and moniliformine could not be detected 77 days	1) 0 (control) (n = 30) 2) 2 mg FB/kg feed (n = 25) 3) 8 mg FB/kg feed (n = 25) 4) 32 mg FB/kg feed (n = 25) 5) 128 mg FB/kg feed (n = 25)	No effect on feed intake or bw gain Increased serum protein, cholesterol, ALP, LDH, ALT, AST (from 32 mg/kg feed). Increase highest after 7–21 days for most parameters Increased serum Sa and Sa/So ratio (from 2 mg/kg feed).	–	NOAEL 8 mg/kg feed Endpoint: Altered serum biochemistry	By gavage Culture material	Tran et al. (2006)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Mule **ducks** from 1 week of age Culture material from *F verticillioides* was mixed in the feed Traces of FB_2 and FB_3, AFB_1, ochratoxin A, zearalenone, trichothecenes, fusarine C, fusaric acid and moniliformine could not be detected 77 days	1) 0 (control) (n = 30) 2) 2 mg FB/kg feed (n = 25) 3) 8 mg FB/kg feed (n = 25) 4) 32 mg FB/kg feed (n = 25) 5) 128 mg FB/kg feed (n = 25)	No effect on feed intake or body weight gain Increased Sa/So ratio in liver and kidney (from 2 mg/kg feed)	No macroscopic lesion Alteration in the centrilobular areas of the fumonisin-fed animals on days 7, 14, 21 and 28, but not on day 77	NOAEL 32 mg/kg feed No adverse effect reported		Tardieu et al. (2006)
One-week-old male **turkey** chicks (BUT-9) (n = 36/dose) Naturally contaminated maize was mixed into the feed. Other mycotoxins (AFB_1, ochratoxin A, ZEN, DON, T-2 toxin below their respective LOD 63 days (on days 7–70)	1) 0 (control) 2) 5 mg FB_1 + FB_2/kg feed 3) 10 mg FB_1 + FB_2/kg feed 4) 20 mg FB_1 + FB_2/kg feed	Increased feed consumption (20 mg FB_1/kg feed) No effect on body weight gain No effect on markers of liver damage Increased Sa/So ratio in liver and kidney from 10 mg/kg. No effects on Sa/So in serum	No changes in organ weights No pathological alterations	NOAEL 20 mg/kg feed No adverse effect reported	Naturally contaminated material	Tardieu et al. (2007)
Male **turkeys** (BUT 9 strain) Culture extracts containing B_1 and B_2, from 22 days of age	Force-fed an oral dose of 10 mg FB_1 + FB_2/kg bw for 21 days	No effects on body weight gain, no mortality Increased Sa and Sa/So ratio in serum, liver and kidney.	No lesions, or organ weight alterations		Oral force feeding	Benlasher et al. (2012)
Female **turkeys** 11 weeks old at start of the experiment Culture material of *F. verticillioides*	15 mg FB (B_1 + B_2) on the feed for 14 days	⇑sphinganine/sphingosine in serum		NOAEL 15 mg/kg feed No adverse effect observed	Only one dose	Masching et al. (2016)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Japanese quail 50 control, 100 exposed, from 1 day old FB_1 given as: verticillioides culture material mixed into feed	1) 0 (control) (n = 50) 2) 300 mg FB_1/kg feed (n = 100)	— 59% mortality — Signs of neurotoxicity — Ruffled feathers — Reduced feed intake and body weight gain — Diarrhoea — Altered clinical chemistry		LOAEL 300 mg/kg feed Endpoint: Reduced feed intake and body weight gain, diarrhoea, clinical chemistry	Only one high dose Culture material used	Asrani et al. (2006)
Young laying Japanese quail days old). Culture material from *F. verticillioides* in the diet. In addition the material contained FB_2 (approximately 33% of FB_1) and FB_3 (approx. 10% of FB_1) 28 days	1) 0 (control) 2) 10 mg FB_1/kg feed 3) 50 mg FB_1/kg feed 4) 250 mg FB_1/kg feed	— Reduced feed intake and body weight gain (from 50 mg/kg feed, reduced feed conversion (from 250 mg/kg feed, reduced egg production (from 250 mg/kg feed), reduced egg weight (from 50 mg/kg feed, thinner egg shells (from 50 mg/kg feed)	No histopathological changes in liver, kidney or heart from any treatment group	NOAEL 10 mg/kg feed LOAEL 50 mg/kg feed Endpoint: Feed intake and body weight gain		Butkeraitis et al. (2004)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Japanese quail from 5 days old. 75/group *F. moniliforme* culture material was mixed into the feed. Birds were infected with *S.* Gallinarum at 21 days of age (exposed for 16 days) 37 days (16 days before infection with *S.* Gallinarum and 21 days after infection	1) 0 (control) 2) 150 mg FB_1/kg feed	— 3 dead birds in FB_1 fed vs none in controls — Reduced feed and water intake — Reduced body weight gain — Increased erythrocyte count — leucocytosis — Diarrhoea, clinical neurological symptoms — More severe and earlier onset of symptoms after infection — Reduced lymphocyte response to infection — Increased mortality after infection		LOAEL 150 mg/kg feed Endpoint: Reduced feed intake and bw gain, haematology and immunology, neurological symptoms, diarrhoea, mortality	Culture material used Only one high dose	Deshmukh et al. (2005a)
Japanese quail from 5 days old. 75/group *F. moniliforme* culture material was mixed into the feed. Birds were infected with *S.* Gallinarum at 21 days of age (exposed for 16 days) 37 days (16 days before infection with *S.* Gallinarum and 21 days after infection	1) 0 (control) 2) 150 mg FB_1/kg feed		Mild to moderate hepatomegaly and pale discoloration of liver Increased pathological alterations in liver after infection with *S.* Gallinarum	LOAEL 150 mg/kg feed Endpoint: Pathological changes in liver after and without infection.	Culture material used Only one high dose	Deshmukh et al. (2005b)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Japanese quail from 5 days old. 75/group *F. moniliforme* culture material was mixed into the feed. Birds were infected with *S.* Gallinarum at 21 days of age (exposed for 16 days) 37 days (16 days before infection with *S.* Gallinarum and 21 days after infection	1) 0 (control) 2) 150 mg FB_1/kg feed	–	Reduced spleen size - depletion of white pulp thinning of cardiomyocytes, lymphoid cell depletion from bursal follicles renal tubular nephrosis lower response in agglutination test to *S.* Gallinarum	LOAEL 150 mg/kg feed Endpoint: Pathological alterations in several organs, lower immune response towards infection	Culture material used Only one high dose	Deshmukh et al. (2007)
Japanese quail from 8 weeks of ageCulture material of *F. verticillioides* mixed into the feed140 days (5 egg laying cycles of 28 days)	1) 0 (control) (n = 48) 2) 10 mg B_1/kg feed (n = 48)	— Reduced feed consumption in cycles 4 and 5, but not 1–3. — Reduced body weight on cycle 5, not 1–4 — No effect on feed efficiency (g feed/g egg) — Reduced egg weight		LOAEL 10 mg B_1/kg feed Endpoint: reduced feed consumption, reduced body weight, reduced egg weight	Culture material Only one dose	Ogido et al. (2004)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Japanese quail from day 1 Culture material of *F. verticillioides* mixed into the feed 35 days	1) 0 (control) 2) 200 mg/kg FB_1	— Ruffled feathers and reduced body weight gain — Increased serum protein, albumin, cholesterol, AST, LDH, creatinine kinase — Reduced mononuclear immunity response — Increased skin thickness		LOAEL 200 mg/kg feed Endpoint: Reduced bw gain, neurological symptoms, altered serum biochemistry, reduced immunological response	Culture material Only one dose Short-term	Sharma et al. (2008)

AFB: aflatoxin B; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; bw: body weight; Chol: total cholesterol; DON: deoxynivalenol; FB: fumonisin B; GGT: gamma-glutamyl transferase; GOT: glutamic-oxaloacetic transaminase; LDH: lactate dehydrogenase; LOAEL: lowest-observed-adverse-effect level; n: number of animals; NOAEL: no-observed-adverse-effect level; Sa/So: sphinganine-to-sphingosine ratio; Tri: triglycerides; ZEN: zearalenone.

Horses

Fumonisins were first isolated and described from cultures of *Fusarium verticillioides* isolated from maize associated with equine leucoencephalomalacia (ELEM) (Marasas, 2001). Clinical signs of ELEM include apathy, drowsiness, pharyngeal paralysis, blindness, circling, staggering, hyperexcitability, and seizures. In some cases, sudden death occurs without any prior signs. A typical finding at necroscopy is necrosis of the white matter in the brain. Fumonisins also damage the cardiovascular system in horses, causing decreased heart rates, lower cardiac output, and ventricular contractility (EFSA, 2005) and these effects are probably linked to the neurological effects.

In the previous opinion, EFSA concluded that horses, together with pigs, were the most sensitive farm animal species (EFSA, 2005). Evaluations of field outbreaks of ELEM in the USA showed that consumption of feed containing more than 10 mg FB_1/kg feed was associated with increased risk of ELEM, while no increased risk was found for feed containing less than 6 mg/kg feed (Ross et al., 1991).

No oral dose–response studies with fumonisins including low doses are available. EFSA based its previous evaluation on a study using iv injection. Horses (3 or 4/group) were given daily injections of 0 (control), 0.01, 0.05, 0.1 or 0.2 mg pure FB_1/kg bw for up to 28 days. Horses considered as unsafe for themselves or the surroundings were euthanised prior to 28 days (Constable et al., 2000; Foreman et al., 2004). The horses were subject to neurologic and cardiovascular examinations. In addition, serum biochemical analysis of liver enzymes creatinine and cholesterol were performed and samples of cerebrospinal fluid were investigated in the euthanised horses. Neurological symptoms such as hindlimb ataxia, delayed forelimb placing reactions, decreased tongue movement, depression, hyperaesthesia and dementia were reported. Two horses died unexpectedly few hours after detection of mild neurological symptoms (at the highest dose 0.2 mg pure FB_1/kg bw). Cardiovascular effects like decreased heart rate, cardiac contractility arterial pulse pressure, venous blood pH and increased systemic vascular resistance were reported from horses with neurological symptoms. The symptoms were more severe and occurred more rapidly with increasing doses. No neurological or cardiovascular effects were reported from horses given 0.01 mg/kg bw per day. Increased serum creatinine, AST, ALP and GGT activity and increased bile acids, total bilirubin and cholesterol concentrations were found in all treated horses. Based on these findings, the authors concluded that 0.01 mg/kg bw was a LOAEL for horses, which was also used by EFSA in 2005. Both the authors and EFSA assumed an oral bioavailability of 5% and estimated that 0.01 mg/kg bw corresponds to an oral dose of 0.2 mg/kg bw per day or 8 mg/kg feed (Foreman et al., 2004; EFSA, 2005).

No later oral feeding studies with horses were identified.

In more recent field reports of ELEM in horses, the syndrome has been associated with feed for horses containing 6.6 mg FB_1/kg feed in Brazil (dos Santos et al., 2013) and 12.5 mg/kg in feed in Argentina (Giannitti et al., 2011). In Serbia, 21 out of 100 horses in a stable were diagnosed with ELEM based on clinical observations. Pathological examinations performed on one of the horses revealed findings consistent with fumonisin intoxications. One sample of each of the feed ingredients were collected. The samples of milled maize collected at the time of diagnosis contained 6.0 mg FB_1/kg and 2.4 mg FB_2/kg, while the maize bran contained 6.05 mg/kg FB_1 and 1.68 mg/kg FB_2 (Jovanović et al., 2015), but there are no description of the sampling procedure or any information of levels in the previous feed batch. These field reports do not contain details such as feed consumption. It is therefore not possible to establish safe limits based on these reports.

The EFSA evaluation from 2005 was based on a preliminary report from UDSA (Constable et al., 2000). Parts of the findings have been published in other papers (Smith et al., 2002; Foreman et al., 2004), but the effects on serum biochemistry have not been published in peer-reviewed journals. Furthermore, the preliminary report provided is uncomplete and the actual data are lacking. The CONTAM Panel could therefore not derive a reference point based on the effects on serum biochemical parameters. EFSA therefore consider an i.v dose of 0.01 mg FB_1/kg bw per day for a NOAEL based on neurological and cardiovascular effects (Smith et al., 2002; Foreman et al., 2004). Assuming a 5% bioavailability, this would correspond to 0.2 mg/kg bw per day.

Using the consumption value in Appendix C.1, this corresponds to feed contaminated at 8.8 mg/kg feed.

Table 6: Adverse effects in horses

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Healthy horses between 6 months and 6 years of age (252–524 kg, breed and gender not specified) were given pure (purity not specified) FB_1 for 28 days	I.v. injection of 0 (control), 0.01, 0.05, 0.1 or 0.2 mg/kg bw	Hindlimb ataxia, delayed forelimb placing reactions, decreased tongue movement, depression, hyperaesthesia and dementia, decreased heart rate, cardiac contractility arterial pulse pressure, venous blood pH and increased systemic vascular resistance		NOAEL of 200 μg/kg bw per day (neurological effects) Corresponding to 8.8 mg/kg feed	Pivotal study used in EFSA (2005), FAO/WHO (2001) I.v. injection Limited number of horses	Smith et al. (2002), Foreman et al. (2004)

bw: body weight; i.v.: intravenous; FB: fumonisin B; NOAEL: no-observed-adverse-effect level.

Rabbits

No LOAEL or NOAEL was identified for rabbits in the previous EFSA opinion (EFSA, 2005). New studies were reported since and data from the study of Ewuola (2009) indicates a LOAEL of 5 mg FB_1/kg diet (130 μg FB_1/kg bw) based on decreased performance, biochemical alterations in serum (total protein, liver enzymes) and blood composition. These results are supported by the findings of Ewuola and Egbunike (2008) showing moderate to severe alterations in liver at the same concentration (5 mg FB_1/kg feed).

In the present opinion, the studies without a control group were excluded.

Based on studies published after the last EFSA opinion, it appears that the effect of Fumonisin on rabbit performance was time and dose-dependent. For example, Szabo et al. (2014) reported that 10 mg FB/kg diet had no effect on feed intake and body weight gain of male rabbits exposed to the toxins for 4 weeks while a decrease of feed intake was observed in rabbits fed diets contaminated with higher doses (12.3 and, respectively, 24.5 mg FB/kg diet) for 5 weeks (Ewuola et al., 2008); in addition, a single dose of 630 mg FB_1/kg feed (31.5 mg/bw) decreased body weight in male rabbits (Orsi et al., 2009).

Serum biochemical analyses revealed that FB_1 decreased serum total protein, albumin, urea and creatinine levels in serum of male rabbits exposed to 5 mg FB_1/kg diet (Ewuola and Egbunike, 2008) or to 1.5 mg FB_1/kg bw per day (Orsi et al., 2007). A decrease in serum total protein concentrations was also observed in pregnant female rabbits fed a diet contaminated with 5 or 10 mg FB_1/kg diet (Gbore and Akele, 2010). By contrast, a dose of 31.5 mg FB_1/kg body weight significantly increased the total protein, urea and creatinine in male rabbits and increased the urinary protein concentrations (Orsi et al., 2009).

Contradictory data were also observed for the albumin/globulin ratio. Concentrations of 7.5 and 10 mg FB_1/kg diet increase the ratio (Ewuola et al., 2008) while 12.3 mg FB/kg diet induce a decrease of the albumin/globulin ratio (Ewuola and Egbunike, 2008).

The majority of the studies have shown that FB increases the activity of hepatic enzymes (ALT, AST, ALP, GGT) (Orsi et al., 2007, 2009; Ewuola and Egbunike, 2008; Gbore and Akele, 2010). Only one study showed no effect of FB on serum biochemical and enzyme parameters (Ewuola et al., 2008). The exposure of New Zealand rabbits to 1.5 mg FB_1/kg bw per day for 21 days increased the Sa level and the Sa/So ratio in urine, serum and liver of rabbits (Orsi et al., 2007). In some of these studies, the feed for control group was contaminated with low doses of FB_1 (Ewuola and Egbunike, 2008, 2010a,b; Ewuola et al., 2008).

Some studies showed that concentrations of 5–10 mg FB_1/kg diet (12 weeks of exposure) decreased the packed cell volume, haemoglobin concentration and erythrocytes number in rabbits (Ewuola and Egbunike, 2008; Gbore and Akele, 2010). These alterations were accompanied by the increase of white blood cells count and of the lymphocyte number (Ewuola and Egbunike, 2008; Gbore and Akele, 2010). However, other studies using higher concentration of FB_1 (12.3 and, respectively, 24.56 mg FB_1/kg diet) during 5 weeks of exposure showed no effect of FB on the mean values of all the haematological variables (PCV, RBC, WBC, Hb, MCH, MCV, MCHC) (Ewuola et al., 2008).

FB decrease the relative weight of visceral organs (liver, spleen, kidney, testes) (Orsi et al., 2007, 2009; Ewuola, 2009). Histopathological analyses showed liver congestion after 21 days of exposure to 1.5 mg FB_1/kg bw per day with different degree of liver lesions with moderate vacuolar degeneration (Orsi et al., 2007). Liver necrosis was observed after an exposure to 5 mg/kg feed for 196 days (Ewuola, 2009). Renal congestion associated with hypo pigmented areas were also associated with the exposure to 1.5 mg FB_1/kg bw per day (Orsi et al., 2007). The stomach and small intestine present erosion of the tunica mucosa in rabbits exposed to 7.5 and 10 mg FB_1/kg bw (Ewuola, 2009). Gross pathological profile of kidney of intoxicated rabbits is characterised by renal congestion associated with hypopigmented areas (Orsi et al., 2007).

Mild-to-moderate lesions and Sertoli cell degeneration were observed in testis of rabbits exposed to 0.13, 5 and 7.5 mg FB_1/kg diet (Ewuola, 2009) for 196 days. FB_1 impaired spermatogenesis and decrease the sperm reserves in testis, caput, corpus and caudal epididymis (Ogunlade et al., 2006; Ewuola and Egbunike, 2010a). FB_1 delay the onset of puberty (Ewuola and Egbunike, 2010b).

In summary, data available from the study of Gbore and Akele (2010), Ewuola (2009) and Ewuola and Egbunike (2010a) indicates a LOAEL of 5 mg FB_1/kg feed (0.2 mg FB_1/kg bw) based on mild moderate to severe alterations in liver and impairment of reproductive capacity. However, it is to be mentioned that the feed of control group was contaminated with a low dose of toxin (0.13 mg FB_1/kg diet) in this study.

Table 7: Adverse effects in rabbits

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
				No pivotal study to derive NOAELs/LOAELs		EFSA CONTAM Panel (2014)
N = 30, adult male rabbits, 25 weeks of age) Average weight 1.88 kg bw 5 weeks of exposure	1) Control 0.35 mg FB/kg diet (N = 10) 2) 12.3 mg FB/kg diet (N = 10) 3) 24.6 mg FB/kg diet (N = 10)	Impaired spermatogenesis	↓gonadal sperm reserves of matured rabbits	LOAEL 24.6 mg FB/kg diet Endpoint: ↓ caput and caudal epididymides weight	Feed contaminated with no purified *(F. verticillioides)* cultured maize grains No data on feed intake –no correspondence in μg/kg bw for LOAEL Control group contaminated with low dose of fumonisin	Ogunlade et al. (2006)
N = 16, New Zealand rabbits Average weight 1.7 kg 21 days of exposure	1) Control, 0 mg FB_1/kg bw per day (N = 8) 2) 1.5 mg FB_1/kg bw per day (N = 8)	No effect on body weight ↓ total protein, albumin, urea and creatinine levels and an increase in AP, AST, ALT and GGT ↑ Sa level and the Sa/So ratio in urine, serum	↓ liver weight Gross pathological profile characterised by hepatic Renal congestion associated with hypopigmented areas Moderate vacuolar degeneration of the liver ↑ Sa level and the Sa/So ratio in liver	LOAEL 1.5 mg FB_1/kg bw per day Endpoint: ↑ Sa level and the Sa/So ratio in urine, serum and liver ↓ in biochemical parameters Histological effects, liver degeneration	Feed contaminated with purified FB_1 Only one dose No data about feed intake No correspondence in μg/kg bw for LOAEL	Orsi et al. (2007)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 48, 49-day-old New Zealand White × Chinchilla male rabbits Average weight 757.50 g; 12 weeks of exposure	1) Control, 0.1 mg FB_1/kg diet (N = 12) 2) 5.0, mg FB_1/kg diet (N = 12) 3) 7.5 mg FB_1/kg diet (N = 12) 4) 10 mg FB_1/kg diet (N = 12)	7.5 and 10 mg FB_1/kg diet ↓the packed cell volume, haemoglobin concentration and RBC number ↑ WBC count and the lymphocyte number ↓ total serum protein, albumin, albumin-globulin ratio 7.5 and 10 mg FB_1/kg diet ↑ ALT, AST and ALP	–	LOAEL 5 mg FB_1/kg diet Endpoint: Decrease in biochemical parameters Modulation of haematological parameters	Feed contaminated with not purified fungal (*F. verticillioides)* culture material No data on feed intake – no correspondence in μg/kg bw for LOAEL Control group contaminated with low dose of fumonisin	Ewuola and Egbunike (2008)
N = 30, 22–24 week of age, matured crossbred male rabbits Average weight 1.36 kg 5 weeks of exposure	1) Control, 0.35 mg FB/kg diet (low dose) (N = 10) 2) 12.30 mg FB/kg diet (medium dose) (N = 10) 3) 24.56 mg FB/kg diet (high dose) (N = 10)	↓the dry matter intake no effect on the mean values of all the haematological variables (PCV, RBC, WBC, Hb, MCH, MCV, MCHC) or on the serum biochemical and enzyme parameter Medium dose of FB_1↑the albumin/globulin ratio		LOAEL 12.30 mg FB/kg diet Endpoint: decrease in feed intake	Feed contaminated with not purified *F. verticillioides* cultured maize grains No data on feed intake; no LOAEL calculated in μg/kg bw Control group contaminated with low dose of fumonisin	Ewuola et al. (2008)
N = 18, white New Zealand male rabbits, 50-day-old Average weight 1.7 kg A single dose of purified FB_1	1) Control 0 mg FB_1/kg bw (N = 6) 2) 31.5 mg FB_1/kg bw, corresponding to about 630 mg FB_1/kg diet (N = 12)	↓ body and liver weight. ↑total protein, AP, AST, ALT, GGT, urea and creatinine ↑ urinary protein concentrations	–	LOAEL 31.5 mg FB_1/kg Endpoint: alteration of reproductive system Decrease in performance and increase in biochemical parameters	Purified toxin Only one dose Oral administration (Gavage)	Orsi et al. (2009)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 48, 49-day old New Zealand white × Chinchilla male rabbits Average weight 757.50 g 196 day of exposure	1) Control, 0.13 mg FB_1/kg diet (N = 12) 2) 5.0 mg FB_1/kg diet (N = 12) 3) 7.5 mg FB_1/kg diet (N = 12) 4) 10.0 mg FB_1/kg diet (N = 12)	↓ the relative weight of visceral organs (liver, spleen, kidney, testes)	FB_1 > 5 mg/kg diet induces mild moderate to severe liver necrosis/lesions FB_1 concentrations higher than 7.5 mg/kg diet induces mild–moderate lesions and sertoli cell degeneration in testis FB_1 > 7.5 mg/kg diet induces tunica mucosa erosion in the stomach and small intestine	LOAEL 199 μg FB_1/kg bw corresponding to 5 mg FB_1/kg diet LOAEL reported in the study Endpoint: mild moderate to severe liver necrosis/ lesions	Feed contaminated with no purified fungal (*F. verticillioides)* culture material Control group contaminated with low dose of fumonisin	Ewuola (2009)
N = 48, 7- week-old New Zealand White × Chinchilla Male rabbits Average weight 757.50 g 28 weeks of exposure	1) Control, 0.13 mg FB_1/kg diet (N = 12) 2) 5.0 mg FB_1/kg diet (N = 12) 3) 7.5 mg FB_1/kg diet (N = 12) 4) 10.0 mg FB_1/kg diet (N = 12)	FB_1 decrease the daily sperm production ↑ the epididymal weight ↓the sperm reserves in testis, caput, corpus and caudal epididymis	↓ the sperm reserves in testis, caput, corpus and caudal epididymis	LOAEL 5 mg FB_1/kg diet Endpoint: changes in reproductive system	Feed contaminated with no purified fungal (*F. verticillioides*) culture material No data on feed intake – no correspondence in μg/kg bw for LOAEL Control group contaminated with Low dose of fumonisin	Ewuola and Egbunike (2010a)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 24, normal matured crossbred female rabbits Average weight 1.82 kg bw Six weeks of exposure	1) Control, 0 mg FB/kg diet (N = 8) 2) 5 mg FB/kg diet (N = 8) 3) 10 mg FB/kg diet (N = 8)	↓ daily dry matter intake and final live weight ↓ serum total protein concentrations in pregnant female rabbits ↑ the serum enzymes ALT, AST (low and high dose) ALP (high dose) ↓ the haemoglobin values and ↑the leukocyte values of the pregnant female rabbits ↓ the RBC counts and packed cell volume only at 10 mg of FB_1	–	LOAEL 130 μg FB/kg bw, corresponding to 5 mg FB/kg diet Endpoint: modulation of serum biochemical parameters	Feed contaminated with no purified fungal *(F. verticillioides)* cultured maize grains	Gbore and Akele (2010)
N = 40 Male New Zealand White × Chinchilla male rabbits, 49 day old Average weight 757.50 g 175 days of exposure	1) Control 0.13 mg FB_1/kg diet (N = 10) 2) 5.0 mg FB_1/kg diet (N = 10) 3) 7.5 mg FB_1/kg diet (N = 10) 4) 10.0 mg FB_1/kg diet (N = 10)	7.5 and 10.0 mg FB_1/kg diet delay the onset of puberty	–	LOAEL 7.5 mg FB_1/kg diet Endpoint: delay the onset of puberty	Feed contaminated with no purified fungal *(F. verticillioides)* cultured maize grains No purified culture material No data about feed intake- no correspondence in μg/kg bw for LOAEL Control group contaminated with low dose of fumonisin	Ewuola and Egbunike (2010b)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
N = 20 male rabbits 35 day old Average weight 949.8 g (control) 998.8 g (FB_1) 4 weeks of exposure	1) Control, 0 mg FB_1/kg diet (N = 10) 2) 10 mg FB_1/kg diet (N = 10)	No significant bw differences FB_1 significantly increased the RBC Na+/K+ ATPase activity Minor alterations on the RBC membrane fatty acid (FA) composition No effect on the haematological profile	No effect on organ (heart, liver, kidney, spleen) weight	LOAEL 10 mg FB_1/kg diet Endpoint: increase ATPase activity in RBC	Feed contaminated with not purified fungal (*F. verticillioides* strain MRC 826) culture material Only one dose	Szabo et al. (2014)

AP: alkaline phosphatase; AFB: aflatoxin B; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; bw: body weight; Chol: total cholesterol; DON: deoxynivalenol; FA: fatty acid; FB: fumonisin B; GGT: gamma-glutamyl transferase; GOT: glutamic-oxaloacetic transaminase; Hb: haemoglobin concentration; LDH: lactate dehydrogenase; LOAEL: lowest-observed-adverse-effect level; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; MCV: mean cell volume; N: number of animals; NOAEL: no-observed-adverse-effect level; PCV: packed cell variable; RBC: red blood cell; Sa/So: sphinganine-to-sphingosine ratio; Tri: triglycerides; WBC: white blood cell; ZEN: zearalenone.

Fish

The available database from feeding studies giving fumonisins to fish is limited as only two feeding experiments with carp and one with each of channel catfish, African catfish and Nile tilapia have been identified. Fumonisins reduced the body weight gain of all species.

EFSA (2005) concluded that the available data at that time indicated a LOAEL of 10 mg FB/kg feed for carp, based on a study where 1-year-old carps (mean weight 127 g) were given feed containing 10 or 100 mg FB_1/kg feed for 42 days (Petrinec et al., 2004). The diet was prepared by mixing *Fusarium* culture material into the feed. Pathological alterations in liver, endocrine and exocrine pancreas, kidney, heart and brain were reported from fish receiving the low dose feed.

In another feeding study, 1-year old carp (120–140 g) were given FB_1 purified from *Fusarium* culture material mixed into the feed and given feed corresponding to 0.5 or 50 mg/kg bw per day (feed concentration not given). The exposure resulted in a loss of body weight gain and alterations of haematological and biochemical parameters, indicating liver and kidney damage (Pepeljnak et al., 2003).

One additional study from the same group has been published since the EFSA opinion. One-year old carps were given 10 or 100 mg FB_1/kg feed using the same experimental design as in the studies above (Kovacić et al., 2009). Histopatholgical examinations revealed reduced weight gain, and vacuolated, degenerated or necrotic neural cells around damaged brain capillaries in both dose groups.

A LOAEL of 10 mg FB_1/kg feed, corresponding to 0.5 mg/kg bw per day, could be derived for carp based on the available studies.

EFSA concluded in 2005 that available data indicated a NOAEL of 20 mg/kg feed for catfish, based on a study by Lumlertdacha et al. (1995). In this study, catfish were fed diets containing *Fusarium* culture material with final FB_1 concentrations of 20, 80, 320 or 720 mg/kg feed for 10 weeks to 1-month-old fish (n = 50/group) or for 14 weeks to 1-year-old fish (n = 30/group). The mortality increased from 320 mg/kg feed in both age groups. In the 1-month-olds, the weight gain was decreased in fish given from 20 mg FB_1/kg feed, while in the 1-year-old fish, the body weight gain decreased from 80 mg/kg feed. Haematocrit, erythrocyte and leucocyte counts were reduced in 1-month-old fish given from 80 mg FB_1/kg feed and from 320 mg FB_1/kg feed in 1-year-old fish. Microscopic examinations revealed liver lesions in fish given from 20 mg FB_1/kg feed or more in both age groups.

There are no new feeding studies with channel catfish available since then and the LOAEL for Nile tilapia is 10 mg FB_1/kg feed.

EFSA also concluded that the data at that time indicated a NOEL of 20 mg FB_1/kg feed for catfish and Nile tilapia (EFSA, 2005). This was based on a study where groups of Nile tilapia (n = 20/group) (*Oreochromis niloticus*) had been given feed containing 0, 10, 40, 70 or 150 mg FB_1/kg feed prepared by mixing culture material into the feed (Tuan et al., 2003). The body weight gain was reduced in fish receiving from 40 mg/kg feed. The Sa/So ratio in liver increased dose dependently and no histopathological lesions were found. No new studies with Nile tilapia has been found and 10 mg/kg feed, corresponding to 0.4 mg/kg bw per day, is still considered as a NOAEL for Nile tilapia.

African catfish (*Clarias gariepinus*, 17.35 ± 1.26 g size) were fed a diet where maize culture material of *F. verticillioides*, were mixed into the feed in different rations to give feed concentrations of 0 (control), 5.0 mg, 10.0 or 15.0 mg B_1/kg feed for 6 weeks. There were 16 tanks with 20 fish in each treatment (Gbore et al., 2010). Feed intake and weight gain was reduced in all groups exposed to fumonisins compared to the control. Due to limitations in experimental design and reporting from the studies, the study could not be used to establish a safe limit for catfish.

Table 8: Adverse effects in fish

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
Carp (*Cyprinus carpio L*), 1 year old, mean weight 127 g, n = 8/ group, (gender not specified), Purified fumonisin (purity not specified) 42 days	1) Control (n = 8) 2) 10 mg FB_1/kg feed (n = 8) 3) 100 mg FB_1/kg feed (n = 8)	No mortality. Reduced body weight gain in treated groups, but no difference between dose groups, erythrodermatitis cyprini lesions	Pathological and histopathological alterations in several organs including liver, pancreas, head and trunk kidneys, gall bladder, pericardium	LOAEL 10 mg/kg feed Endpoint: bw gain, pathological alterations	– Pivotal study in EFSA 2005 for carp – kept in separate cages immersed in one pond – fed once daily, (FB_1 may partly dissolve in water but pelleted feed) Only 1 cage/ treatment	Petrinec et al. (2004)
Carp (*Cyprinus carpio L*), 1 year old, mean weight 127 g (gender not specified) Purified fumonisin (purity not specified) 42 days	1) Control (n = 8) 2) 10 mg FB_1/kg feed (n = 8) 3) 100 mg FB_1/kg feed (n = 8)	–	Vacuolated, degenerated or necrotic neural cells, around damaged brain blood capillaries and the periventricular area	LOAEL 10 mg/kg feed Endpoint: Reduced weight gain, neuronal apoptosis in brain	– Kept in separate cages immersed in one pond – fed once daily, (FB_1 may partly dissolve in water but pelleted feed) Only 1 cage/ treatment	Kovacić et al. (2009)
Channel catfish *(Ictalurus punctatus)* one year old (1.2 g) or 2 year old (31 g) *F. moniliforme* culture material 10 or 14 weeks	1) 0.3 mg FB_1/kg feed (control) (n = 50 × 4 for 1 year old, 30 × 4 for 2 year old) 2) 20 mg FB_1/kg feed (n = 50 × 4 for 1 year old, 30 × 4 for 2 year old) 3) 80 mg FB_1/kg feed (n = 50 × 4 for 1 year old, 30 × 4 for 2 year old) 4) 320 mg FB_1/kg feed (n = 50 × 4 for 1 year old, 30 × 4 for 2 year old) 5) 720 mg FB_1/kg feed (n = 50 × 4 for 1 year old, 30 × 4 for 2 year old)	Increased mortality (from 320 mg/kg feed) Decreased body weight gain (from 20 mg/kg feed Decreased haematocrit, red blood cell counts and white blood cell (from 80 mg/kg feed	Liver lesions (from 20 mg/kg feed	LOAEL 20 mg/kg feed Endpoint: bw gain, liver pathology	Pivotal study from channel catfish in EFSA (2005) Culture material also contains FB_2	Lumlertdacha et al. (1995)

Study design breed, age, gender, exposure period, animal weight	Doses or feed concentration	Clinical signs/ biochemical changes	Pathological findings	NOAEL/LOAEL and endpoint	Remarks source and nature of the toxin	Reference
African catfish (*Clarias gariepinus*), 17.35±1.26 g size. Maize cultured with *F. verticillioides* For 6 weeks	1) control (n = 4 × 20) 2) 5.0 mg B_1/kg feed (n = 4 × 20) 3) 10.0 mg B_1/kg feed (n = 4 × 20) 4) 4)15.0 mg B_1/kg feed (n = 4 × 20)	All doses had reduced feed intake and weight gain compared to Decreased haematocrit, erythrocytes, haemoglobin, MCV and MCH. Increased leucocyte counts. Reduced serum protein levels		LOAEL 5 mg/kg feed Endpoint: Reduced weight gain and reduced levels of haematological parameters	Fungal culture material used Surplus feed removed only once/day Method for measuring feed consumption not given Increased levels of ammonia in water Decreased DO_2	Gbore et al. (2010)
Nile tilapia *(Oreochromis niloticus)* 2.7 g *F. moniliforme* culture material 8 weeks	1) 0 (n = 3 × 40) 2) 10 (n = 3 × 40) 3) 40 (n = 3 × 40) 4) 70 (n = 3 × 40) 5) 150 (n = 3 × 40)	Reduced body weight gain (from 40 mg/kg feed) Increased FCR (from 40 mg/kg feed) Reduced haematocrit (from 150 mg/kg feed) Increased Sa/So ratio (from 150 mg/kg feed	No histological abnormalities found in internal organs	NOAEL of 10 mg/kg feed (0.4 mg/kg bw per day)	Stated in EFSA as NOAEL of 20 mg/kg feed in Nile tilapia Fungal culture material used	Tuan et al. (2003)

bw: body weight; FB: fumonisin; FCR: feed conversion ratio; LOAEL: lowest-observed-adverse-effect level; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; MCV: mean cell volume; n: number of animals; NOAEL: no-observed-adverse-effect level; PCV: packed cell variable; RBC: red blood cell; Sa/So: sphinganine-to-sphingosine ratio.

Cats

No information could be retrieved on the adverse effects of fumonisins and modified forms in cats.

Dogs

No information could be retrieved on the adverse effects of fumonisins and modified forms in dogs.

Farmed mink

Only one study on the effect of fumonisins on farmed mink was published since the last EFSA evaluation. In this study conducted by Bursian et al. (2004), male adult mink were exposed for 14 days to a basal diet contaminated with fungal (*F. verticillioides*) culture material resulting in 200 mg FB_1 + 34 mg FB_2/kg feed concentration. FB_1 had no effect on feed consumption and body weight. Only the sphinganine concentration in urine was significantly higher, but sphingosine concentration as well as the urinary Sa/So ratio were unaffected by the FB exposure. The addition of a mycotoxin adsorbent did not reduce the increased urinary sphinganine concentration. Because cereal grains are important components of mink diets more information is needed on the effect of fumonisins on mink to derive reference points for this species. For the sum of fumonisin B_1 and B_2, guidance value is 50 mg/kg for mink (EFSA CONTAM Panel, 2014, Commission Recommendation 2016/1319/EC)[2,13].

3.1.3.2. Modified forms of Fumonisins

Only one study has investigated the effect of modified forms of Fumonisin in farm and companion animals. This study compared the toxicity of HFB_1 to the one of FB_1 in piglets (Grenier et al., 2012). Animals were exposed by gavage for 2 weeks to 2.8 μmol FB_1 or HFB_1/kg body weight per day (corresponding to 2.0 mg FB_1/kg bw per day and equimolar concentration of HFB_1). In contrast to FB_1, HFB_1 did not trigger hepatotoxicity as indicated by lesion score, level of several biochemical analytes and expression of inflammatory cytokines. Similarly HFB_1 did not alter the morphology and villus height of the different segments of the small intestine and slightly modified the mRNA level in the intestine and the mesenteric lymph nodes (increased 12p40 mRNA expression in the mid- and distal small intestine, increased IFN-γ in the distal small intestine, decreased TNF-α· and IL-6 in the mesenteric lymph nodes). This low toxicity of HFB_1 correlated with a weaker increased of the sphinganine/sphingosine ratio in the liver and in the plasma, when compared to FB_1.

This low toxicity of HFB_1 is supported by several feeding trial performed in pigs and in poultry, in which the feed was supplemented with enzyme hydrolysing FB_1 to HFB_1 (Grenier et al., 2013; Masching et al., 2016).

3.1.3.3. Conclusions – Adverse effects

There are rather limited data available on oral toxicity in livestock species, horses, fish and dogs, especially studies using purified toxins. Only a few of these are suitable for the derivation of NOAELs and LOAELs. Table 9 summarises the adverse effects observed in cattle, pigs, poultry, horse, rabbit, and fish. Sheep and goats would not seem to be more susceptible to fumonisins than cattle. Except for horses, the NOAEL and/or LOAEL value were obtained from studies using feed contaminated with fixed levels of toxins and calculation were made to convert the reference value in μg/kg bw per day. No suitable data were available to derive NOAEL or LOAEL for dog, cats and fur animals.

The adverse effects observed in the different animal species upon exposure to FBs are summarised in Table 9. The main targets organs are the liver (cattle, pig, chickens, ducks, rabbits, channel catfish) the lung (pig) and the brain (horse, carp). The immune and cardiovascular systems were also a target for cattle and horses, respectively.

Pigs was the most sensitive species to FBs as evidenced by a low NOAEL (1 mg FB_1/kg feed corresponding to 40 μg/kg bw per day) and LOAEL (5 mg FB_1/kg feed corresponding to 40 μg/kg bw per day).

Rabbits and horses were quite sensitive to FBs. For rabbits, a LOAEL of 5 mg FB_1/kg feed (corresponding to 130 μg FB_1/kg bw per day) was derived. For horses the NOAEL was 8.8 mg FB_1/kg feed (derived from i.v dosing and calculated into 0.2 mg FB_1/kg bw per day).

Poultry were more resistant to FBs; however, large variation was observed between duck and chicken or turkey. The NOAELs were 8 mg FBs/kg feed for ducks and 20 mg FBs/kg feed for chickens

[13] Commission Recommendation 2006/576/EC 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. OJ L 229, 23.8.2006, p. 7–9.

and turkeys. A similar variation was observed among fish species with LOAEL ranging from 10 mg FBs/kg feed for carp; 20 mg FBs/kg feed for channel cat fish and 40 mg FBs/kg feed for Nile tilapia.

Ruminants appear quite resistant to FBs; however, it was only possible to derive a reference point for cattle. The NOAEL for cattle was 31 mg FBs/kg feed corresponding to 600 μg FBs/kg bw per day.

Table 9: Relevant fumonisin toxicity studies with ruminants, pigs, poultry, horse, rabbit and fish to possibly set NOAELs/LOAELs for fumonisins

Species	No observed adverse effect levels (NOAEL)	Lowest observed adverse effect level (LOAEL)	Adverse effects observed (type of study)	References	Comments
Cattle	31 mg FBs (FB_1 + FB_2)/kg feed (corresponding to 600 μg FBs/ kg bw per day)	N/A	Biochemical alterations of serum enzymes and cholesterol, suggesting alteration of liver function, lymphocyte blastogenesis	Osweiler et al. (1993)	From EFSA (2005)
Pig	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) 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)	Mentioned in EFSA (2005)
Chicken	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)	From EFSA (2005)
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	0.2 mg FB_1/kg bw per day (8.8 mg/kg feed)	1 mg FB_1/kg bw per day (44 mg kg/feed)	Neurological abnormalities Cardiovascular effects	From EFSA (2005)	
Rabbits		5 mg FB_1/kg feed (corresponding to 130 μg FB_1/ kg bw per day)	Decreased performance and biochemical alteration (Serum protein, enzymes) Altered blood formula	Gbore and Akele (2010)	Supported by other studies, i.e. Ewuola (2009)

Species	No observed adverse effect levels (NOAEL)	Lowest observed adverse effect level (LOAEL)	Adverse effects observed (type of study)	References	Comments
Fish (Carp)		10 mg FB_1/kg feed (corresponding to 0.5 mg/kg bw per day)	Reduced weight gain, neuronal apoptosis in brain	Kovacić et al. (2009), Petrinec et al. (2004)	Same values as EFSA (2005)
Other fish: Channel catfish	20 mg FB_1/kg feed		Reduced weight gain, liver lesions	Lumlertdacha et al. (1995)	From EFSA (2005)
Other fish: Nile tilapia	10 mg FB_1/kg feed (corresponding to 0.4 mg FB_1/kg bw per day)	40 mg FB_1/kg feed	Reduced weight gain	Tuan et al. (2003)	From EFSA (2005)

ALP: alkaline phosphatase; AST: aspartate aminotransferase; bw: body weight; FB: fumonisin B; GGT: gamma-glutamyl transferase; GOT: glutamic-oxaloacetic transaminase; LDH: lactate dehydrogenase; LOAEL: lowest-observed-adverse-effect level; N/A: not applicable; NOAEL: no-observed-adverse-effect level; Sa/So: sphinganine-to-sphingosine ratio.

3.2. Feed occurrence data

3.2.1. Previously reported feed occurrence data in the open literature

Data reported in the literature about occurrence of fumonisins in raw materials and feed are mainly based on the determination of FB_1 and FB_2 by HPLC or ELISA methods, while only in the more recent years LC-MS/MS analysis enables the collection of occurrence data for FB_3. Consistently, data are commonly reported as the sum of FB_1 and FB_2, also in agreement with current regulation. Data on the occurrence of FB_4 in feed were not identified in the literature.

Surveys are generally addressed to raw materials, while small scale studies may cover specific animal feed categories.

The main global survey for mycotoxin contamination in feed was reported by Schatzmayr and Streit (2013), and further analysed with a focus on European countries by Streit et al. (2013).

The survey covered 19,757 samples collected worldwide, among them 11,439 considered for fumonisin occurrence. Overall, 54% of the samples were found positive for fumonisins (as the sum of FB_1, FB_2 and FB_3), with a mean of 1,674 μg/kg. More in details, 70% of samples from South Europe and 33% from Eastern Europe were found to be positive, while no positive sample was identified in Northern Europe.

Similar results were described by Griessler et al. (2010), who analysed compound feeds and ingredients collected in EU between 2005 and 2009. Samples were grouped on the base of the analytical method used. Overall, fumonisins (sum of FB_1 and FB_2) were found in 33 out of 43 samples analysed by HPLC, with a mean concentration of 1,411 μg/kg (range: 25–7,714 μg/kg), and in 26 out of 46 samples analysed by ELISA, with a mean concentration of 6,260 μg/kg (range: 373–36,390 μg/kg). The highest contamination levels were associated with samples from Italy, Portugal and Spain.

These findings are consistent with data reported over years for fumonisin occurrence in maize from Italy (Berardo et al., 2011; Pietri et al., 2012), underlying a strong frequency of positive samples at high concentration levels. Camardo Leggieri et al. (2015) reported on the strong occurrence of FB_1 and FB_2 in maize from Italy in 2012 (mean concentration: 3,040 μg/kg; max concentration: 10,604 μg/kg; n = 46), in 2010 (mean concentration: 3,781 μg/kg; max concentration: 12,637 μg/kg; n = 48) and in 2011 (mean concentration: 2181 μg/kg; max concentration: 21,007 μg/kg; n = 46). The authors underlined the significant correlation between climate factors and fumonisin incidence in maize.

Surveys performed in Poland showed a significant influence of the environmental condition on the contamination levels. Kosicki et al. (2016) reported on the occurrence of fumonisin B_1 and B_2 in maize harvested in 2011–2014 in Poland, with mean concentration levels in the range 53–324 μg/kg feed, and 33–1,063 μg/kg for finished feed. A similar study was performed on animal feed from Poland by Grajewski et al. (2012), showing concentrations in the range 28–1,030 μg/kg for corn grains and

15–2,260 μg/kg for silages. Czembor et al. (2015) reported an incidence of 100% in samples collected from Poland in 2011–2012, with a mean FB_1 concentration of 373 μg/kg.

Data on fumonisin occurrence in wheat from Europe have not been identified, with the only exception of a study from Western Romania (Alexa et al., 2013). The authors reported for FB_1 a 15% of frequency in wheat, with a contamination range of 960–1,180 μg/kg. Similar data have been obtained for Argentinian wheat, demonstrating the possible occurrence of FB_1 and FB_2 at lower concentration levels than those commonly reported in maize (Cendoya et al., 2014).

Considering other feed ingredients, Batatinha et al. (2007) investigated the presence of FB_1 in spent brewers' grains from barley as dairy cattle feed, and found a mean contamination of 44–500 μg/kg.

Almeida et al. (2011) described the incidence of FB_1 and FB_2 in feed for sows, with a frequency of 8.7% and a concentration range of 50–200 μg/kg.

A number of studies have been recently performed on companion animal's feed. Bohm et al. (2010) investigated the occurrence of FB_1 and FB_2 in dry dog feed. Overall, 42% of the samples (n = 76) were found positive at low levels, with the mean and maximum concentration 178 μg/kg and 568 μg/kg, respectively. Extruded dog feed was considered by Gazzotti et al. (2015), indicating a 85% of positive samples (n = 48) with a mean and maximum concentration of 67 μg/kg and 350 μg/kg, respectively. In contrast, dry dog feed from the market was analysed by Pagliuca et al. (2011), showing higher contamination levels. In particular, premium complete (n =16) and standard complete (n = 16) feed were found in the range 150–3,050 μg/kg and 20–5,190 μg/kg, respectively. In addition, complementary feed (n = 9) was found in the range 230–8,800 μg/kg.

Liesener et al. (2010) described the possible occurrence of FB_1 and FB_2 in commercial horse feed (n = 62). Overall, 94% of the samples were found contaminated, in a range of 2–2,200 μg/kg.

Results for swine feed were reported by Martins et al. (2012), who performed a survey over the years 2007–2010 (n = 278) with an incidence of contamination < 10% in the concentration range 53–3,815 μg/kg.

Nácher-Mestre et al. (2015) described the possible occurrence of FB_1, FB_2 and FB_3 in feed for Atlantic salmon and gilthead sea bream. A very low contamination was found in wheat gluten (mean 13.2 μg/kg), while higher levels were reported for corn gluten (range: 11–4,901 μg/kg).

Hidden fumonisins are commonly determined after alkaline hydrolysis of the sample. Dall'Asta et al. (2012) investigated the occurrence of hidden fumonisins in maize harvested in 2009 and 2010 in Italy. The total fumonisins detected after hydrolysis and expressed as $FB_{1–3}$ equivalents, were found to exceed the free $FB_{1–3}$ of about 60% in both years. Similar results were confirmed by Giorni et al. (2015).

More comprehensive studies on the accumulation and distribution of hidden fumonisins in maize and its milling fractions, were reported by Bryła et al. (2014, 2015, 2016, 2017). The authors confirmed the significant occurrence of hidden fumonisins in maize, and pointed out that the both particle size and starch amount may affect the distribution of hidden fumonisins. Also in these studies, the hidden fraction was in the range 30–100% compared to the parent compounds, although the average additional factor was about 59%.

The occurrence of hidden fumonisins was investigated in ensiled maize by Latorre et al. (2015), indicating that hidden FB_1 accounted in average for an additional 64%. The same average additional factor was reported by Oliveira et al. (2015) by analysing 72 maize samples from Brazil for fumonisins (the sum of FB_1 and FB_2) by alkaline hydrolysis.

Oliveira et al. (2015) reported higher concentration values for hidden fumonisins. Overall, after hydrolysis the total fumonisin content in raw maize (n = 72) was up to 3.8 times higher than before hydrolysis. Concerning modified forms of fumonisins, the Panel identified no occurrence data in feed in the open literature.

3.2.2. Feed Occurrence data submitted to EFSA

3.2.2.1. Fumonisins

Out of the 18,273 analytical results submitted by Member States, 133 results were excluded from the present analysis due to the following reasons: duplicates, suspected samples, analytical method not provided, or outliers (i.e. 2 results > 3,000 mg/kg in compound feed, not confirmed by the Member State laboratory).

Thus, the final data set included 18,140 analytical results from 7,970 samples on fumonisins in feed collected between 2003 and 2016 from 19 European countries available for the assessment.

The major contributing countries were the Netherlands (42%), France (18%), Belgium (12%) and Bulgaria (11%) (Table 10). Occurrence data on FB_1 were provided by all countries, FB_2 by all but one countries, whereas data on FB_3 were provided by three countries, namely Belgium, the UK and the Netherlands. It should be noted that the origin of the samples was not always the European country.

Table 10: Frequency distribution of analytical results of fumonisins in feed per sampling country (2003–2016)

Country	Abbreviations			Total	% of total
	FB_1	FB_2	FB_3		
Belgium	741	741	674	2,156	12
Bulgaria	970	969	–	1,939	11
Cyprus	20	20	–	40	0
Czech Republic	437	435	–	872	5
Estonia	24	24	–	48	0
Spain	1	1	–	2	0
France	1,596	1,596	–	3,192	18
United Kingdom	95	95	34	224	1
Croatia	37	–	–	37	0
Hungary	76	69	–	145	1
Ireland	6	6	–	12	0
Italy	193	170	–	363	2
Lithuania	39	39	–	78	0
Luxembourg	14	14	–	28	0
Netherlands	2,869	2,870	1,958	7,697	42
Norway	44	44	–	88	0
Portugal	415	415	–	830	5
Slovenia	158	159	–	317	2
Slovakia	36	36	–	72	0
Total	**7,771**	**7,703**	**2,666**	**18,140**	**100**

FB: fumonisin B.

Analytical methods

Only occurrence data with information on the analytical method and on LOD/LOQ levels that fulfilled the inclusion criteria for the present analysis were included. The CONTAM Panel considered only quantitative methods able to return a confirmation of the analyte identification and with an adequate sensitivity (Table 11). MS-based methods (Group 1, 68%) were mostly used.

Table 11: Distribution of analytical results by analytical method

Analytical method group[(a)]	FB_1	FB_2	FB_3	N	%
Methods based on mass spectrometry	5,228	5,202	1,969		
Methods based on spectroscopic detection	2,491	2,486	697	5,674	31
Gas-chromatographic methods	15	15	–	30	0
ELISA	37	–	–	37	0
Total	**7,771**	**7,703**	**2,666**	**18,140**	**100**

ELISA: enzyme-linked immunosorbent assay; FB: fumonisin B.

(a): Methods based on mass spectrometry: LC–MS/MS, LC–MS, LC–MS quadrupole, HPLC-ESI-MS. Chromatographic methods based on spectroscopic detection: HPLC-FD, HPLC-UV, HPLC with standard detection methods, HPLC-CF →. Gas-chromatographic methods: GC–MS.

The data set included 77% of left-censored data (results below the LOD/LOQ), of which 50% below LOD and 27% between LOD and LOQ. LOQs were reported for 54% of the samples. Samples where the LOQ value was not reported either referred to a sample with quantifiable levels or to a sample with residues below the LOD. Table B.1 of Appendix B gives the distribution of LOD and LOQ for the

different feed categories and compound feed. Seven samples with LOQ values above 2,000 μg/kg were considered outliers and were not included in the data set used for this assessment.

Occurrence data on feed by feed group

Table B.2 of Appendix B gives occurrence levels of the feed samples classified according to the catalogue of feed materials described in Commission Regulation 68/2013. Overall, 77% of the results were below the LOD or LOQ, accounting for 67% for FB_1, 80% for FB_2 and 96% for FB_3. Most of the analytical results were on 'cereal grains, their products and by products' (47%), 'compound feed' (23%) and 'forages and roughages, and products derived thereof' (16%). The highest number of reported samples in cereal grains were 'maize' (n = 4,655), 'wheat' (n = 1,504) and 'barley' (n = 687). Other feed groups that were well represented were 'complementary/complete feed' (n = 3,643), forages and roughage (n = 2,280), sunflower seed (n = 438) and toasted soya (beans) (n = 1,199).

High fumonisins concentrations were reported mainly in cereal grains in maize grains (mean LB/UB ranged from 20 to 2,037.7 μg/kg), wheat (mean LB/UB ranged from 0.4 to 2,482.5 μg/kg) and compound feed (mean LB/UB ranged from 0.3 to 1,678.1 μg/kg). Fumonisins at lower concentrations were also found in forages, land animal products, legume seeds, minerals, oil seeds and tubers. Concentration levels higher than 2,000 μg/kg were reported for compound feed, different types of maize, including maize gluten feed, maize flakes, and maize bran, and plants by-products from spirits production.

About 15% of the samples of the data set were analysed for all the three fumonisins, whereas more than 90% of the samples were analysed for both FB_1 and FB_2. Therefore, in order to estimate the concentrations of all fumonisins in each feed sample, the following approach was used. For samples in which the compound was analysed, but not quantified, the substitution method was used to estimate the LB and the UB (see Section 2.1). For samples in which any of the compounds were not analysed, the levels were estimated by using the mean concentration of the closest feed group available.

3.2.2.2. Hidden fumonisins

The occurrence of hidden fumonisins has been often reported in raw maize and maize-derived products. Their contribution to the overall occurence is usually obtained through the application of an alkaline hydrolysis treatment to the sample.

According to the previous studies reported in the literature, hidden fumonisins contribute to the overall fumonisins occurrence by an additional amount ranging from 40% to 70% of the parent compounds, and in few cases may reach an additional 100% (See Appendix D). The presence of hidden fumonisins is dependent on the climate conditions during the growing season, on the maize genotype, and on the processing (Dall'Asta and Battilani, 2016). All these factors may affect not only the overall occurrence, but also the ratio between parent and hidden forms. As a general observation, the ratio of modified fumonisins is higher when the overall contamination is low, while it is lower in highly contaminated samples (Dall'Asta and Battilani, 2016). Although this percentage can vary depending on the processing, different factors cannot be derived for single products, due to the lack of sufficient data from the literature.

In order to evaluate the contribution due to hidden forms in the risk assessment, an additional factor of 1.6 was derived from calculation based on data provided by three research groups located in Italy, Poland and Brazil. Occurrence data provided by the groups were obtained over several harvest years and in different geographical area. From a statistical analysis, the average additional contribution due to hidden forms to the overall contamination was about 60% in the EU-based area, while in South America the contribution was higher. Taking into account that EFSA risk assessment is based on European foods and feeds, and that different agronomic and climate conditions apply in the EU, the CONTAM Panel considered it appropriate to apply an additional factor of 60% with respect to the parent compound for an exposure assessment. However, this should be considered as an uncertainty.

The distribution of the mean, median, and P95 LB and UB concentrations of the sum of $FB_1 + FB_2 + FB_3$ (with and without 1.6 RPFs applied) in feed materials and species-specific compound feeds used to estimate exposures for farmed livestock and companion animals are provided in Appendix B (Tables B.3 and B.4).

3.2.3. Feed processing

Prior to processing, cereal grains are cleaned which removes broken kernels and those having mould growth, together with fine materials with particle size < 3 mm. It was demonstrated that this step can reduce the fumonisin amount from 26% to 69% (Sydenham et al., 1994).

Dry milling of grain is mainly utilised for feed manufacturing, separating the grain into four distinct physical components: flour (200–300 μm), medium and fine grits (300–1,000 μm), coarse and flaking grits (1,000–5,000 μm), other products (i.e. germ, bran, broken grains, meal). The effects of dry milling on fumonisin distribution in maize fractions have been reported (Brera et al., 2004, 2006; Bullerman and Bianchini, 2007; Vanara et al., 2009) with consistent results.

Fumonisins occurring in maize kernels are not degradated by the milling process, although they may undergo redistribution among milling fractions. In particular, levels of fumonisins are slightly reduced in maize flour and significantly lowered in grits (up to 70%) compared to raw materials, while they are increased in bran and middlings. According to Pietri et al. (2009) FB_1 tends to accumulate in the small particles intended for animal consumption (maize-milling fractions). This observation is in agreement with the possible fractionation of fumonisins according to particle size fractions (Brera et al., 2004). Fumonisin concentration is significantly reduced by extrusion, although reductions vary depending on the matrix (whole corn, grits, flour, etc.), formulation and specific process conditions. In the absence of added sugar or salt, reported reductions have ranged from 2% to 99% (Humpf and Voss, 2004; Jackson et al., 2012). Reduction of FB_1 in corn grits by extrusion is enhanced by glucose addition, due to the possible formation of Maillard-type modified forms such as NDF-FB_1 or NCM-FB_1 (Bullerman et al., 2008; Jackson et al., 2011). Extrusion cooking resulted in greater apparent loss of fumonisin B_1 (degradation product and/or binding not reported) with mixing screws than with non-mixing screws (Castelo et al., 1998).

No information has been identified by the CONTAM Panel on the effects on fumonisin levels of other stages in the chain for feed production. However, it should be underlined that the addition of sugar-rich ingredients, such as sugar beet pulp and molasses, may favour the formation of modified fumonisins due to Maillard-type reaction between the different forms and reducing sugars.

In food production, several studies have demonstrated that fumonisins are removed from corn during nixtamalisation by a combination of extraction and conversion to their hydrolysed forms (Voss et al., 2001; Palencia et al., 2003; Burns et al., 2008). However, the CONTAM Panel is not aware of these processes being applied to animal feed.

For many livestock, compound feeds represent part or all of the daily ration. One of the final stages in the compound feed manufacturing process is the production of feed pellets, which results in an increase in temperature of the feed. The extent of the temperature rise will depend on a number of factors, including the types of ingredients used in the formulation, the amount of moisture added and the equipment used, but pellets generally leave the die at temperatures ranging between 60°C and 95°C (Thomas et al., 1997). Fumonisin appears to be relatively stable at these temperatures (Bullerman et al., 2002) and therefore compound feed manufacturing is unlikely to affect concentrations in the finished product.

For many ruminant livestock, maize silage is an important component of the daily ration, and typically represents between 30 and 50% of the daily ration, although it may be fed up to approximately 80% of the diet, especially to beef cattle. Fumonisin degrading microorganisms have been isolated from silage (Camilo et al., 2000), but it is not known if this degradation is of any significance in reducing the fumonisin concentrations in maize silage.

3.3. Exposure assessment

3.3.1. Previously reported exposure assessments in animals

In 2005, EFSA published an Opinion on fumonisins as undesirable substances in animal feed (EFSA, 2005).

Subsequently, EFSA published 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).

In the 2014 Opinion, the highest level of exposure to fumonisins were for fattening chickens (broilers) (12.6 and 18.3 μg/kg bw per day for LB and UB, respectively, at the mean level) and for laying hens (11.1 and 16.1 μg/kg bw per day for LB and UB, respectively, at the mean level). However, the Opinion also noted exposure by dairy cows could reach similar levels (8.2 and 17.7 μg/kg bw per day for LB and UB, respectively, at the mean level) when fed maize silage-based diets. The lowest level of exposure 0.1 and 1.7 μg/kg bw per day for LB and UB, respectively, at the mean level) was estimated for horses. A more detailed comparison between estimates of exposure in this Scientific Opinion and EFSA 2014 (EFSA CONTAM Panel, 2014) is shown in Table 6.

The CONTAM Panel have not identified any other previously reported estimates of exposure by livestock.

3.3.2. Dietary exposure assessment for farm and companion animals

Two scenarios have been considered in estimating exposure for farm and companion animals. Scenario 1 represents the sum of fumonisins (FB_1, FB_2, FB_3), while Scenario 2 includes the sum of fumonisin and the hidden forms. Scenario 2 has been achieved by multiplying exposures derived in Scenario 1 by 1.6. This scenario does not include the modified forms, for which we have no data concerning both the occurrence or the toxicity.

For all species, P95 and mean exposures have been estimated based on the 95th percentile and the mean LB and UB concentrations, respectively. According to EFSA (2010b), caution is needed when calculating chronic exposure (95th percentile) where data on less than 60 samples are available, since the results may not be statistically robust. Therefore, in this Opinion, there are no acute exposure estimations where data on < 60 samples are available. Furthermore, EFSA (2010b) has indicated that estimates of chronic exposure based on data for < 10 samples are unreliable, and therefore, no data on less than 10 samples have been provided, these have not been used to estimate the mean LB and UB exposures.

For many livestock in Europe, feeds are supplied in the form of commercially produced species-specific blends or compound feeds, and where these data were available, mean exposures have been calculated using the concentrations reported and assumed intakes given in Appendix C, Table C.6.

For those livestock categories for which insufficient data on species-specific compound feeds were provided, the CONTAM Panel identified example diets and feed inclusion rates (see Appendix C for details), and used concentrations of fumonisins in individual feed materials to estimate P95 and mean exposure.

As reported in Appendix C, a wide range of feeds and feeding systems are used for livestock in Europe. It must be stressed that the feed intakes or diet compositions used in estimating exposures in this scientific opinion are not 'average' diets, nor are they an attempt to describe 'worst-case' scenarios. Rather, they are intended to provide an indication of likely exposure to fumonisins across a range of feeding systems in Europe.

For ruminants and horses, forages – fed either fresh or conserved - are essential dietary ingredients. The data submitted to EFSA confirm the presence of fumonisins in certain forages (Table C.3). Fresh grass and grass silage are important feeds for ruminants and horses, but since no information on the level of fumonisins in these feeds was available it has not been possible to estimate their contribution to the exposure. However, data have been provided to EFSA on levels of fumonisins and their hidden forms in grass hay, maize silage and cereal straws (see Appendix B), and these have been used to estimate exposure in those ruminant feeding systems where these are the main forages.

In the tables below, the dietary concentrations are presented on a dry matter basis (as μg/kg dry matter). However, these estimates have been converted to an as-fed (or fresh weight) basis in Tables 17 and 18 to bring the data in line with the NOAEL/LOAEL values identified in this Opinion.

3.3.2.1. Estimated exposure by farm and companion animals (cats and dogs) to fumonisins, and to the sum of fumonisins and the hidden form

Ruminants and horses

For high yielding dairy cows, fattening beef cattle and horses, sufficient data were available to allow exposure to be made from species-specific compound feeds. For these, forages are an important component of their diets, and therefore exposure has been estimated in which grass hay is the sole forage. In practice, this probably represents a minority of feeding conditions (except for horses) but insufficient data were available for the more common forages, e.g. grazed grass or silages (grass, arable or maize) to permit reliable estimates to be made.

Estimated P95 and mean exposures are given below for ruminants and horses to fumonisins (Table 12a) and the hidden forms (Table 12b).

Table 12a: Estimated P95 and mean exposure to the sum of fumonisins (imputed) for ruminants and horses derived from LB and UB concentrations in species-specific compound feeds

Animal species	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Estimates derived from LB and UB concentrations in species-specific compound feeds							
Dairy cows: high yielding	LB	136	53.8	2,815	1,114	4.33	1.71
	UB	341	125	7,057	2,579	10.9	3.97
Beef: fattening	LB	–(a)	66.6	–(a)	639	–(a)	1.60
	UB	–(a)	124	–(a)	1,188	–(a)	2.97
Horses	LB	21.7	21.7	195	196	0.43	0.43
	UB	223	203	2,011	1,826	4.47	4.06
Estimates derived from LB and UB concentrations in feed materials and their relative proportions in diets							
Dairy cows: maize silage-based diet	LB	1,783	368	48,875	10,043	74.9	15.5
	UB	1,894	507	51,710	13,845	79.6	21.3
Beef cattle: cereal-based diet	LB	754	172	7,543	1,716	18.9	4.29
	UB	964	337	9,639	3366	24.1	8.42
Beef cattle: maize silage-based diet	LB	597	120	3,939	793	13.1	2.64
	UB	674	233	4,452	1,537	14.8	5.12
Beef cattle: straw-based diet	LB	39.8	14.3	318	114	1.06	0.38
	UB	270	210	2,160	1,679	7.20	5.60
Lactating sheep(b)	LB	41.6	30.1	116	84.4	1.45	1.05
	UB	206	152	579	425	7.23	5.32
Lactating goats(b)	LB	20.8	20.9	71.0	71.0	1.18	1.18
	UB	187	187	638	638	10.6	10.6
Fattening goats(b)	LB	612	25.2	918	37.8	22.9	0.95
	UB	716	133	1,074	200	26.8	5.01

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.
(a): Insufficient samples available to estimate P95 exposure.
(b): Exposures assume that grass hay is the sole forage.

Table 12b: Estimated P95 and mean exposure to the sum of fumonisins and the hidden forms

Animal species	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw	
		P95	Mean	P95	Mean	P95	Mean
Estimates derived from LB and UB concentrations in species-specific compound feeds							
Dairy: high yielding	LB	218	86	4,504	1783	6.93	2.74
	UB	545	199	11,291	4126	17.37	6.35
Beef: fattening	LB	–(a)	107	–(a)	1023	–(a)	2.56
	UB	–(a)	198	–(a)	1901	–(a)	4.75
Horses	LB	34.8	34.8	312	313	0.70	0.70
	UB	358	325	3,218	2,921	7.15	6.49
Estimates derived from LB and UB concentrations in feed materials and their relative proportions in diets							
Dairy cows: maize silage-based diet	LB	2,853	589	77,879	16,068	120	24.7
	UB	3,031	811	82,736	22,153	127	34.1
Beef cattle: cereal-based diet	LB	1,207	275	12,069	2,746	30.2	6.87
	UB	1,542	539	15,422	5,386	38.6	13.5
Beef cattle: maize silage-based diet	LB	955	192	6,303	1,269	21.0	4.23
	UB	1,079	373	7,123	2,459	23.7	8.2

Animal species	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw	
		P95	Mean	P95	Mean	P95	Mean
Beef cattle: straw-based diet	LB	63.6	22.9	509	183	1.70	0.61
	UB	432	336	3,456	2,686	11.5	8.95
Lactating sheep[(b)]	LB	66.5	48.2	186	135	2.33	1.69
	UB	330	243	926	681	11.6	8.51
Lactating goats[(b)]	LB	33.3	33.4	113	113	1.89	1.89
	UB	300	300	1,022	1,020	17.0	17.0
Fattening goats[(b)]	LB	979	40.3	1,469	60.5	36.7	1.51
	UB	1,146	213	1,719	320	42.9	8.02

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.
(a): Insufficient samples were available to estimate P95 exposure.
(b): Exposures assume that grass hay is the sole forage.

Pigs and poultry

Estimates of P95 and mean exposures by pigs and poultry to fumonisins, and to the sum of fumonisins, and the hidden forms are given in Tables 13a and 13b, respectively. For pigs, these were derived from data for species-specific compound feeds; for poultry, insufficient data on species-specific compound feeds were available, and therefore, exposures have been estimated using example rations and concentrations in individual feed materials (see Appendix C Table C.1 for details of rations used).

Table 13a: Estimates of P95 and mean exposure to fumonisin for pigs and poultry derived from LB and UB concentrations

Animal species	LB/UB	Diet concentration µg/kg dry feed matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Pigs: Estimates derived from LB and UB concentrations in species-specific compound feeds							
Pigs: starter	LB	770	159	770	154	38.5	7.69
	UB	943	413	943	413	47.2	20.7
Pigs: growing and fattening	LB	568	164	1,705	492	17.0	4.92
	UB	756	321	2,267	963	22.7	9.63
Lactating sow	LB	_[(a)]	23.2	_[(a)]	139	_[(a)]	0.70
	UB	_[(a)]	70.2	_[(a)]	421	_[(a)]	2.11
Poultry: Estimates derived from LB and UB concentrations in feeds and their relative proportions in diets							
Fattening chickens[(a)]	LB	1,521	367	182	44.1	91.3	22.1
	UB	1,749	575	209	69.0	104	34.5
Laying hens[(a)]	LB	1,394	331	167	39.7	83.6	19.9
	UB	1,674	556	201	66.8	100	33.4
Fattening turkeys[(a)]	LB	72.3	58.4	28.9	23.3	2.41	1.95
	UB	384	273	154	109	12.8	9.09
Fattening ducks[(a)]	LB	78.8	77.8	11.0	10.9	3.68	3.63
	UB	452	310	63.4	43.5	21.1	14.5

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.
(a): Insufficient samples were available to estimate P95 exposure.

Table 13b: Estimates of P95 and mean exposure to fumonisins, and the hidden form for pigs and poultry derived from LB and UB concentrations

		Diet concentration µg/kg dry feed matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Pigs: Estimates derived from LB and UB concentrations in species-specific compound feeds							
Pigs: starter	LB	1,232	246	1,232	246	61.6	12.3
	UB	1,509	661	1,509	661	75.4	33.1
Pigs: growing and fattening	LB	909	263	2,727	788	27.3	7.88
	UB	1,209	514	3,627	1,541	36.3	15.4
Lactating sow	LB	_(b)	37.1	_(b)	223	_(b)	1.11
	UB	_(b)	112	_(b)	674	_(b)	3.37
Poultry: Estimates derived from LB and UB concentrations in feeds and their relative proportions in diets							
Fattening chickens(a)	LB	2,434	588	292	70.6	146	35.3
	UB	2,799	920	336	110	168	55.2
Laying hens(a)	LB	2,230	529	267.6	63.5	134	31.8
	UB	2,679	890	321	107	161	53.4
Fattening turkeys(a)	LB	116	93.3	46.3	37.3	3.86	3.11
	UB	615	436	246	174	20.5	14.5
Fattening ducks(a)	LB	126	124	17.6	17.4	5.88	5.80
	UB	724	497	101	69.6	33.8	23.2

bw: body weight; LB: lower bound; P95: 95_{th} percentile; UB: upper bound.
(a): Insufficient species-specific samples were provided to allow reliable estimates of exposure to be made, and therefore example diets have been used (see Appendix C).
(b): Insufficient samples were available to estimate P95 exposure.

Farmed fish (salmonids, carp), rabbits and mink

In the absence of reliable data on concentrations of fumonisin and their hidden forms in species-specific compound feeds, estimates of exposure were made by using example rations and concentrations in individual feed materials (see Appendix C, Table C.2 for details of rations used) and are reported in Tables 14a (fumonisins) and 14 (the sum of fumonisins and the hidden forms). Although NOAEL and NOAEL values have been identified for catfish and Nile tilapia, insufficient data on diet composition for these species were available to allow estimates of exposures to be calculated.

Table 14a: Estimated P95 and mean exposure to fumonisins for rabbits, farmed fish and mink derived from LB and UB concentrations in individual feed materials and their relative proportions in diets

Animal species	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Salmonids	LB	976	229	39.0	9.16	19.5	4.58
	UB	1,110	310	44.4	12.4	22.2	6.20
Carp	LB	421	121	9.26	2.66	9.26	2.66
	UB	803	370	17.7	8.15	17.7	8.15
Rabbits	LB	19.4	6.89	2.91	1.03	1.45	0.52
	UB	296	233	44.4	35.0	22.2	17.5
Mink	LB	241	58.3	18.1	4.37	8.73	2.11
	UB	260	84.1	19.5	6.31	9.43	3.05

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.

Table 14b: Estimated P95 and mean exposure to fumonisins and the hidden forms for rabbits, farmed fish and mink

Animal species	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Salmonids	LB	1,562	366	62.5	14.7	31.2	7.33
	UB	1,776	496	71.0	19.8	35.5	9.92
Carp	LB	673	193	14.8	4.25	14.8	4.25
	UB	1,284	592	28.2	13.0	28.2	13.0
Rabbits	LB	31.0	11.0	4.65	1.65	2.33	0.83
	UB	474	373	71.0	56.0	35.5	28.0
Mink	LB	385	93.2	28.9	6.99	14.0	3.38
	UB	416	135	31.2	10.1	15.1	4.88

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.

Companion animals (dogs and cats)

Few data on levels of fumonisins and their hidden forms in proprietary feeds for dogs and cats were available, and therefore exposure was estimated using example rations (see Appendix C for details) and concentrations of these toxins in individual feed materials. The exposures are reported in Table 15a and 15b for fumonisins and for the sum of fumonisins and the hidden forms, respectively.

Table 15a: Estimated P95 and mean exposure to fumonisins by companion animals (dogs and cats)

Companion animal	LB-UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Cats	LB	1,626	365	97.5	21.9	24.4	5.47
	UB	1,765	465	106	27.9	26.5	6.98
Dogs	LB	1,501	338	540	122	21.6	4.86
	UB	1,634	441	588	159	23.5	6.35

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.

Table 15b: Estimated P95 and mean exposure to fumonisins and the hidden forms by companion animals (dogs and cats)

Companion animal	LB/UB	Diet concentration µg/kg dry matter		Exposure µg/day		Exposure µg/kg bw per day	
		P95	Mean	P95	Mean	P95	Mean
Cats	LB	2,601	583	156	35.0	39.0	8.75
	UB	2,824	745	169	44.7	42.4	11.2
Dogs	LB	2,402	540	865	194	34.6	7.78
	UB	2,614	705	941	254	37.6	10.2

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.

3.3.2.2. Concluding remarks

The mean LB and UB exposures to fumonisins and the hidden forms for all species were 6.8/15.0 µg/kg bw per day, while the LB and UB for the 95th percentile were and 31.0/40.9, respectively. However, there was considerable variation in the estimated exposure by farmed livestock and companion animals. The lowest exposure to Fumonisins expressed as µg/kg bw per day, was for horses, both at the mean (LB = 0.70, UB = 6.49) and 95th percentile (LB = 0.70, UB = 7.15) levels. Overall, the highest estimated exposure was for poultry, and within this category the highest estimates were for fattening chickens (broilers), with LB and UB estimates of 35.3/55.2 and 146/168 µg/kg bw per day for chronic and P95 estimates, respectively. Estimated exposure for laying hens were only marginally lower.

For ruminants, the highest estimated exposure was for dairy cows on maize silage-based diets, and intensively reared beef cattle on cereal-based diets.

The estimates of exposure for cats and dogs are based on example diets provided by the Pet Food Manufacturers Association. Although these frequently include cereals and oilseed-based feeds, their diets – and those of farmed mink – may include products of animal origin. However, no data on levels of fumonisins in these feed materials of animal origin were available, and therefore no estimates of exposure from these feeds could have been calculated.

Overall, the differences between the different livestock categories were a reflection of the higher levels of fumonisins in cereals or maize silage and the levels of inclusions of these feeds in their diets.

As discussed above, estimates of exposure were previously published by EFSA (EFSA CONTAM Panel, 2014). A comparison of these with those estimated in this Opinion is given in Table 16.

Table 16: Comparison of estimates of exposure (μg/kg bw per day) reported in this Scientific Opinion and in EFSA CONTAM Panel (2014)

Animal species	LB/UB	This Opinion		EFSA CONTAM Panel (2014)	
		P95	Mean	P95	Mean
Dairy: high yielding	LB	4.33	1.71	–[(a)]	8.2
	UB	10.86	3.97	–[(a)]	17.7
Horses	LB	0.43	0.43	–[(a)]	0.0
	UB	4.47	4.06	–[(a)]	1.0
Beef cattle: cereal-based diet	LB	18.9	4.29	–[(a)]	0.6
	UB	24.1	8.42	–[(a)]	8.2
Lactating sheep	LB	1.45	1.05	14.5	2.7
	UB	7.23	5.32	16.2	4.0
Lactating goats	LB	1.18	1.18	33.3	6.3
	UB	10.6	10.6	37.2	9.1
Fattening goats	LB	22.9	0.95	15.8	3.0
	UB	26.8	5.01	17.7	4.3
Pigs: starter	LB	38.5	7.69	17.6	3.7
	UB	47.2	20.7	22.5	10.3
Pigs: growing and fattening	LB	17.0	4.92	–[(a)]	7.4
	UB	22.7	9.63	–[(a)]	11.1
Lactating sow	LB	–[(a)]	0.71	29.1	4.6
	UB	–[(a)]	2.11	32.1	11.9
Fattening chickens[(a)]	LB	91.3	22.1	67	12.6
	UB	104	34.5	74.6	18.3
Laying hens[(a)]	LB	83.6	19.9	58.9	11.1
	UB	100	33.4	65.6	16.1
Fattening turkeys[(a)]	LB	2.41	1.95	32.7	6.2
	UB	12.8	9.09	36.4	8.9
Fattening ducks[(a)]	LB	3.68	3.63	50.7	9.5
	UB	21.1	14.5	56.5	13.9
Rabbits	LB	1.45	0.52	40.7	7.7
	UB	22.2	17.5	45.4	11.2
Cats	LB	24.4	5.47	12.4	2.3
	UB	26.5	6.98	13.9	3.4
Dogs	LB	21.6	4.86	14.1	2.7
	UB	23.5	6.35	15.7	3.9

bw: body weight; LB: lower bound; P95: 95th percentile; UB: upper bound.
(a): Insufficient samples were available to estimate P95 exposure.

The CONTAM Panel noted the differences between the two assessments. In general, exposure estimates by EFSA CONTAM Panel (2014) were higher than for this Opinion (based on mean LB and UB levels for all species, at both the mean and P95 levels). There were no consistent differences between the two studies, although marked differences for individual species were observed at both LB and UB levels. However, a comparison of the database used in these two studies reveals large differences; in particular, the 2014 assessment was based on fewer feed samples, while in that database the differences between the LB and UB values were larger, particularly for the 95th percentile data.

3.4. Risk characterisation

There is limited knowledge on the effects of Fumonisins and their modified and hidden forms on farm and companion animals. Furthermore, there is no comprehensive database on feed consumption by livestock in the EU. It has therefore not been possible to fully assess the risks of FBs and its modified and hidden forms for farm and companion animal health. Risk characterisation of the modified forms of FBs was not performed as no data concerning their occurence and toxicity was available.

However, for a number of farm livestock and companion animal categories the chronic exposure of fumonisins (expressed as the sum of FB_1, FB_2 and FB_3) in feed could be estimated at the mean and 95th percentile concentrations in animal diets based on expected feed intakes and example diets. Exposure to the sum of fumonisins and hidden forms was calculated by applying a 1.6 multiplying factor as described in Section 3.2.2.2. These exposures to fumonisin and to the sum of fumonisin and their hidden forms have been compared with identified reference points (NOAELs and LOAELs, expressed as mg/kg feed) in farm and companion animals. The identified NOAELs or LOAELs for cattle, pigs, poultry, fish, rabbit and horses were used for risk characterisation. For cats, dogs and mink the health risk from the exposure to FBs could not be assessed as no NOAELs or LOAELs have been identified. For sheep and goats, a very limited data set indicate a sensitivity similar to cattle.

In Tables 17 and 18, exposure estimates (UB mean and 95th percentile) are presented together with NOAELs/LOAELs for the different farm and companion animal species. Exposure is expressed as a percentage of the NOAEL in the right-hand columns. When a NOAEL is lacking, the LOAEL is used instead but provides a less conservative basis for comparison with exposure. The estimates of exposure to FBs and the sum of FBs and their hidden forms are presented in Section 3.3.

Table 17: Comparison of estimated FBs exposure levels and NOAELs/LOAELs for different farm and companion animal species

Animal species	NOAEL (mg FBs/kg feed)	LOAEL (mg FBs/kg feed)	Estimated exposure (mg FBs/kg feed)[a]		Estimated exposure, % of NOAEL or LOAEL	
			P95 (UB)	Mean (UB)	P95 (UB)	Mean (UB)
Cattle[b]	31	–	1.57	0.11	5.01	0.35
Pig	1	5	0.83	0.36	83.0	36.3
Chicken	20	40	1.54	0.51	7.70	2.53
Turkeys[c]	20	–	0.34	0.24	1.69	1.20
Ducks[c]	8	32	0.40	0.27	4.98	3.41
Horses	8.8	44	0.20	0.18	2.23	2.03
Rabbit	–	5	0.26	0.20	5.20	4.10
Fish (carp)	–	10	0.71	0.33	7.07	3.26

bw: body weight; FB: fumonisin B; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; 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 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.

(c): The exposures for turkeys and ducks were calculated for fattening animals. whereas the LOAELs and NOAELs were obtained from younger birds.

For FBs alone, for **cattle** the highest calculated chronic exposure was used (Table 17), with the UB mean and UB 95th percentile being 0.35% and 5.01% of the identified NOAEL, respectively. This NOAEL was based on lymphocytes blastogenesis and biochemical alterations. The Panel concluded that the risk of adverse health effects of feed containing FBs was very low for cattle.

Sheep and goats are also considered resistant to fumonisins and thus the risk was also considered as very low.

For **poultry**, (chickens, fattening turkeys and ducks), the estimated exposures of FBs at the UB mean or the 95th percentile ranged from 1.2% to 7.7% of the NOAELs. The NOAELs were based on liver lipid and biochemical alterations for chickens, on zootechnical performances and organ lesions for fattening turkeys and on serum biochemistry indicative of liver damage for fattening ducks. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs was low for poultry.

For **horses**, the calculated chronic exposures at the UB mean and UB 95th percentile were 2.03 and 2.23% of the identified NOAEL, respectively. This NOAEL was based on neurological abnormalities and cardiovascular effects. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs was low for horses.

For **pigs**, the estimated exposures of FBs at the UB mean and 95th percentile were 36.3% and 83.0%, respectively, of the NOAEL. This NOAEL was based on lung alteration. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs was low for pigs exposed to mean levels but of potential concern for animals exposed to the 95th percentile.

For **rabbits**, only a LOAEL was available. The estimated exposures of FBs at the UB mean and 95th percentile were 4.1% and 5.2%, respectively, of the LOAEL. This LOAEL was based on decreased zootechnical performances and alteration of blood haematology and biochemistry. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs was low for rabbit.

For **fish,** LOAEL were available for carp, channel catfish and Nile tilapia, however exposure was only available for salmonid and carp, and therefore carp were used for risk characterisation. The estimated chronic exposures of carp to FBs at the UB mean and 95th percentile were 3.3% and 7.1% of the LOAEL, respectively. This LOAEL was based on reduced weight gain and neuronal apoptosis in the brain. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs was low for fish.

Table 18: Comparison of estimated FBs + hidden forms exposure levels and NOAELs/LOAELs for different farm and companion animal species

Animal species	NOAEL (mg toxins/kg feed)	LOAEL (mg toxin/kg feed)	Estimated exposure (mg toxin/kg feed)[a]		Estimated exposure, % of NOAEL or LOAEL	
			P95 (UB)	Mean (UB)	P95 (UB)	Mean (UB)
Cattle[b]	31	–	2.51	0.17	8.10	0.56
Pig	1	5	1.33	0.58	132.7	58.2
Chicken	20	40	2.46	0.81	12.3	4.01
Turkeys[c]	20		0.54	0.38	2.71	1.92
Ducks[c]	8	32	0.64	0.44	7.96	5.46
Horses	8.8	44	0.31	0.29	3.58	3.25
Rabbit	–	5	0.42	0.33	8.34	6.56
Fish (carp)	–	10	1.13	0.52	11.3	5.21

bw: body weight; FB: fumonisin B; NOAEL: no–observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; 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 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.

(c): The exposures for turkeys and ducks were calculated for fattening animals. whereas the LOAELs and NOAELs were obtained from younger birds.

Risk characterisation for FBs and their hidden forms (Table 18) was based on UB exposure. The estimated exposures were compared with the NOAELs/LOAELs identified for FBs, as hidden forms can be disrupted leading to FBs.

For $FB_{1–3}$ and their hidden forms, for **cattle** the highest calculated mean exposure was used, with the UB mean and UB 95th percentile were 0.56% and 8.1% of the identified NOAEL, respectively. The Panel concluded that the risk of adverse health effects of feed containing FBs and hidden forms was very low for cattle.

Sheep and goats are also considered resistant to fumonisins and thus the risk was also considered as very low.

For **poultry** (chickens, fattening turkeys and ducks), the estimated exposures to FBs and their hidden forms at the UB mean or the 95th percentile ranged between 1.9% and 12.3% of the NOAELs. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs and hidden forms was low for poultry.

For **horses** the calculated chronic exposures at the UB mean and UB 95th percentile were 3.3% and 3.9% of the identified NOAEL, respectively. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs and their hidden forms was low for horses.

For **pig**, the estimated exposures of FBs at the UB mean and the 95th percentile were 58% and 133%, respectively, of the NOAEL. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs and their hidden forms was low for starter pigs exposed to mean levels but of concern for animals exposed to the 95th percentile.

For **rabbits**, only a LOAEL was available. The estimated exposures of FBs and hidden forms at the UB mean and 95th percentile were 6.6% and 8.3%, respectively, of the LOAEL. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs and hidden forms was low for rabbit.

For **fish,** LOAEL were available for carp, channel catfish and Nile tilapia; however exposure was only available for salmonid and carp, and therefore carp were used for risk characterisation. The estimated chronic exposures of carp to FBs and their hidden forms at the UB mean and 95th percentile were 5.2% and 11% of the LOAEL, respectively. The Panel concluded that the estimated risk for chronic adverse health effects from feed containing FBs and their hidden forms was low for fish.

3.5. Uncertainty analysis

Sections 3.5.1–3.5.3 present in more detail the uncertainties affecting different parts of the risk assessment. It includes a qualitative assessment of whether each source of uncertainty leads to over/underestimation of the resulting risk. Table 19 lists the main sources of uncertainty identified by the Panel.

3.5.1. Uncertainty associated with analytical chemistry

- Fumonisins exhibit a strong interaction with matrix macroconstituents. Therefore, a matrix-dependent recovery has been often reported. Extraction yield is affected by the matrix composition and by the extraction parameters. Slight changes in the extraction protocol may lead to relevant changes in the final outcome.
- The determination of hidden forms through alkaline hydrolysis may likely include not only the release of non-covalently bound fumonisins from the matrix, but also to the cleavage of modified forms. Therefore, the occurrence of hidden fumonisins may lead to an overestimation.

3.5.2. Uncertainty associated with occurrence and exposure

The CONTAM Panel considered it important to estimate the occurrence and the animal exposure to the total concentration of fumonisins for which data were available (i.e. FB_1, FB_2, FB_3) through feed. However, estimating the occurrence and exposure with high number of left censored data leads to a high uncertainty.

An additional factor of 1.6 was applied to the occurrence data, taking into account the possible occurrence of hidden forms. This factor was derived from the literature, considering data obtained for maize. However, in this opinion, the 1.6 factor was applied to all feed categories. Although maize is the main contributor in animal diet, this can lead to an overestimation.

Occurrence

The amount of occurrence data submitted differs considerably depending on feed category and reporting data provider, with most of the samples (~ 70%) collected in only three Member States, mostly from northern Europe, and ~ 40% originating from one single Member State. There is therefore uncertainty on whether possible country-based differences in the levels of fumonisins in diverse feed commodities are well represented. More than 85% of the data available were on FB_1 and FB_2, whereas only 15% were on FB_3.

Another uncertainty regarding the occurrence data refers to the high number of left censored data (about 80%). Estimating the occurrence and exposure with a high number of left censored data can lead to an underestimation of the LB and an overestimation of the UB. Moreover, the total

concentration of fumonisins was calculated by summing up the analytical concentrations of FB_1, FB_2, FB_3 for each sample. This information was available for a small proportion of the analytical samples. Thus, the levels were estimated by using the mean concentration of the closest feed group available and therefore adding additional uncertainty.

Fumonisin occurrence is strongly related to climatic conditions, geographical area, and maize genotype. All these factors may affect not only the overall occurrence, but also the ratio between parent and hidden forms. Due to the lack of appropriate models, this should be considered as a factor of uncertainty.

The Panel noted that the occurrence data in the EFSA database, used in the exposure assessment, were mainly from Northern Europe, where occurrence is generally lower than southern Europe. This could lead to a potential underestimation of exposure.

Exposure

- In estimating exposure to fumonisins various assumptions have been made, particularly in respect of the types and amounts of feed consumed by livestock and companion animals, and this will contribute to the uncertainty associated with the estimates of exposure. The main areas of uncertainty/concern relate to the extent to which the feeds reported are representative of feeds used for livestock and companion animals in the EU, the composition of the diets assumed for each of the livestock species/companion animals, and the estimates of feed consumed (*possible over/underestimation*).
- Horses appear to be particularly susceptible to fumonisins. Although data on complementary feeds for horses were available, the lack of data on forages meant that a reliable estimate of exposure could not be made (*possible underestimation*).

Feed composition

- *Representativeness of feeds analysed:* As described above, there is a wide discrepancy in the geographical spread of samples reported (*possible over/underestimation*).
- *Feed data – concentrate feeds:* There were limited or no data available on some key ingredients, e.g. oilseed meals. The formulations therefore assume no exposure from these feeds (*possible underestimation*). Fumonisins occur mainly in maize (corn) and wheat and for these feeds there were sufficient sample with which to assess exposure, but there was a lack of data on the by-products of these feeds (*possible underestimation*).
- *Feed data – forages:* For ruminants and horses, forages are a major constituent of their diets. Although data on 888 samples of forages were reported in the category "Forages and roughages", these were not sufficiently characterised (e.g. as fresh, ensiled or dried grass, maize silage or legumes) to allow them to be used to assess exposure. However, levels of fumonisins this general category were higher than in the categories maize silage, grass hay and cereal straw that were used to estimate exposure (*possible underestimation*).
- *Diet formulations:* Single diet formulations have been assumed for each species, although there are large differences in feeding systems and diet formulations for livestock and companion animals in the EU (*possible over/underestimation*).

Feed intakes

- A single level of feed intake has been assumed for each livestock species/companion animal, but in practice this will vary for a given live weight or level of activity/productivity (*possible over/underestimation*).
- Single levels of production or activity have been assumed, but these can vary markedly resulting in greater or lesser amounts of feed required or consumed (*possible over/under estimation*).

3.5.3. Uncertainty on the studies used for evaluation of the adverse effect in farm and companion animals

- No toxicological data are available for farmed mink, cats and dogs; for other animals, such as goats and sheep, the toxicological data were too limited to allow the establishment of reference point for FBs
- There is scant information about the FBs adverse effects in ruminants and fish
- For fish, there is no data for salmonids which is the main aquaculture species in Europe. The only toxicological data were obtained for carp, Nile tilapia and channel catfish
- No studies involving the oral administration are available for horses

- No data were available on the effect of sex and age on the toxicity of FBs. For all the animal species taken into consideration, no data were available on the possible difference of the different breeds. This contributed to the overall uncertainty.
- The factor of 1.6 in order to include occurrence of hidden forms might not be appropriate for all species, the enteric hydrolysis being possibly subjected to interspecies variation
- Concerning the modified forms of FBs, the toxicological data were either lacking or very limited. For the different animal species, it was not possible to identify any reference point for any modified form of FBs.
- For most animal species, the key studies were performed with naturally contaminated maize for which the level of FB_3 and other mycotoxin was not reported.

3.5.4. Summary of uncertainties

In Table 19, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the source of uncertainty leads to over/underestimation of the resulting risk.

Table 19: Summary of the qualitative evaluation of the impact of uncertainties on the assessment

Sources of uncertainty	Direction[(a)]
Extraction yield is affected by the matrix composition and by the extraction parameters. Small changes may have strong effects	–
Use of alkaline hydrolysis for hidden fumonisins determination	+
Extrapolation of the occurrence data mainly from Northern Europe to the whole of Europe	–
No occurrence data for modified forms in EFSA database	–
The number of samples were not equally distributed across all feed groups	+/–
Large proportion of left censored data in the final data set	+/–
Using the substitution method at the lower bound (LB)	–
Using the substitution method at the upper bound (UB)	+
Imputation of missing results for the calculation of the sum of fumonisins	+/–
Application of 1.6 factor derived from maize to all feed components	+
Applicability of the 1.6 to account for hidden forms to different animal species with different metabolism	+/–
No toxicological/no robust data for some animal species	+/–
Toxicity data with naturally contaminated material (usually containing other mycotoxins)	+/–
No data on salmonid, extrapolation from other fish species	+/–
No data on differences between ages, sexes and breed	+/–
The number of samples were not equally distributed across all feed groups	+/–
Effect of variation between countries, between sampling methods and over time, and uncertainty about moisture content, on extrapolation from occurrence data to 95th percentile for the EU	+/–
High variability of feedstuffs used and feeding systems for livestock	+/–
Example animal diets used to calculate animal exposure	+/–

(a): + = uncertainty with potential to cause overestimation of exposure/risk; – = uncertainty with potential to cause underestimation of exposure/risk, +/– = extent of potential over/underestimation might differ in direction.

The CONTAM Panel noted that the FBs modified forms were not considered due to very limited occurrence and toxicity data.

The impact of the uncertainties in the risk assessment of farm and companion animals is large.

4. Conclusions

Fumonisins are mycotoxins produced predominantly by *F. verticillioides* and *F. proliferatum.*

In terms of chemical structure, fumonisins are long-chain aminopolyols with two TCA side chains. The most relevant compounds are the B-type fumonisins $FB_{1–3}$ which differ in the number and position of hydroxy-groups at the backbone. The most relevant modified forms are HFBs and pHFBs. Fumonisins may react during food processing, giving rise to the formation of Maillard-type modified forms, such as NCM-FBs and NDF-FBs.

Due to the chemical structure, fumonisins may strongly interact through non covalent binding with the matrix macroconstituents, giving rise to the so-called hidden fumonisins. Hidden forms may be disrupted released upon digestion, contributing to the total amount of leading to the release of the unchanged parent forms of fumonisins in the gastrointestinal tract.

Methods of analysis

Analytical methods for $FB_{1–3}$ are well established and are mainly based on MS. Modified forms of FB_1 are commonly analysed under the same conditions as their parent compound. However, the strong physical interaction of fumonisins with the feed matrix, which is well documented in the literature, may significantly affect the analytical performance in a matrix-related way. For the determination of hidden fumonisins, the food/feed matrix is usually treated under alkaline conditions prior to the analysis.

Only $FB_{1–3}$ are available on the market as calibrant solutions. Except for HFB_1, analytical standards for modified forms are not commercially available.

Hazard identification and characterisation

Toxicokinetics in farm and companion animals

Fumonisins

- There is poor information on $FB_{1–3}$ ADME in farm animal species and the available studies are almost limited to FB_1.
- In orally exposed animals, $FB_{1–3}$ are in general poorly bioavailable, rapidly distributed mainly to liver and kidney, extensively biotransformed and rapidly excreted mostly via the faecal route.
- Hydrolytic biotransformations largely prevail; the main metabolites are $pHFB_1$ and HFB_1; both may be found in limited amounts in tissues.
- Unlike in rats, no further metabolites (e.g. *N*-acyl derivatives of FB_1 and its hydrolysed forms) have been isolated in farm and companion animals.
- A very limited excretion of fumonisins in milk and a negligible excretion in eggs have been documented.
- No information on $FB_{1–3}$ kinetics could be identified for farmed rabbits, fish, horses, farmed mink, dogs and cats.

Ruminants

- The scant information available indicates poor oral bioavailability and an extensive biotransformation to the hydrolysed $pHFB_1$ and HFB_1.
- Hydrolytic biotransformations appear not to occur in rumen or liver.
- Milk excretion has been investigated and documented in cows only.

Pigs

- In pigs, $FB_{1–3}$ are poorly bioavailable but extensively hydrolysed to $pHFB_1$ and HFB_1 in the enteric tract. The bioavailability of FB_2 is likely to be much lower than that of FB_1.
- Measurable amounts of the toxin and of both hydrolysed metabolites are present in liver and kidneys up to several days after treatment cessation.
- The faecal excretion largely outweighs the urinary one; the extent of biliary excretion might vary according to the dose and the duration of the exposure.

Poultry

- There is very limited knowledge on FB kinetics in avian species, with no information on FB_1 biotransformations.
- Oral bioavailability is poor and in the order turkey>duck>chicken.
- Kinetic studies point to a more rapid elimination in ducks and chickens than in turkeys.
- In birds fed with feed at, or approaching the EU recommended guidance level, residues were detected only in liver.
- The kinetics of FB_2 in ducks and turkeys is similar to that of FB_1, with evidence of a lower bioavailability.

Mode of action

- FBs are structural analogues of sphingoid bases and they inhibit ceramide synthase. This induces a disruption of sphingolipid metabolism and pathological changes.

- Even if the disruption of the sphingolipid metabolism at an early stage is closely related with fumonisin toxicity, there is no evidence that fumonisin-induced ceramide synthase inhibition is in itself an adverse effect. Therefore, reference points for fumonisins have been derived using endpoints other than the sole alteration of sphingolipid ratio in serum or organs.
- The implication of the disruption of sphingolipid metabolism in some of the observed critical adverse effects still remains to be established.
- At the cellular level FB_1, FB_2 and FB_3 have the same mode of action and are considered as having similar toxicological profiles and potencies.

Adverse effects in farm and companion animals

Ruminants

- Based on a limited data set, ruminants are considered less sensitive than horses and pigs.
- Gross and histopathological lesions, as well as changes in serum enzymes and biochemistry indicate an impairment of liver and possibly kidney function
- A NOAEL (31 mg $FB_{1–3}$/kg feed) was identified only for cattle based on the increase in serum enzymes, cholesterol and bilirubin as well as the decrease in lymphocyte blastogenesis.
- Sheep and goats would not seem to be more susceptible to fumonisins than cattle.

Pigs

- Porcine pulmonary oedema syndrome is the specific effect produced by FB in pigs and cardiovascular toxic effects of FBs could play a role in the development of this abnormality.
- Increased 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 was reported in many studies.
- A NOAEL of 1 mg FB_1/kg feed and a LOAEL of 5 mg/kg feed based on lung lesions after 8 weeks feeding of FB_1 were identified.

Poultry

- Fumonisins affect the liver, feed intake and the immune system in poultry species. A decreased feed intake and body weight gain were reported from feeding studies with ducks and Japanese quail, but not from studies with chickens and turkeys.
- Increased Sa and Sa/So levels in both tissues and serum have also been reported from low feed concentrations in investigated poultry species.
- A NOAEL of 8 mg/kg feed based on alterations of liver enzymes indicative of liver toxicity was identified for ducks.
- A NOAEL of 20 mg/kg feed was identified for chickens on the basis of an increase in liver lipids. This was considered as an adverse effect taking the observed liver toxicity in all investigated species into consideration.
- A NOAEL of 20 mg/kg feed was also identified for turkeys, the highest dose tested.

Horses

- A NOAEL of 0.2 mg FB_1/kg bw per day, recalculated from an i.v study, (corresponding to 8.8 mg FB_1 kg/feed) was estimated for horses, based on neurological and cardiovascular effects. This NOAEL was supported by field studies.

Rabbit

- Decreased performance, alterations in serum biochemistry and blood composition, liver and kidney congestion, impaired spermatogenesis and delay of the onset of puberty, as well as increased Sa level and the Sa/So ratio in urine, serum and liver were associated with the exposure to FBs.
- A LOAEL of 5 mg FB_1/kg feed was identified based on alterations in liver.

Fish

- There is limited information available from feeding studies with fish. There is no information available on the effects of fumonisins on salmonids.

- Observed effects of fumonisins in fish species includes pathological damages in several organs, reduced body weight gain and haematological and immunological alterations.
- A NOAEL of 10 mg FB_1/kg feed was identified for Nile tilapia based on reduced weight gain.
- A LOAEL of 10 mg FB_1/kg feed was identified for carp, based on pathological alterations, alterations of haematological parameters and reduced body weight gain.
- A NOAEL of 20 mg FB_1/kg feed was identified for catfish. This was based on reduced body weight gain and microscopic liver lesions.

Companion animals

- No data could be identified concerning the effects of FBs in cats and dogs.

Farmed mink

- No data could be identified concerning the effects of FBs in farmed mink.

Adverse effects and identification of reference points for risk characterisation in farm and companion animals for modified forms of fumonisins

- No data were available to set up reference points for any modified form of fumonisins.

Occurrence and exposure

- The dietary exposure was estimated using a final data set of 18,140 feed samples on fumonisins (i.e. FB_1, FB_2 and FB_3) representing most of the feed commodities with potential presence of fumonisins.
- Samples were collected between 2003 and 2016 in 19 different European countries, but most of them from four Member States.
- The total concentration of FBs was estimated by summing available concentrations for each sample. For samples for which no concentration was available, the levels were imputed by using the mean concentration of available data.
- The percentage of left-censored data reported (results below limit of detection and/or limit of quantification) was high (~ 80%). The highest number of reported analytical results corresponded to the feed group 'Cereal grains' (~ 47%) and in particular to maize, wheat and barley. Other represented feed groups included forages, animal products, legume seeds, minerals, oil seeds, and tubers.
- High quantified values were reported for maize, wheat and compound feed. The compound feeds with highest levels were for unspecified species and were therefore not used for the exposure assessment.
- The animal exposure was presented as dietary concentrations because the animal risk assessment was carried out on a feed concentration basis.
- Exposure to FBs and the hidden forms is primarily from the consumption of maize (corn), and its by-products. Except for forage maize, and maize silage produced from it, levels on forages are generally low.
- The highest estimated dietary concentrations to FBs by cattle was for lactating dairy cows on a maize silage-based diet (mean LB = 368 and 95th percentile UB = 1,894 μg/kg feed), reflecting both the high levels of FBs in forage maize and the inclusion of cereal grains in the complementary compound feeds.
- For other cattle, the lowest overall dietary concentration was for beef cattle on a straw-based ration (LB mean = 14, UB P95 = 270 μg/kg feed).
- For sheep and goats, the calculated lowest LB to highest UB mean dietary concentrations of FBs were 25 and 187 μg/kg feed, respectively, while at the 95th percentile the range was from 42 (LB) to 716 (UB) μg/kg feed.
- For horses, the calculated mean LB and UB diet concentrations of FBs were 22 and 203 μg/kg feed, respectively, while for the 95th percentile the range (LB to UB) was 22 to 223 μg/kg feed.
- The calculated mean LB and UB exposures to FBs by pigs, derived from data for species-specific compound feeds, ranged from 23 to 417 μg/kg feed, respectively, while the 95th percentile exposures ranged from 568 (LB) to 943 (UB) μg/kg feed.
- For poultry, the calculated mean exposure ranged from 58 (LB) to 575 (UB) μg/kg feed, based on levels in individual feeds and their inclusion in diets. The equivalent range for the 95th percentile estimates of exposure was 72 and 1,749 μg/kg feed, respectively.

- For farmed salmonids and carp, the calculated mean LB and UB for dietary concentrations ranged from 121 to 370 μg/kg feed, respectively. At the 95th percentile, LB and UB estimates dietary concentrations ranged from 421 (LB) to 1,110 (UB) μg/kg feed.
- The calculated mean diet concentration for farmed rabbits ranged from 7.0 (LB) to 233 (UB) μg/kg DM, while the equivalent range for the 95th percentile was from 20 to 296 μg/kg DM.
- The mean calculated diet concentration for farmed mink ranged from 58 (LB) to 84 (UB) μg/kg DM, while the equivalent range for the 95th percentile was 241 and 260 μg/kg DM.
- For companion animals (cats and dogs), the calculated LB and UB mean diet concentrations of FBs were 365 and 465 μg/kg DM, respectively while at the 95th percentile the range was from 1,501 (LB) to 1,765 (UB) μg/kg feed.
- Fumonisins hidden forms are assumed to be 60% of the dietary concentrations for FBs. The sum of FBs plus the hidden forms may be calculated by multiplying the values given above (for FBs) by 1.6.

Farm and companion animal health risk characterisation

- The risk characterisation of exposure to fumonisins is evaluated taking into consideration the comparison between the exposure of the sum of FB_1, FB_2 and FB_3, and the identified NOAELs/LOAELs for chronic adverse effects.
- The risk characterisation of exposure to FBs and their hidden forms is evaluated based on the comparison between the exposure of FBs and their hidden forms (exposure to FBs multiplied by a factor of 1.6), and the identified NOAELs/LOAELs for chronic adverse effects of FBs.
- For dogs, cats and mink, the health risk from the exposure to FBs and to FBs and their hidden forms could not be assessed as no NOAEL or LOAEL have been identified.
- For cattle, the risk of adverse health effect of feed containing FBs was considered very low. It is expected that sheep and goat have similar sensitivity to FBs as cattle and the risk was considered very low also for those species.
- For poultry, horse, rabbits and fish, the risk of adverse health effect of feed containing FBs was considered low.
- For pigs, the risk of adverse health effect of feed containing FBs was considered low for pigs exposed to mean levels but of potential concern for animals exposed to the 95th percentile.
- The same conclusions apply to the sum of FBs and their hidden forms except for pigs for which the risk of adverse health effect of feed containing FBs was considered low for pigs exposed to mean levels and of concern for animals exposed to the 95th percentile.

5. Recommendations

- More studies are needed to reach a consensus method for the analytical determination of hidden fumonisins under routine conditions.
- Occurrence data using analytical methods with lower LOQs are needed.
- More information on occurrence of FB_{2-3} and modified forms in feed are needed.
- More data on the occurrence of hidden forms of FBs are needed in order to refine the exposure estimates.
- More information is needed on ADME of FBs and their modified forms especially for horses, farmed rabbits, farmed mink, fish and companion animals.
- More information on the adverse effects of FBs in farm and companion animals are needed especially for horse, salmonids, cats and dogs.
- Studies on the adverse effects of modified forms of FBs, especially hydrolysed and *N*-acyl derivatives, are needed in all farm and companion animals.

Documentation provided to EFSA

Data on fumonisins occurrence (specifically to evaluate the impact of the hidden fumonisins in the total fumonisins) used for the modelling in Appendix D were submitted to EFSA by:

- Bryła, M (Department of Food Analysis Prof. Waclaw Dabrowski Institute of Agricultural, Warsaw, Poland) on 17 July 2017.
- Mallmann, CA (Universidade Federal de Santa Myaria, Laboratório de Análises Micotoxicológicas – LAMIC Santa Maria, Brasil) on 11 October 2017.

- Dall'Asta, C (Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli studi di Parma, Italy) on 1 February 2018.

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Abbreviations

AChE	acetylcholinesterase
ADME	absorption, distribution, metabolism and excretion
AFB_1	aflatoxin B1
AFRC	Agricultural and Food Research Council
AKLP or ALP	alkaline phosphatase
ALT	alanine aminotransferase
AOAC	Association of Analytical Chemists
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATP	adenosine triphosphate

AUC	area under the curve
BALF	bronchoalveolar lavage fluid
BUN	blood urea nitrogen
bw	body weight
Ca	calcium
CerS	ceramide synthases
CI	confidence interval
Chol	total cholesterol
CONTAM	EFSA Panel on Contaminants in the Food Chain
CYP	cytochrome P450
DATA Unit	EFSA Evidence Management Unit
DM	dry matter
DON	deoxynivalenol
DWG	daily weight gain
ELEM	equine leucoencephalomalacia
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionisation
ETEC	enterotoxigenic *E. coli*
FA	fatty acid
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FBs	fumonisins of the B type
FCR	feed conversion ratio
FEDIAF	European Pet Food Industry Federation
FSA	Food Standards Agency
FWC	Framework Contract
GC	gas chromatography
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GIT	gastrointestinal tract
GM	geometric mean
GOT	glutamic-oxaloacetic transaminase
GST	glutathione *S*-transferase
Hb	haemoglobin concentration
HBGV	health-based guidance value
HFB	hydrolysed fumonisin B
HPLC	high-performance liquid chromatography
HPLC-FLD	high-performance liquid chromatography coupled with fluorescence detection
HRMS	high-resolution mass spectrometry
IAC	immunoaffinity chromatography
IARC	International Agency for Research on Cancer
IFN	interferon
Ig	immunoglobulin
IL	interleukin
i.p.	intraperitoneal
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
JECFA	Joint FAO/WHO Committee on Food Additives
LB	lower bound
LC	liquid chromatography/left-censored
LC–MS/MS	LC coupled to tandem mass spectrometry
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LOD	limit of detection
LOQ	limit of quantification
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration

MCV	mean cell volume
ML	maximum level
mRNA	messenger Ribonucleic Acid
MRM	multiple reaction monitoring
MRT	mean residence time
MS	mass spectrometry
MW	molecular weight
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-Operation and Development
OPA	*o*-phthaldialdehyde
PCV	packed cell variable
pHFB	partially hydrolysed fumonisin B
RBC	red blood cell
RPF	relative potency factor
Sa/So	sphinganine-to-sphingosine ratio
SAChE	specific acetylcholinesterase
SCF	Scientific Committee on Food
SD	standard deviation
SOP	Standard Operating Procedure
$t_{1/2el}$	elimination half-life
T_{max}	time to maximal plasma concentration
TCA	tricarballylic acid
TDI	tolerable daily intake
TK	toxicokinetics
TLC	thin-layer chromatography
TNF	tumour necrosis factor
Tri	triglycerides
UB	upper bound
UV	ultraviolet
Vd	volume of distribution
WBC	white blood cells
WG	working group
WHO	World Health Organization
ZEN	zearalenone

Appendix A – EFSA guidance documents applied for the risk assessment

- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 31 pp. https://doi.org/10.2903/j.efsa.2005.282
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Appendix B – Occurrence data received by EFSA

Table B.1: LOD and LOQ of the concentrations (micrograms/kg) of fumonisins in feed samples

Feed category	Fumonisin	LOD			LOQ		
		Mean	Min	Max	Mean	Min	Max
Cereal grains, their products and by-products	FB_1	56	0	300	106	0.03	1,000
	FB_2	59	0	300	115	0.3	1,000
	FB_3	52	0	100	49	10	50
Compound feed	FB_1	37	0.07	1,000	56	0.03	1,000
	FB_2	58	0.07	1,000	63	0.3	1,000
	FB_3	25	25	25	50	50	50
Forages and roughage, and products derived thereof	FB_1	100	20	300	48	2	1,000
	FB_2	100	20	300	50	3	1,000
	FB_3	100	100	100	.	.	.
Land animal products and products derived thereof	FB_1	.	.	.	10	10	10
	FB_2	.	.	.	20	20	20
Legume seeds and products derived thereof	FB_1	92	20	100	20	10	50
	FB_2	97	30	100	28	20	50
	FB_3	100	100	100	.	.	.
Minerals and products derived thereof	FB_1	68	20	100	50	50	50
	FB_2	88	50	100	100	100	100
	FB_3	100	100	100	.	.	.
Miscellaneous	FB_1	107	20	300	525	50	1,000
	FB_2	106	30	300	448	50	1,000
	FB_3	100	100	100	.	.	.
Oil seeds, oil fruits, and products derived thereof	FB_1	102	0	300	94	0.03	1,000
	FB_2	102	0	300	94	3	1,000
	FB_3	99	0	100	19	10	50
Other seeds and fruits, and products derived thereof	FB_1	99	50	100	10	10	10
	FB_2	100	100	100	20	20	20
	FB_3	100	100	100	.	.	.
Tubers, roots, and products derived thereof	FB_1	103	100	300	339	7	1,000
	FB_2	103	100	300	343	8	1,000
	FB_3	100	100	100	.	.	.

LOD: limit of detection; LOQ: limit of quantification.

Table B.2: Statistical description of the concentrations (μg/kg dry matter)[(a),(b)] of fumonisins in feed samples classified according to the Catalogue of feed materials specified in Commission Regulation (EU) No 68/2013[(c)]

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
Cereal grains, their products and by-products	Barley	Barley, unspecified	FB_1	266	74	12.7	65.5	0.0	44.0	53.0	142.0
			FB_2	264	83	9.0	66.6	0.0	50.0	20.3	101.4
			FB_3	131	99	0.8	64.4	0.0	50.0	0.0	100.0
		Barley middlings	FB_1	3	100	0.0	33.3	0.0	25.0	–	–
			FB_2	3	67	40.0	73.3	0.0	50.0	–	–
			FB_3	2	100	0.0	50.0	0.0	50.0	–	–
		Barley protein feed	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
		Malt rootlets	FB_1	7	29	6.4	13.5	2.0	10.1	–	–
			FB_2	7	29	9.6	23.8	3.0	20.3	–	–
			FB_3	2	100	0.0	50.0	0.0	50.0	–	–
	Buckwheat	Buckwheat, unspecified	FB_1	4	100	0.0	48.9	0.0	48.9	–	–
			FB_2	4	100	0.0	48.9	0.0	48.9	–	–
			FB_3	4	100	0.0	48.9	0.0	48.9	–	–
	Cereal grains, their products and by-products, unspecified	Cereal grains, their products and by-products, unspecified	FB_1	84	62	347.4	367.9	0.0	26.5	826.0	826.0
			FB_2	83	86	64.7	100.0	0.0	50.0	160.0	160.0
			FB_3	37	95	3.5	57.5	0.0	50.0	–	–
	Grains as crops	Grains as crops	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
	Maize	Maize bran	FB_1	2	50	1,400.5	1,450.5	1,400.5	1,450.5	–	–
			FB_2	2	50	293.5	343.5	293.5	343.5	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
		Maize fibre	FB_1	5	0.00	444.6	444.6	100.0	100.0	–	–
			FB_2	5	80	20.0	60.0	0.0	50.0	–	–
			FB_3	4	100	0.0	50.0	0.0	50.0	–	–
		Maize flakes	FB_1	7	43	907.5	924.7	33.9	76.5	–	–
			FB_2	7	86	64.0	92.5	0.0	32.8	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
		Maize germ	FB_1	4	0.00	899.7	899.7	614.4	614.4	–	–
			FB_2	2	0.00	121.6	121.6	121.6	121.6	–	–
		Maize germ expeller	FB_1	3	67	40.0	73.3	0.0	50.0	–	–
			FB_2	3	100	0.0	66.7	0.0	50.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
		Maize germ meal	FB_1	4	25	159.5	172.0	160.0	160.0	–	–
			FB_2	4	25	52.8	65.3	55.5	55.5	–	–
			FB_3	1	100	0.0	50.0	0.0	50.0	–	–
		Maize gluten	FB_1	3	0.00	2,037.7	2,037.7	2,678.3	2,678.3	–	–
			FB_2	1	0.00	126.8	126.8	126.8	126.8	–	–
		Maize gluten feed	FB_1	110	14	1,078.1	1,090.3	271.5	271.5	5,465.8	5,465.8
			FB_2	108	31	378.5	406.1	164.0	164.0	1,700.0	1,700.0
			FB_3	36	61	129.4	189.2	0.0	100.0	–	–
		Maize middlings	FB_1	9	22	270.2	275.2	52.3	52.3	–	–
			FB_2	9	56	115.0	160.9	0.0	56.6	–	–
		Maize screenings	FB_1	1	100	0.0	21.9	0.0	21.9	–	–
			FB_2	1	100	0.0	21.9	0.0	21.9	–	–
		Maize, unspecified	FB_1	1,978	54	496.7	549.8	0.0	100.0	2,600.0	2,600.0
			FB_2	1,941	70	165.8	229.3	0.0	88.0	841.7	861.5
			FB_3	399	84	44.2	119.1	0.0	100.0	260.0	260.0
		Sweet corn silage	FB_1	2	100	0.0	54.7	0.0	54.7	–	–
			FB_2	2	100	0.0	49.2	0.0	49.2	–	–
	Millet	Millet	FB_1	14	79	19.5	80.5	0.0	100.0	–	–
			FB_2	14	100	0.0	75.3	0.0	76.1	–	–
			FB_3	13	100	0.0	73.4	0.0	52.2	–	–
	Mixed grains	Brewers' grains	FB_1	18	83	83.3	158.3	0.0	100.0	–	–
			FB_2	18	83	51.1	128.9	0.0	100.0	–	–
			FB_3	16	88	26.3	107.5	0.0	100.0	–	–
		Distillers' dark grains; [Distillers' dried grains and solubles]	FB_1	27	11	421.9	424.7	210.0	210.0	–	–
			FB_2	27	41	105.9	126.2	64.0	64.0	–	–
			FB_3	19	84	9.8	52.0	0.0	50.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
		Distillers' dried grains	FB_1	2	50	524.5	674.5	524.5	674.5	–	–
			FB_2	2	50	177.2	327.2	177.2	327.2	–	–
		Grain flour	FB_1	1	0.00	141.5	141.5	141.5	141.5	–	–
			FB_2	1	0.00	59.9	59.9	59.9	59.9	–	–
		Mixed grains, unspecified	FB_1	31	94	17.9	50.8	0.0	10.0	–	–
			FB_2	31	97	0.7	36.8	0.0	10.0	–	–
			FB_3	7	100	0.0	100.0	0.0	100.0	–	–
	Oats	Oat feed	FB_1	61	100	0.0	15.0	0.0	15.0	0.0	15.0
			FB_2	61	100	0.0	15.0	0.0	15.0	0.0	15.0
		Oat groats (Feed)	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
		Oats, unspecified	FB_1	78	67	15.7	61.9	0.0	44.0	90.0	100.0
			FB_2	78	74	7.8	58.2	0.0	50.0	20.0	100.0
			FB_3	48	100	0.0	70.9	0.0	50.0	–	–
	Rice, broken	Rice bran	FB_1	6	83	4.2	79.2	0.0	100.0	–	–
			FB_2	6	83	1.7	76.7	0.0	100.0	–	–
			FB_3	4	100	0.0	100.0	0.0	100.0	–	–
		Rice middlings	FB_1	2	100	0.0	25.0	0.0	25.0	–	–
			FB_2	2	100	0.0	50.0	0.0	50.0	–	–
			FB_3	2	100	0.0	50.0	0.0	50.0	–	–
		Rice, broken, unspecified	FB_1	196	99	0.5	45.8	0.0	44.1	0.0	44.1
			FB_2	196	100	0.0	45.5	0.0	44.1	0.0	44.1
			FB_3	196	100	0.0	45.5	0.0	44.1	0.0	44.1
		Rice, milled	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
	Rye	Rye, unspecified	FB_1	25	88	0.9	52.6	0.0	50.0	–	–
			FB_2	25	84	7.1	51.1	0.0	50.0	–	–
			FB_3	18	100	0.0	52.8	0.0	50.0	–	–
		Rye middlings	FB_1	2	50	22.5	72.5	22.5	72.5	–	–
			FB_2	2	100	0.0	75.0	0.0	75.0	–	–
			FB_3	2	100	0.0	75.0	0.0	75.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
	Sorghum; [Milo]	Sorghum; [Milo]	FB_1	15	100	20.3	95.9	0.0	100.0	–	–
			FB_2	15	100	7.3	95.9	0.0	100.0	–	–
			FB_3	12	100	0.0	95.8	0.0	100.0	–	–
	Spelt	Spelt	FB_1	19	47	66.9	82.7	10.2	25.0	–	–
			FB_2	19	84	3.2	48.0	0.0	50.0	–	–
			FB_3	15	100	0.0	53.3	0.0	50.0	–	–
	Triticale	Triticale	FB_1	35	54	20.8	67.5	0.0	83.0	–	–
			FB_2	35	0.60	10.8	59.3	0.0	50.0	–	–
			FB_3	13	100	0.0	80.8	0.0	100.0	–	–
	Wheat	Vital wheat gluten[(d)]	FB_1	2	0.00	2,482.5	2,482.5	2,482.5	2,482.5	–	–
			FB_2	2	0.00	1,417.0	1,417.0	1,417.0	1,417.0	–	–
		Wheat, unspecified	FB_1	347	65	76.2	116.6	0.0	34.0	100.0	100.9
			FB_2	347	79	66.3	117.1	0.0	50.0	30.0	100.9
			FB_3	158	99	0.4	67.8	0.0	50.0	0.0	100.0
		Wheat bran (Feed)	FB_1	164	95	122.9	171.2	0.0	50.0	2.0	50.0
			FB_2	166	96	120.7	171.1	0.0	50.0	0.0	50.0
			FB_3	11	100	0.0	59.1	0.0	50.0	–	–
		Wheat feed	FB_1	109	93	7.9	56.5	0.0	50.0	30.0	100.0
			FB_2	109	95	3.1	54.2	0.0	50.0	0.0	100.0
			FB_3	10	100	0.0	75.0	0.0	75.0	–	–
		Wheat germ (Feed)	FB_1	2	100	0.0	62.5	0.0	62.5	–	–
			FB_2	2	100	0.0	75.0	0.0	75.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
		Wheat gluten feed	FB_1	7	57	26.0	61.3	0.0	58.0	–	–
			FB_2	7	100	0.0	61.7	0.0	50.0	–	–
			FB_3	4	75	22.0	59.5	0.0	50.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
		Wheat middlings	FB_1	21	95	4.8	89.6	0.0	100.0	–	–
			FB_2	21	95	4.8	93.2	0.0	100.0	–	–
			FB_3	11	100	0.0	86.4	0.0	100.0	–	–
		Wheat starch containing protein, partially de-sugared	FB_1	1	100	0.0	25.0	0.0	25.0	–	–
			FB_2	1	100	0.0	25.0	0.0	25.0	–	–
			FB_3	1	100	0.0	50.0	0.0	50.0	–	–
Compound feed	Complementary/ Complete feed	Breeding pigs	FB_1	32	66	15.1	34.7	0.0	10.0	–	–
			FB_2	32	75	5.3	27.1	0.0	10.0	–	–
		Calves	FB_1	15	67	81.7	110.5	0.0	50.0	–	–
			FB_2	15	87	7.8	47.2	0.0	50.0	–	–
		Complementary feed (incomplete diet)	FB_1	139	28	314.7	323.9	57.0	58.0	1,179.6	1,179.6
			FB_2	139	94	53.3	101.7	0.0	50.0	230.0	300.0
			FB_3	121	99	0.5	50.1	0.0	50.0	0.0	50.0
		Complete feed	FB_1	290	49	225.5	237.8	1.8	25.0	240.0	240.0
			FB_2	285	84	65.6	103.8	0.0	50.0	86.0	86.0
			FB_3	196	99	0.3	50.1	0.0	50.0	0.0	50.0
		Dairy cows	FB_1	160	44	49.5	99.0	1.7	50.0	194.0	300.0
			FB_2	146	67	29.2	84.0	0.0	50.0	50.0	300.0
		Fattening calves	FB_1	6	50	167.5	190.2	11.8	48.6	–	–
			FB_2	6	67	47.9	95.5	0.0	64.7	–	–
		Fattening cattle	FB_1	31	52	212.7	265.0	0.0	100.0	–	–
			FB_2	31	81	28.0	116.8	0.0	50.0	–	–
		Fattening chickens	FB_1	11	64	54.2	193.1	0.0	117.3	–	–
			FB_2	11	82	10.4	113.3	0.0	58.7	–	–
		Fattening ducks/Complete feed	FB_1	9	0.00	309.1	309.1	148.4	148.4	–	–
			FB_2	9	56	68.3	90.1	0.0	39.1	–	–
		Fattening rabbits	FB_1	2	100	0.0	30.0	0.0	30.0	–	–
			FB_2	2	100	0.0	30.0	0.0	30.0	–	–
		Fattening sheep	FB_1	2	100	0.0	97.8	0.0	97.8	–	–
			FB_2	2	100	0.0	195.6	0.0	195.6	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
		Fattening turkeys/Complete feed	FB_1	2	50	220.0	268.9	220.0	268.9	–	–
			FB_2	2	50	65.0	109.0	65.0	109.0	–	–
		Fish/Complete feed	FB_1	6	33	306.0	406.0	200.6	345.6	–	–
			FB_2	6	67	50.5	159.0	0.0	151.4	–	–
		Fur animals/Complete feed	FB_1	1	0.00	365.0	365.0	365.0	365.0	–	–
			FB_2	1	0.00	115.0	115.0	115.0	115.0	–	–
		Goat (kids) (weaning diets)/ Complementary feed	FB_1	1	0.00	424.7	424.7	424.7	424.7	–	–
			FB_2	1	100	0.0	70.8	0.0	70.8	–	–
		Growing/fattening pigs	FB_1	119	58	119.8	182.0	0.0	47.2	401.1	405.0
			FB_2	119	75	24.6	100.5	0.0	58.7	104.2	300.0
		Horses	FB_1	115	96	9.0	104.3	0.0	97.8	0.0	97.8
			FB_2	115	98	2.8	192.1	0.0	195.6	0.0	195.6
		Lactating/dairy sheep	FB_1	7	86	27.0	99.0	0.0	118.0	–	–
			FB_2	7	100	0.0	111.2	0.0	50.0	–	–
		Lambs	FB_1	1	0.00	112.0	112.0	112.0	112.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
		Laying hens	FB_1	18	44	168.6	243.1	2.1	108.7	–	–
			FB_2	17	65	46.5	177.6	0.0	74.3	–	–
		Pet food, birds	FB_1	18	6	66.4	69.1	19.6	21.6	–	–
			FB_2	18	6	39.3	42.1	39.1	39.1	–	–
		Pet food, dogs	FB_1	4	75	53.8	102.7	0.0	78.2	–	–
			FB_2	4	100	0.0	58.7	0.0	58.7	–	–
		Poultry (starter diets)	FB_1	151	39	203.7	221.2	25.0	50.0	1,145.0	1,145.0
			FB_2	151	68	44.8	71.8	0.0	50.0	287.1	287.1
		Rabbits/Complete feed	FB_1	3	33	83.4	86.8	19.6	19.6	–	–
			FB_2	3	67	13.0	35.9	0.0	39.1	–	–
		Sows/Complete feed	FB_1	13	54	173.2	200.5	0.0	60.3	–	–
			FB_2	13	62	58.8	107.2	0.0	65.1	–	–
		Unspecified Complementary/ Complete feed	FB_1	117	44	86.0	98.0	10.0	30.0	290.0	290.0
			FB_2	117	62	43.2	59.8	0.0	15.0	155.0	170.0

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
		Weaning pigs	FB_1	400	83	120.6	196.6	0.0	97.8	641.4	667.3
			FB_2	400	95	14.7	167.0	0.0	195.6	0.0	199.5
	Compound feed	Compound feed[(e)]	FB_1	229	41	1,657.5	1,678.1	81.0	81.0	9,250.5	9,250.5
			FB_2	227	56	454.8	482.1	0.0	50.0	2,554.8	2,554.8
			FB_3	1	100	0.0	50.0	0.0	50.0	–	–
Forages and roughage, and products derived thereof	Cereals straw	Cereal straw, treated	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
		Cereals straw, unspecified	FB_1	42	100	0.0	50.0	0.0	50.0	–	–
			FB_2	42	100	0.0	50.0	0.0	50.0	–	–
	Clover meal	Clover meal	FB_1	2	100	0.0	75.0	0.0	75.0	–	–
			FB_2	2	50	38.0	88.0	38.0	88.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Forage meal; [Grass meal]; [Green meal]	Forage meal; [Grass meal]; [Green meal]	FB_1	61	100	0.0	99.7	0.0	100.0	0.0	100.0
			FB_2	61	100	0.0	99.7	0.0	100.0	0.0	100.0
			FB_3	47	100	0.0	100.0	0.0	100.0	–	–
	Forages and roughage, and products derived thereof, unspecified	Forages and roughage, and products derived thereof, unspecified	FB_1	887	76	276.1	422.1	0.0	100.0	1,357.0	1,357.0
			FB_2	888	90	53.6	234.4	0.0	100.0	250.0	411.2
			FB_3	505	99	2.0	100.7	0.0	100.0	0.0	100.0
	Grass, field dried, [Hay]	Grass, field dried, [Hay] unspecified	FB_1	35	20	11.2	28.3	9.6	19.1	–	–
			FB_2	35	20	15.3	32.4	19.1	19.1	–	–
		Grass, herbs, legume plants, [green forage]	FB_1	20	0.00	30.4	30.4	40.3	40.3	–	–
			FB_2	20	0.00	38.6	38.6	40.3	40.3	–	–
	Lucerne; [Alfalfa]	Lucerne field dried; [Alfalfa field dried]	FB_1	6	100	0.0	100.0	0.0	100.0	–	–
			FB_2	6	100	0.0	100.0	0.0	100.0	–	–
			FB_3	6	100	0.0	100.0	0.0	100.0	–	–
		Lucerne meal; [Alfalfa meal]	FB_1	20	100	0.0	101.3	0.0	100.0	–	–
			FB_2	20	100	0.0	101.3	0.0	100.0	–	–
			FB_3	18	100	0.0	100.0	0.0	100.0	–	–
		Lucerne, high temperature dried; [Alfalfa, high temperature dried]	FB_1	1	0.00	17.6	17.6	17.6	17.6	–	–
			FB_2	1	0.00	17.6	17.6	17.6	17.6	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
	Maize silage	Maize silage	FB_1	46	26	106.4	127.1	38.8	38.8	–	–
			FB_2	46	30	34.2	56.1	38.8	38.8	–	–
	Pea Straw	Pea Straw	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
Land animal products and products derived thereof	Animal by-products	Animal by-products	FB_1	1	0.00	9.1	9.1	9.1	9.1	–	–
			FB_2	1	0.00	18.2	18.2	18.2	18.2	–	–
Legume seeds and products derived thereof	Carob, dried	Carob pods, dried	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
		Dried carob pod meal, micronised	FB_1	1	0.00	10.0	10.0	10.0	10.0	–	–
			FB_2	1	0.00	20.0	20.0	20.0	20.0	–	–
	Horse beans	Horse beans	FB_1	1	0.00	10.0	10.0	10.0	10.0	–	–
			FB_2	1	0.00	20.0	20.0	20.0	20.0	–	–
	Mung beans	Mung beans	FB_1	4	100	0.0	87.5	0.0	100.0	–	–
			FB_2	4	100	0.0	100.0	0.0	100.0	–	–
			FB_3	3	100	0.0	100.0	0.0	100.0	–	–
	Peas	Peas	FB_1	14	100	0.0	98.0	0.0	100.0	–	–
			FB_2	14	100	0.0	98.0	0.0	100.0	–	–
			FB_3	5	100	0.0	100.0	0.0	100.0	–	–
	Sweet lupins	Sweet lupins	FB_1	4	75	2.5	57.5	0.0	60.0	–	–
			FB_2	4	75	5.0	62.5	0.0	65.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Vetches	Vetches	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
Minerals and products derived thereof	Minerals and products derived thereof	Minerals and products derived thereof	FB_1	4	75	42.5	90.8	0.0	73.3	–	–
			FB_2	4	100	0.0	96.6	0.0	96.6	–	–
			FB_3	2	100	0.0	100.0	0.0	100.0	–	–
Miscellaneous	Miscellaneous	Miscellaneous	FB_1	2	100	0.0	101.8	0.0	101.8	–	–
			FB_2	2	100	0.0	101.8	0.0	101.8	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Products from the bakery and pasta industry	Feed beer	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
		Plants by-products from spirits production	FB_1	6	17	1,203.3	1,206.7	190.0	190.0	–	–
			FB_2	6	50	238.3	263.3	35.0	80.0	–	–
		Products from the bakery and pasta industry, unspecified	FB_1	27	100	0.0	119.5	0.0	100.0	–	–
			FB_2	27	100	0.0	119.5	0.0	100.0	–	–
			FB_3	18	100	0.0	100.0	0.0	100.0	–	–
	Starch	Starch	FB_1	3	100	0.0	83.3	0.0	100.0	–	–
			FB_2	3	100	0.0	100.0	0.0	100.0	–	–
Oil seeds, oil fruits, and products derived thereof	Cocoa husks	Cocoa hulls	FB_1	2	100	0.0	100.0	0.0	100.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–
		Cocoa husks	FB_1	3	33	6.7	13.3	10.0	10.0	–	–
			FB_2	3	33	13.3	23.3	20.0	20.0	–	–
	Cotton seed	Cotton seed, unspecified	FB_1	3	0.00	7.4	7.4	10.0	10.0	–	–
			FB_2	3	0.00	14.4	14.4	20.1	20.1	–	–
		Cotton seed expeller	FB_1	1	0.00	10.0	10.0	10.0	10.0	–	–
			FB_2	1	0.00	20.1	20.1	20.1	20.1	–	–
	Groundnut expeller, partially decorticated	Groundnut expeller, partially decorticated unspecified	FB_1	10	100	0.0	100.0	0.0	100.0	–	–
			FB_2	10	100	0.0	100.0	0.0	100.0	–	–
			FB_3	7	100	0.0	100.0	0.0	100.0	–	–
		Groundnut meal, decorticated	FB_1	2	100	0.0	100.0	0.0	100.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–
			FB_3	2	100	0.0	100.0	0.0	100.0	–	–
		Groundnut meal, partially decorticated	FB_1	2	100	0.0	100.0	0.0	100.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
			FB_3	2	100	0.0	100.0	0.0	100.0	–	–
	Linseed	Linseed, unspecified	FB_1	6	100	0.0	98.4	0.0	100.0	–	–
			FB_2	6	100	0.0	98.4	0.0	100.0	–	–
			FB_3	4	100	0.0	100.0	0.0	100.0	–	–
		Linseed expeller	FB_1	4	75	25.0	99.3	0.0	100.0	–	–
			FB_2	4	100	0.0	99.3	0.0	100.0	–	–
			FB_3	3	100	0.0	100.0	0.0	100.0	–	–
	Niger seed	Niger seed	FB_1	2	100	0.0	75.0	0.0	75.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Oil seeds, oil fruits, and products derived thereof	Oil seeds, oil fruits, and products derived thereof	FB_1	1	100	0.0	25.0	0.0	25.0	–	–
			FB_2	1	100	0.0	50.0	0.0	50.0	–	–
			FB_3	1	100	0.0	50.0	0.0	50.0	–	–
	Palm kernel expeller	Palm kernel expeller, unspecified	FB_1	78	100	0.0	100.0	0.0	100.0	0.0	100.0
			FB_2	78	100	0.0	100.0	0.0	100.0	0.0	100.0
			FB_3	55	100	0.0	100.0	0.0	100.0	–	–
		Palm kernel meal	FB_1	3	100	0.0	82.9	0.0	100.0	–	–
			FB_2	3	100	0.0	99.2	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Rape seed	Rape seed, unspecified	FB_1	21	95	0.5	82.5	0.0	100.0	–	–
			FB_2	21	95	1.0	83.4	0.0	100.0	–	–
			FB_3	10	100	0.0	100.0	0.0	100.0	–	–
		Rape seed meal	FB_1	7	14	6.3	13.5	10.0	10.0	–	–
			FB_2	7	14	12.3	19.5	20.1	20.1	–	–
		Rape seed, expeller	FB_1	17	82	15.4	93.6	0.0	100.0	–	–
			FB_2	17	88	1.4	77.7	0.0	100.0	–	–
			FB_3	12	100	0.0	100.0	0.0	100.0	–	–
		Rape seed, extruded	FB_1	35	97	5.7	103.0	0.0	100.0	–	–
			FB_2	35	100	0.0	100.1	0.0	100.0	–	–
			FB_3	19	100	0.0	100.0	0.0	100.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
	Safflower seed	Safflower seed	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Sunflower seed	Sunflower seed, unspecified	FB_1	145	99	0.4	70.8	0.0	50.0	0.0	100.0
			FB_2	145	99	0.1	71.5	0.0	96.9	0.0	100.0
			FB_3	61	100	0.0	83.7	0.0	100.0	0.0	100.0
		Sunflower seed expeller	FB_1	34	97	2.3	58.2	0.0	50.0	–	–
			FB_2	34	100	0.0	57.4	0.0	50.0	–	–
		Sunflower seed meal	FB_1	8	63	2.4	64.9	0.0	50.0	–	–
			FB_2	7	71	5.5	77.0	0.0	50.0	–	–
		Sunflower seed meal, dehulled	FB_1	2	0.00	9.7	9.7	9.7	9.7	–	–
			FB_2	2	0.00	19.4	19.4	19.4	19.4	–	–
	Toasted soya (beans)	Soya (bean) expeller	FB_1	16	88	1.3	51.8	0.0	50.0	–	–
			FB_2	16	94	1.3	52.4	0.0	50.0	–	–
		Soya (bean) hulls	FB_1	14	100	0.0	99.8	0.0	100.0	–	–
			FB_2	14	100	0.0	99.8	0.0	100.0	–	–
			FB_3	9	100	0.0	100.0	0.0	100.0	–	–
		Soya (bean) meal	FB_1	97	96	1.6	108.7	0.0	100.0	0.0	300.0
			FB_2	95	98	0.1	110.5	0.0	100.0	0.0	300.0
			FB_3	58	100	0.0	100.0	0.0	100.0	–	–
		Soya (bean) meal, dehulled	FB_1	5	20	6.4	66.4	10.0	10.0	–	–
			FB_2	5	20	12.6	72.6	19.9	19.9	–	–
		Soya (bean) protein concentrate	FB_1	3	67	3.3	33.6	0.0	45.4	–	–
			FB_2	3	67	6.6	36.9	0.0	45.4	–	–
			FB_3	2	100	0.0	45.4	0.0	45.4	–	–
		Soya beans, extruded	FB_1	306	98	5.6	103.8	0.0	100.0	0.0	100.0
			FB_2	306	99	2.0	100.4	0.0	100.0	0.0	100.0
			FB_3	234	100	0.0	100.0	0.0	100.0	0.0	100.0
		Toasted soya (beans), unspecified	FB_1	8	100	0.0	81.0	0.0	99.7	–	–
			FB_2	8	100	0.0	81.0	0.0	99.7	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
	Vegetable oil and fat	Vegetable oil and fat	FB_1	2	100	0.0	100.0	0.0	100.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
Other seeds and fruits, and products derived thereof	Buckwheat	Buckwheat	FB_1	2	100	0.0	74.4	0.0	74.4	–	–
			FB_2	2	100	0.0	98.9	0.0	98.9	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Citrus pulp	Citrus pulp	FB_1	60	98	12.9	109.8	0.0	98.5	0.0	100.0
			FB_2	60	98	6.0	102.9	0.0	98.5	0.0	100.0
			FB_3	23	100	0.0	100.0	0.0	100.0	–	–
	Fruit kernels	Fruit pulp, dried	FB_1	2	0.00	8.8	8.8	8.8	8.8	–	–
			FB_2	2	0.00	17.6	17.6	17.6	17.6	–	–
	Grape pips	Grape pips	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Other seeds and fruits, and products derived thereof	Other seeds and fruits, and products derived thereof	FB_1	10	100	0.0	98.9	0.0	100.0	–	–
			FB_2	10	100	0.0	98.9	0.0	100.0	–	–
			FB_3	7	100	0.0	100.0	0.0	100.0	–	–
	Perilla seed	Perilla seed	FB_1	1	100	0.0	50.0	0.0	50.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
	Pine nut	Pine nut	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
Tubers, roots, and products derived thereof	Potatoes	Potato protein	FB_1	2	100	0.0	100.0	0.0	100.0	–	–
			FB_2	2	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
		Potato pulp	FB_1	4	100	0.0	100.0	0.0	100.0	–	–
			FB_2	4	100	0.0	100.0	0.0	100.0	–	–
			FB_3	2	100	0.0	100.0	0.0	100.0	–	–

Feed category			Fumonisin	N	% LC	Mean		Median		P95	
						LB	UB	LB	UB	LB	UB
	Sugar beet	Dried (sugar) beet pulp	FB_1	23	96	0.5	106.2	0.0	102.4	–	–
			FB_2	23	96	0.9	106.7	0.0	102.4	–	–
			FB_3	6	100	0.0	100.0	0.0	100.0	–	–
		Sugar beet, unspecified	FB_1	30	97	3.7	97.2	0.0	100.0	–	–
			FB_2	30	100	0.0	96.9	0.0	100.0	–	–
			FB_3	22	100	0.0	100.0	0.0	100.0	–	–
	Sweet potato	Sweet potato	FB_1	1	100	0.0	100.0	0.0	100.0	–	–
			FB_2	1	100	0.0	100.0	0.0	100.0	–	–
			FB_3	1	100	0.0	100.0	0.0	100.0	–	–
	Tubers, roots, and products derived thereof	Tubers, roots, and products derived thereof	FB_1	21	100	0.0	103.8	0.0	100.0	–	–
			FB_2	21	100	0.0	103.8	0.0	100.0	–	–
			FB_3	12	100	0.0	100.0	0.0	100.0	–	–

N: number of samples; LC: left censored; LB: lower bound; UB: upper bound.
(a): The 95th percentile with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(b): Values were rounded to 1 decimal place.
(c): Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials Text with EEA relevance. OJ L 29, 16.1.2013, p. 1–64.
(d): Protein fraction.
(e): The livestock species for which these were intended were not specified.

Table B.3: Mean, median and P95 LB and UB concentrations of the sum of FB_1 + FB_2 + FB_3 (without 1.6 Factor applied) in feed materials and species-specific compound feeds used to estimate exposures for farmed livestock and companion animals[(a),(b)]

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
Cereal grains, their products and by-products	Barley	Barley	295	22.5	196.4	0.8	139.9	67.8	300.0
		Barley middlings	3	40.0	156.7	0.0	150.0	–	–
		Barley protein feed	1	0.0	100.0	0.0	100.0	–	–
		Malt rootlets	7	15.9	87.4	5.1	80.4	–	–
	Buckwheat	Buckwheat	4	0.0	146.7	0.0	146.7	–	–
	Cereal grains, their products and by-products	Cereal grains, their products and by-products	85	415.5	525.3	3.5	145.0	1,041.5	1,095.5
	Grains as crops	Grains as crops	1	0.0	100.0	0.0	100.0	–	–
	Maize and Corn	Maize bran	2	1,694.0	1,894.0	1,694.0	1,894.0	–	–
		Maize fibre	5	464.6	554.6	200.0	250.0	–	–
		Maize flakes	10	971.5	1,017.1	595.1	628.8	–	–
		Maize germ	4	1,021.3	1,021.3	756.6	756.6	–	–
		Maize germ expeller	3	40.0	240.0	0.0	200.0	–	–
		Maize germ meal	4	212.3	287.3	240.0	290.0	–	–
		Maize gluten	3	2,164.5	2,164.5	2,805.1	2,805.1	–	–
		Maize gluten feed	111	1,586.1	1,685.5	585.4	652.2	7,320.0	7,400.0
		Maize middlings	9	385.2	436.0	183.4	183.4	–	–
		Maize screenings	2	0.0	43.7	0.0	43.7	–	–
		Maize and Corn	2,035	707.7	899.2	44.2	319.1	3,391.7	3,466.6
		Sweet corn silage	2	0.0	103.9	0.0	103.9	–	–
	Millet	Millet	14	19.5	229.3	0.0	241.7	–	–
	Mixed grains	Brewers' grains	18	160.7	394.7	0.0	300.0	–	–
		Distillers' dark grains; [Distillers' dried grains and solubles]	27	537.6	602.8	210.0	310.0	–	–
		Distillers' dried grains	2	701.8	1,001.8	701.8	1,001.8	–	–
		Grain flour	1	201.4	201.4	201.4	201.4	–	–
		Mixed grains	31	18.5	187.6	0.0	120.0	–	–

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Oats	Oat feed	61	0.0	30.0	0.0	30.0	0.0	30.0
		Oat groats (Feed)	1	0.0	100.0	0.0	100.0	–	–
		Oats	78	23.5	191.1	0.0	132.0	97.0	300.0
	Rice, broken	Rice bran	7	5.8	255.8	0.0	300.0	–	–
		Rice middlings	2	0.0	125.0	0.0	125.0	–	–
		Rice, broken	196	0.5	136.7	0.0	132.2	0.0	132.2
		Rice, milled	1	0.0	100.0	0.0	100.0	–	–
	Rye	Rye	25	8.0	156.5	0.0	150.0	–	–
		Rye middlings	2	22.5	222.5	22.5	222.5	–	–
	Sorghum; [Milo]	Sorghum; [Milo]	15	27.3	285.5	0.0	300.0	–	–
	Spelt	Spelt	19	70.1	184.0	30.6	125.0	–	–
	Triticale	Triticale	36	31.5	207.6	15.8	217.0	–	–
	Wheat	Vital wheat gluten	2	3,899.5	3,899.5	3,899.5	3,899.5	–	–
		Wheat	376	142.9	301.6	0.4	177.9	130.0	300.0
		Wheat bran (Feed)	166	243.5	401.4	0.0	159.1	5.0	159.1
		Wheat feed	109	10.9	185.7	0.0	175.0	30.3	300.0
		Wheat germ (Feed)	2	0.0	237.5	0.0	237.5	–	–
		Wheat gluten feed	7	48.0	182.5	22.0	171.0	–	–
		Wheat middlings	21	9.5	269.1	0.0	288.2	–	–
		Wheat starch containing protein, partially de-sugared	1	0.0	100.0	0.0	100.0	–	–
Compound feed	Complementary/ Complete feed	Breeding pigs	33	20.4	61.8	0.0	20.0	–	–
		Calves	15	89.6	157.7	0.0	100.0	–	–
		Complementary feed (incomplete diet)	139	368.4	475.6	57.0	165.0	1,651.9	1,701.5
		Complete feed	290	291.5	391.7	4.9	125.0	270.0	370.0
		Dairy cows	160	78.7	182.9	2.4	100.0	241.9	600.0
		Fattening calves	6	215.4	285.7	35.4	109.0	–	–
		Fattening cattle	31	240.7	381.9	40.0	151.0	–	–
		Fattening chickens	11	64.6	306.4	0.0	176.0	–	–
		Fattening ducks/Complete feed	9	377.4	399.2	148.4	187.5	–	–

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
		Fattening rabbits	2	0.0	60.0	0.0	60.0	–	–
		Fattening sheep	2	0.0	293.3	0.0	293.3	–	–
		Fattening turkeys/Complete feed	2	285.0	377.9	285.0	377.9	–	–
		Fish/Complete feed	6	356.4	564.9	200.6	600.0	–	–
		Fur animals/Complete feed	1	480.0	480.0	480.0	480.0	–	–
		Goat (kids) (weaning diets)/ Complementary feed	1	424.7	495.4	424.7	495.4	–	–
		Growing/fattening pigs	128	144.4	282.5	15.0	117.6	500.0	664.9
		Horses	115	11.8	296.3	0.0	293.3	0.0	293.3
		Lactating/dairy sheep	7	27.0	210.1	0.0	224.1	–	–
		Lambs	1	112.0	162.0	112.0	162.0	–	–
		Laying hens	18	215.1	420.7	21.5	185.5	–	–
		Pet food, birds	18	105.7	111.2	58.7	58.7	–	–
		Pet food, dogs	4	53.8	161.3	0.0	136.9	–	–
		Poultry (starter diets)	175	248.4	293.0	79.8	110.0	1,230.0	1,230.0
		Rabbits/Complete feed	3	96.5	122.7	58.7	58.7	–	–
		Sows/Complete feed	16	232.0	307.6	146.1	220.7	–	–
		Unspecified Complementary/ Complete feed	141	129.1	157.8	43.2	97.5	400.0	420.0
		Weaning pigs	411	135.3	363.7	0.0	293.3	677.6	829.9
	Compound feed	Compound feed	231	2,112.4	2,210.2	90.7	190.7	11,867.3	11,917.3
Forages and roughage, and products derived thereof	Cereals straw	Cereal straw, treated	1	0.0	100.0	0.0	100.0	–	–
		Cereals straw	42	0.0	100.0	0.0	100.0	–	–
	Clover meal	Clover meal	2	38.0	263.0	38.0	263.0	–	–
	Forage meal; [Grass meal]; [Green meal]	Forage meal; [Grass meal]; [Green meal]	61	0.0	299.4	0.0	300.0	0.0	300.0
	Forages and roughage, and products derived thereof	Forages and roughage, and products derived thereof	888	331.7	757.1	2.0	300.7	1,600.0	1,910.0
	Grass, field dried, [Hay]	Grass, field dried, [Hay]	35	26.5	60.7	28.6	38.2	–	–
		Grass, herbs, legume plants, [green forage]	20	69.0	69.0	80.5	80.5	–	–

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Lucerne; [Alfalfa]	Lucerne field dried; [Alfalfa field dried]	6	0.0	300.0	0.0	300.0	–	–
		Lucerne meal; [Alfalfa meal]	20	0.0	302.6	0.0	300.0	–	–
		Lucerne, high temperature dried; [Alfalfa, high temperature dried]	1	35.2	35.2	35.2	35.2	–	–
	Maize silage	Maize silage	46	140.7	183.2	68.8	77.5	–	–
	Pea straw	Pea straw	1	0.0	300.0	0.0	300.0	–	–
Land animal products and products derived thereof	Animal by-products	Animal by-products	1	27.3	27.3	27.3	27.3	–	–
Legume seeds and products derived thereof	Carob, dried	Carob pods, dried	1	0.0	300.0	0.0	300.0	–	–
		Dried carob pod meal, micronised	1	30.0	30.0	30.0	30.0	–	–
	Horse beans	Horse beans	1	30.0	30.0	30.0	30.0	–	–
	Mung beans	Mung beans	4	0.0	287.5	0.0	300.0	–	–
	Peas	Peas	14	0.0	296.0	0.0	300.0	–	–
	Sweet lupins	Sweet lupins	4	7.5	220.0	0.0	225.0	–	–
	Vetches	Vetches	1	0.0	150.0	0.0	150.0	–	–
Minerals and products derived thereof	Minerals and products derived thereof	Minerals and products derived thereof	4	42.5	287.4	0.0	269.9	–	–
Miscellaneous	Miscellaneous	Miscellaneous	2	0.0	303.7	0.0	303.7	–	–
	Products from the bakery and pasta industry	Feed beer	1	0.0	100.0	0.0	100.0	–	–
		Plants by-products from spirits production	6	1,441.7	1,470.0	225.0	270.0	–	–
		Products from the bakery and pasta industry	27	0.0	339.1	0.0	300.0	–	–
	Starch	Starch	3	0.0	183.3	0.0	200.0	–	–

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
Oil seeds, oil fruits, and products derived thereof	Cocoa husks	Cocoa hulls	2	0.0	200.0	0.0	200.0	–	–
		Cocoa husks	3	20.0	36.7	30.0	30.0	–	–
	Cotton seed	Cotton seed	3	21.7	21.7	30.1	30.1	–	–
		Cotton seed expeller	1	30.1	30.1	30.1	30.1	–	–
	Groundnut expeller, partially decorticated	Groundnut expeller, partially decorticated	10	0.0	300.0	0.0	300.0	–	–
		Groundnut meal, decorticated	2	0.0	300.0	0.0	300.0	–	–
		Groundnut meal, partially decorticated	2	0.0	300.0	0.0	300.0	–	–
	Linseed	Linseed	6	0.0	296.8	0.0	300.0	–	–
		Linseed expeller	4	25.0	298.5	0.0	300.0	–	–
	Niger seed	Niger seed	2	0.0	275.0	0.0	275.0	–	–
	Oil seeds, oil fruits, and products derived thereof	Oil seeds, oil fruits, and products derived thereof	1	0.0	125.0	0.0	125.0	–	–
	Palm kernel expeller	Palm kernel expeller	78	0.0	300.0	0.0	300.0	0.0	300.0
		Palm kernel meal	3	0.0	282.2	0.0	300.0	–	–
	Rape seed	Rape seed	21	1.4	265.9	0.0	300.0	–	–
		Rape seed meal	7	18.6	32.9	30.1	30.1	–	–
		Rape seed, expeller	17	16.7	271.3	0.0	300.0	–	–
		Rape seed, extruded	35	5.7	303.1	0.0	300.0	–	–
	Safflower seed	Safflower seed	1	0.0	300.0	0.0	300.0	–	–
	Sunflower seed	Sunflower seed	145	0.5	226.1	0.0	229.1	0.0	300.0
		Sunflower seed expeller	34	2.3	115.5	0.0	100.0	–	–
		Sunflower seed meal	8	8.0	141.9	0.0	100.0	–	–
		Sunflower seed meal, dehulled	2	29.1	29.1	29.1	29.1	–	–

Feed category			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Toasted soya (beans)	Soya (bean) expeller	16	2.5	104.2	0.0	100.0	–	–
		Soya (bean) hulls	14	0.0	299.7	0.0	300.0	–	–
		Soya (bean) meal	97	1.7	319.2	0.0	300.0	0.1	700.0
		Soya (bean) meal, dehulled	5	18.9	138.9	29.9	29.9	–	–
		Soya (bean) protein concentrate	3	10.0	115.8	0.0	136.1	–	–
		Soya beans, extruded	306	7.6	304.2	0.0	300.0	0.0	300.0
		Toasted soya (beans)	8	0.0	162.1	0.0	199.3	–	–
	Vegetable oil and fat	Vegetable oil and fat	2	0.0	300.0	0.0	300.0	–	–
Other seeds and fruits, and products derived thereof	Buckwheat	Buckwheat	2	0.0	252.9	0.0	252.9	–	–
	Citrus pulp	Citrus pulp	60	18.9	312.6	0.0	297.1	0.0	300.0
	Fruit kernels	Fruit pulp, dried	2	26.4	26.4	26.4	26.4	–	–
	Grape pips	Grape pips	1	0.0	300.0	0.0	300.0	–	–
	Other seeds and fruits, and products derived thereof	Other seeds and fruits, and products derived thereof	10	0.0	297.7	0.0	300.0	–	–
	Perilla seed	Perilla seed	1	0.0	150.0	0.0	150.0	–	–
	Pine nut	Pine nut	1	0.0	300.0	0.0	300.0	–	–
Tubers, roots, and products derived thereof	Potatoes	Potato protein	2	0.0	300.0	0.0	300.0	–	–
		Potato pulp	4	0.0	300.0	0.0	300.0	–	–
	Sugar beet	Dried (sugar) beet pulp	23	1.3	312.9	0.0	304.7	–	–
		Sugar beet	30	3.7	294.1	0.0	300.0	–	–
	Sweet potato	Sweet potato	1	0.0	300.0	0.0	300.0	–	–
	Tubers, roots, and products derived thereof	Tubers, roots, and products derived thereof	21	0.0	307.6	0.0	300.0	–	–

N: number of samples; LB: lower bound; UB: upper bound.
(a): The 95th percentile with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(b): Values were rounded to 1 decimal place.

Table B.4: Mean, median and P95 LB and UB concentrations of the sum of FB_1 + FB_2 + FB_3 (with 1.6 Factor applied) in feed materials and species-specific compound feeds used to estimate exposures for farmed livestock and companion animals[(a),(b)]

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
Cereal grains, their products and by-products	Barley	Barley	295	36.0	314.3	1.3	223.8	108.5	480.0
		Barley middlings	3	64.0	250.7	0.0	240.0	–	–
		Barley protein feed	1	0.0	160.0	0.0	160.0	–	–
		Malt rootlets	7	25.5	139.8	8.1	128.7	–	–
	Buckwheat	Buckwheat	4	0.0	234.7	0.0	234.7	–	–
	Cereal grains, their products and by-products	Cereal grains, their products and by-products	85	664.8	840.5	5.6	232.0	1,666.4	1,752.9
	Grains as crops	Grains as crops	1	0.0	160.0	0.0	160.0	–	–
	Maize and Corn	Maize bran	2	2,710.4	3,030.4	2,710.4	3,030.4	–	–
		Maize fibre	5	743.4	887.4	320.0	400.0	–	–
		Maize flakes	10	1,554.4	1,627.4	952.1	1,006.1	–	–
		Maize germ	4	1,634.1	1,634.1	1,210.6	1,210.6	–	–
		Maize germ expeller	3	64.0	384.0	0.0	320.0	–	–
		Maize germ meal	4	339.6	459.6	384.0	464.0	–	–
		Maize gluten	3	3,463.2	3,463.2	4,488.1	4,488.1	–	–
		Maize gluten feed	111	2,537.8	2,696.8	936.7	1,043.5	11,712.0	11,840.0
		Maize middlings	9	616.3	697.6	293.5	293.5	–	–
		Maize screenings	2	0.0	70.0	0.0	70.0	–	–
		Maize_&_Corn	2,035	1,132.3	1,438.7	70.7	510.5	5,426.7	5,546.5
		Sweet corn silage	2	0.0	166.2	0.0	166.2	–	–
	Millet	Millet	14	31.2	366.8	0.0	386.7	–	–
	Mixed grains	Brewers' grains	18	257.1	631.6	0.0	480.0	–	–
		Distillers' dark grains; [Distillers' dried grains and solubles]	27	860.2	964.6	336.0	496.0	–	–
		Distillers' dried grains	2	1,122.8	1,602.8	1,122.8	1,602.8	–	–
		Grain flour	1	322.3	322.3	322.3	322.3	–	–
		Mixed grains	31	29.7	300.1	0.0	192.0	–	–

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Oats	Oat feed	61	0.0	48.0	0.0	48.0	0.0	48.0
		Oat groats (Feed)	1	0.0	160.0	0.0	160.0	–	–
		Oats	78	37.6	305.7	0.0	211.2	155.2	480.0
	Rice, broken	Rice bran	7	9.3	409.3	0.0	480.0	–	–
		Rice middlings	2	0.0	200.0	0.0	200.0	–	–
		Rice, broken	196	0.8	218.7	0.0	211.5	0.0	211.5
		Rice, milled	1	0.0	160.0	0.0	160.0	–	–
	Rye	Rye	25	12.8	250.4	0.0	240.0	–	–
		Rye middlings	2	36.0	356.0	36.0	356.0	–	–
	Sorghum; [Milo]	Sorghum; [Milo]	15	43.6	456.9	0.0	480.0	–	–
	Spelt	Spelt	19	112.2	294.3	49.0	200.0	–	–
	Triticale	Triticale	36	50.4	332.1	25.2	347.2	–	–
	Wheat	Vital wheat gluten	2	6,239.2	6,239.2	6,239.2	6,239.2	–	–
		Wheat	376	228.7	482.5	0.7	284.7	208.0	480.0
		Wheat bran (Feed)	166	389.7	642.2	0.0	254.5	8.1	254.5
		Wheat feed	109	17.4	297.2	0.0	280.0	48.4	480.0
		Wheat germ (Feed)	2	0.0	380.0	0.0	380.0	–	–
		Wheat gluten feed	7	76.8	292.0	35.2	273.6	–	–
		Wheat middlings	21	15.2	430.6	0.0	461.1	–	–
		Wheat starch containing protein, partially de-sugared	1	0.0	160.0	0.0	160.0	–	–
Compound feed	Complementary/ Complete feed	Breeding pigs	33	32.6	98.9	0.0	32.0	–	–
		Calves	15	143.3	252.3	0.0	160.0	–	–
		Complementary feed (incomplete diet)	139	589.4	760.9	91.2	264.0	2,643.1	2,722.4
		Complete feed	290	466.4	626.8	7.9	200.0	432.0	592.0
		Dairy cows	160	126.0	292.7	3.8	160.0	387.1	960.0
		Fattening calves	6	344.6	457.1	56.6	174.4	–	–
		Fattening cattle	31	385.2	611.0	64.0	241.6	–	–
		Fattening chickens	11	103.4	490.3	0.0	281.6	–	–
		Fattening ducks/Complete feed	9	603.9	638.7	237.5	300.1	–	–

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
		Fattening rabbits	2	0.0	96.0	0.0	96.0	–	–
		Fattening sheep	2	0.0	469.3	0.0	469.3	–	–
		Fattening turkeys/Complete feed	2	456.0	604.6	456.0	604.6	–	–
		Fish/Complete feed	6	570.3	903.9	320.9	960.0	–	–
		Fur animals/Complete feed	1	768.0	768.0	768.0	768.0	–	–
		Goat (kids) (weaning diets)/ Complementary feed	1	679.5	792.7	679.5	792.7	–	–
		Growing/fattening pigs	128	231.0	452.0	24.0	188.2	800.0	1,063.8
		Horses	115	18.9	474.1	0.0	469.3	0.0	469.3
		Lactating/dairy sheep	7	43.2	336.2	0.0	358.6	–	–
		Lambs	1	179.2	259.2	179.2	259.2	–	–
		Laying hens	18	344.2	673.1	34.4	296.9	–	–
		Pet food, birds	18	169.1	178.0	93.9	93.9	–	–
		Pet food, dogs	4	86.0	258.1	0.0	219.0	–	–
		Poultry (starter diets)	175	397.5	468.8	127.7	176.0	1,968.0	1,968.0
		Rabbits/Complete feed	3	154.4	196.3	93.9	93.9	–	–
		Sows/Complete feed	16	371.1	492.2	233.7	353.2	–	–
		Unspecified Complementary/ Complete feed	141	206.6	252.5	69.1	155.9	640.0	672.0
		Weaning pigs	411	216.5	581.8	0.0	469.3	1,084.2	1,327.9
	Compound feed	Compound feed	231	3,379.8	3,536.3	145.1	305.1	18,987.7	19,067.7
Forages and roughage, and products derived thereof	Cereals straw	Cereal straw, treated	1	0.0	160.0	0.0	160.0	–	–
		Cereals straw	42	0.0	160.0	0.0	160.0	–	–
	Clover meal	Clover meal	2	60.8	420.8	60.8	420.8	–	–
	Forage meal; [Grass meal]; [Green meal]	Forage meal; [Grass meal]; [Green meal]	61	0.0	479.1	0.0	480.0	0.0	480.0
	Forages and roughage, and products derived thereof	Forages and roughage, and products derived thereof	888	530.7	1,211.4	3.3	481.0	2,560.0	3,056.0
	Grass, field dried, [Hay]	Grass, field dried, [Hay]	35	42.3	97.2	45.8	61.1	–	–
		Grass, herbs, legume plants, [green forage]	20	110.4	110.4	128.9	128.9	–	–

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Lucerne; [Alfalfa]	Lucerne field dried; [Alfalfa field dried]	6	0.0	480.0	0.0	480.0	–	–
		Lucerne meal; [Alfalfa meal]	20	0.0	484.1	0.0	480.0	–	–
		Lucerne, high temperature dried; [Alfalfa, high temperature dried]	1	56.3	56.3	56.3	56.3	–	–
	Maize silage	Maize silage	46	225.1	293.1	110.0	124.1	–	–
	Pea Straw	Pea Straw	1	0.0	480.0	0.0	480.0	–	–
Land animal products and products derived thereof	Animal by-products	Animal by-products	1	43.7	43.7	43.7	43.7	–	–
Legume seeds and products derived thereof	Carob, dried	Carob pods, dried	1	0.0	480.0	0.0	480.0	–	–
		Dried carob pod meal, micronised	1	48.0	48.0	48.0	48.0	–	–
	Horse beans	Horse beans	1	48.0	48.0	48.0	48.0	–	–
	Mung beans	Mung beans	4	0.0	460.0	0.0	480.0	–	–
	Peas	Peas	14	0.0	473.6	0.0	480.0	–	–
	Sweet lupins	Sweet lupins	4	12.0	352.0	0.0	360.0	–	–
	Vetches	Vetches	1	0.0	240.0	0.0	240.0	–	–
Minerals and products derived thereof	Minerals and products derived thereof	Minerals and products derived thereof	4	68.0	459.8	0.0	431.8	–	–
Miscellaneous	Miscellaneous	Miscellaneous	2	0.0	485.8	0.0	485.8	–	–
	Products from the bakery and pasta industry	Feed beer	1	0.0	160.0	0.0	160.0	–	–
		Plants by-products from spirits production	6	2,306.7	2,352.0	360.0	432.0	–	–
		Products from the bakery and pasta industry	27	0.0	542.5	0.0	480.0	–	–
	Starch	Starch	3	0.0	293.3	0.0	320.0	–	–

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
Oil seeds, oil fruits, and products derived thereof	Cocoa husks	Cocoa hulls	2	0.0	320.0	0.0	320.0	–	–
		Cocoa husks	3	32.0	58.7	48.0	48.0	–	–
	Cotton seed	Cotton seed	3	34.8	34.8	48.2	48.2	–	–
		Cotton seed expeller	1	48.2	48.2	48.2	48.2	–	–
	Groundnut expeller, partially decorticated	Groundnut expeller, partially decorticated	10	0.0	480.0	0.0	480.0	–	–
		Groundnut meal, decorticated	2	0.0	480.0	0.0	480.0	–	–
		Groundnut meal, partially decorticated	2	0.0	480.0	0.0	480.0	–	–
	Linseed	Linseed	6	0.0	474.8	0.0	480.0	–	–
		Linseed expeller	4	40.0	477.6	0.0	480.0	–	–
	Niger seed	Niger seed	2	0.0	440.0	0.0	440.0	–	–
	Oil seeds, oil fruits, and products derived thereof	Oil seeds, oil fruits, and products derived thereof	1	0.0	200.0	0.0	200.0	–	–
	Palm kernel expeller	Palm kernel expeller	78	0.0	480.0	0.0	480.0	0.0	480.0
		Palm kernel meal	3	0.0	451.5	0.0	480.0	–	–
	Rape seed	Rape seed	21	2.3	425.4	0.0	480.0	–	–
		Rape seed meal	7	29.8	52.7	48.2	48.2	–	–
		Rape seed, expeller	17	26.7	434.1	0.0	480.0	–	–
		Rape seed, extruded	35	9.2	485.0	0.0	480.0	–	–
	Safflower seed	Safflower seed	1	0.0	480.0	0.0	480.0	–	–
	Sunflower seed	Sunflower seed	145	0.9	361.7	0.0	366.5	0.0	480.0
		Sunflower seed expeller	34	3.7	184.9	0.0	160.0	–	–
		Sunflower seed meal	8	12.7	227.0	0.0	160.0	–	–
		Sunflower seed meal, dehulled	2	46.5	46.5	46.5	46.5	–	–

Feed group			N	Mean		Median		P95	
				LB	UB	LB	UB	LB	UB
	Toasted soya (beans)	Soya (bean) expeller	16	4.0	166.8	0.0	160.0	–	–
		Soya (bean) hulls	14	0.0	479.5	0.0	480.0	–	–
		Soya (bean) meal	97	2.8	510.6	0.0	480.0	0.1	1,120.0
		Soya (bean) meal, dehulled	5	30.3	222.3	47.8	47.8	–	–
		Soya (bean) protein concentrate	3	15.9	185.3	0.0	217.7	–	–
		Soya beans, extruded	306	12.2	486.8	0.0	480.0	0.0	480.0
		Toasted soya (beans)	8	0.0	259.3	0.0	318.9	–	–
	Vegetable oil and fat	Vegetable oil and fat	2	0.0	480.0	0.0	480.0	–	–
Other seeds and fruits, and products derived thereof	Buckwheat	Buckwheat	2	0.0	404.6	0.0	404.6	–	–
	Citrus pulp	Citrus pulp	60	30.3	500.2	0.0	475.3	0.0	480.0
	Fruit kernels	Fruit pulp, dried	2	42.2	42.2	42.2	42.2	–	–
	Grape pips	Grape pips	1	0.0	480.0	0.0	480.0	–	–
	Other seeds and fruits, and products derived thereof	Other seeds and fruits, and products derived thereof	10	0.0	476.3	0.0	480.0	–	–
	Perilla seed	Perilla seed	1	0.0	240.0	0.0	240.0	–	–
	Pine nut	Pine nut	1	0.0	480.0	0.0	480.0	–	–
Tubers, roots, and products derived thereof	Potatoes	Potato protein	2	0.0	480.0	0.0	480.0	–	–
	Potatoes	Potato pulp	4	0.0	480.0	0.0	480.0	–	–
	Sugar beet	Dried (sugar) beet pulp	23	2.1	500.6	0.0	487.6	–	–
		Sugar beet	30	5.9	470.5	0.0	480.0	–	–
	Sweet potato	Sweet potato	1	0.0	480.0	0.0	480.0	–	–
	Tubers, roots, and products derived thereof	Tubers, roots, and products derived thereof	21	0.0	492.1	0.0	480.0	–	–

N: number of samples; LB: lower bound; UB: upper bound.
(a): The 95th percentile with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(b): Values were rounded to 1 decimal place.

Appendix C – Feed intakes and diet composition (livestock)

This Appendix gives details of the feed intakes, live weights and diet compositions for different livestock, fish and companion animals used as the basis to estimate exposures. These are based on published guidelines on nutrition and feeding (e.g. Carabano and Piquer, 1998; NRC, 2000, 2007a,b; Ewing, 2002; Leeson and Summers, 2008; OECD, 2009; McDonald et al., 2011; EBLEX, 2008, 2012; EFSA, 2012) and information provided by European feed manufacturers. They are therefore estimates of the Panel on Contaminants in the Food Chain (CONTAM Panel), but agree with common practice. In Table C.6 the concentrations of fumonisins and its hidden forms in feeds used to estimate exposure are presented.

C.1. Feed intakes

C.1.1. Cattle, sheep, goats and horses

Dairy cows

The amounts of feed given to lactating dairy cows varies according to the amount and quality of forages and other feeds available, the weight of the cow and its milk yield. In this Opinion, it is assumed that non-forage (i.e. complementary) feeds are fed at the rate of 0.3 kg/kg of milk produced (Nix, 2010). Exposures to fumonisins and the sum of its hidden forms have been estimated for a 650-kg dairy cow, with a milk yield of 40 kg/day. Assumptions on the amounts of forages and non-forage feed are given in Table C.1.

Beef cattle

There are a wide variety of beef production and husbandry systems in Europe. They may be categorised broadly as forage-based or cereal-based systems, although combinations of these systems are commonly found. In this opinion, four feeding systems are considered, in which the forages are (1) grass hay (2) maize silage and (3) cereal straw with, in each case, appropriate supplementation with non-forage feed materials. A fourth system, commonly known as 'cereal beef', is also considered. For exposure estimates, live weights of 300 or 400 kg, and feed intakes of between 6.6 and 10 kg dry matter per day have been assumed, depending on the feeding regime, based on guidelines published by EBLEX (2008, 2012), and details are given in Table C.1.

Sheep and goats

Many breeds and systems of management have been developed for sheep and goats to suit the land, climate and husbandry conditions in the EU. As for other ruminants, forages may be the only feeds used after weaning (NRC, 2007a). Common exceptions to this are pregnant and lactating animals, whose feed is usually supplemented with non-forage feeds or commercial compound (complementary) feeds (AFRC, 1993; NRC, 2007a). In this Opinion, exposure estimates have been made for lactating sheep and goats. The CONTAM Panel has used a daily dry matter intake of 2.8 kg for an 80-kg lactating sheep feeding twin lambs to estimate the exposures. For lactating goats, the CONTAM Panel has used a daily dry matter intakes of 3.3 kg for a 60-kg goat for milking (4 kg milk/day); for fattening goats, a body weight of 40 kg and feed intakes of 1.5 kg DM/day has been assumed, of which 60% is forage (Table C.1).

Horses

Horses are non-ruminant herbivores. They generally consume 2–3.5% of their body weight in feed (dry matter) each day, of which a minimum of 50% should be as forage (pasture grass or hay) (NRC, 2007b). Assumed intakes are given in Table C.1.

Table C.1: Live weights, growth rate/productivity, dry matter intake for cattle, sheep, goats and horses, and the proportions of the diet as non-forage

Animal species	Live weight (kg)	Growth rate or productivity	Dry matter intake (kg/day)	% of diet as non-forage feed	Reference
Dairy cows, lactating[(a)]	650	40 kg milk/day	20.7	40	OECD (2009)
Fattening cattle: beef[(b)]	400	1 kg/day	9.6	15	AFRC (1993)
Fattening cattle: maize silage-based ration	300	1.4 kg/day	6.6	25	Browne et al. (2004)
Fattening cattle: cereal straw-based diet	300	0.9 kg/day	8.0	68	EBLEX (2008)
Fattening cattle: cereal beef	400	1.4 kg/day	10.0	85	EBLEX (2012)
Sheep: lactating	80	Feeding twin lambs	2.8	50	OECD (2009)
Goats: milking	60	6 kg milk/day	3.4	65	NRC (2007a)
Goats: fattening	40	0.3 kg/day	1.5	40	
Horses	450	Moderate activity	9.0	50	NRC (2007b)

(a): Months 2–3 of lactation;
(b): Housed castrate cattle, medium maturing breed.

C.1.2. Non-ruminant animals

Pigs

Although there is a considerable range of pig production systems in Europe, exposure estimates have been made for piglets (pig starter), finishing pigs and lactating sows (using feed intakes proposed by EFSA (2012). Details are given in Table C.2.

Poultry

The CONTAM Panel applied the live weights and feed intakes reported for fattening chickens (broilers), laying hens and turkeys proposed by EFSA FEEDAP Panel (2012) and for ducks by Leeson and Summers (2008) (Table C.2).

Farmed fish (salmonids and carp)

Commercially reared species include Atlantic salmon, rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. In this Scientific Opinion, exposures to fumonisins and their hidden forms have been made for farmed salmon and carp. Details of the body weights and feed intakes used are given in Table C.2.

Table C.2: Live weights and feed intake for pigs, poultry (EFSA FEEDAP Panel, 2012), ducks (Leeson and Summers, 2008) and fish

Species	Live weight (kg)	Feed intake (kg dry matter/day)	Reference
Pigs: starter	20	1.0	EFSA FEEDAP Panel (2012)
Pigs: finishing	100	3.0	EFSA FEEDAP Panel (2012)
Pigs: lactating sows	200	6.0	EFSA FEEDAP Panel (2012)
Poultry: broilers[(a)]	2	0.12	EFSA FEEDAP Panel (2012)
Poultry: laying hens	2	0.12	EFSA FEEDAP Panel (2012)
Turkeys: fattening turkeys	12	0.40	EFSA FEEDAP Panel (2012)
Ducks: fattening ducks	3	0.14	Leeson and Summers (2008)
Salmonids	2	0.04	EFSA FEEDAP Panel (2012)
Carp	1	0.02	Schultz et al. (2012)

(a): Fattening chickens.

Rabbits

Feed intakes of 65–80 g/kg bw per day have been reported (Carabano and Piquer, 1998). For the exposure estimates, the CONTAM Panel have assumed a live weight of 2 kg, and a daily feed intake of 75 g/kg bw (derived from Carabano and Piquer, 1998).

Farmed mink

For estimating exposure, the CONTAM Panel have assumed a live weight of 2.07 kg for a male mink at pelting, and with a feed intake of 227 g fresh weight/day (75 g dry matter) (NRC, 1982).

Companion animals: Dogs and cats

The amount of food consumed is largely a function of the mature weight of the animal, level of activity, physiological status (e.g. pregnancy or lactation) and the energy content of the diet. In this Scientific Opinion, the CONTAM Panel assumed body weights (kg) and feed intakes (g dry matter/day) for dogs and cats of 25/360 and 4/60, respectively (derived from NRC, 2006).

C.2. Diet composition

Many livestock in the European countries are fed proprietary commercial compound feeds. Where sufficient data have been provided on species-specific compound feeds, estimates of exposure have been made using these data (given in Table C.6) together with estimated intakes given in Appendices C.1 and C.2. Where data on proprietary compound feeds were not available, or were available but in insufficient numbers, estimates of exposure have been made using dietary inclusion rates of feed materials given in this section. Levels of fumonisins, and fumonisins + hidden forms in species-specific compound/complementary feeds or feed materials used to estimate exposure are given in Table C.6.

C.2.1. Cattle, sheep, goats and horses

For most ruminants and horses, forages (either fresh or conserved as silage or hay) are essential ingredients in their diet, but they are normally supplemented with non-forage feeds such as cereals, cereal by-products, oilseed meals and by-products of human food production. These may be fed either as individual feeds, mixtures of feed materials, or as species-specific complementary feeds in the form of compound feeds. In some situations, however, forages may represent the total diet.

Fresh (grazed) grass or grass silage are the principal forages for ruminants and horses in the EU. As reported elsewhere in this Opinion (Section 3.3) fumonisins and its modified forms have not been reported in these feeds, and therefore, it has been assumed that where they are fed they make no contribution to exposure. For other forages, however, notably grass hay, maize silage and cereal straw, the presence of fumonisins has been reported. Therefore, two estimates of exposure have been reported for ruminants and horses, the first of which assumes no exposure from forages (i.e. the main forages are fresh grass and/or grass silage). Exposures have also been estimated for diets in which grass hay, maize silage or cereal straw are the forage.

For lactating dairy cows and fattening beef cattle, data for species-specific compound feeds were provided (Table C.6) and these were used to estimate exposure to fumonisins in these diets. AFSSA (2009) have provided example intakes of dairy cows fed maize silage supplemented with maize grain and soybean meal, while example diets of beef cattle on maize silage or cereal straw-based diets are taken from EBLEX (2008, 2012), and these are given in Table C.3.

For lactating sheep and goats, and for fattening goats, levels of fumonisins and its hidden forms in species-specific compound feed data were not available and therefore example diets (Table C.4) and levels of fumonisins and fumonisins + hidden forms in individual feeds (Table C.6) have been used to estimate exposure.

Horses are non-ruminant herbivores, and consequently their diet should contain a minimum of 50% forages. While mature horses with minimal activity can be fed forage alone (NRC, 2007b), for growing and active horses supplementary feeding with cereal grains, cereal by-products (e.g. oats, barley, and wheat bran) and vegetable proteins is necessary. In this Opinion, the CONTAM Panel have used data available on levels of fumonisins in complementary feeds for horses (Table C.6) to estimate exposure.

Table C.3: Assumed diet compositions and feed intake of lactating dairy cows (40 L/day) and fattening beef cattle fed diets based on different forages

Animal species	Quantities of feed consumed (kg dry matter/day)					Reference
	Forage	Maize grain	Soybean meal	Barley grain	Rapeseed meal	
Lactating dairy cows: maize silage-based diet	15.0	9.5	2.8	ni	ni	AFSSA (2009)
Fattening beef cattle: maize silage-based diet	4.9	ni	ni	ni	1.5	EBLEX (2012)
Fattening beef cattle: cereal straw-based diet	2.5	ni	ni	4.1	1.4	EBLEX (2008)
Fattening beef cattle: intensive cereal-based diet	1.5	ni	ni	5.5	1.5	EBLEX (2008)

ni: not included in the diet formulations.

For lactating sheep, milking goats and fattening goats, no information on levels of fumonisins or its hidden forms in species-specific compound feed were available and therefore example diets have been used to estimate exposure (Table C.4).

Table C.4: Assumed diet compositions (%) for lactating sheep and goats, and fattening goats, and the calculated mean lower bound and upper bound concentrations of fumonisins and the sum of fumonisins + hidden forms in these diets

Non-forage feed materials	Lactating sheep	Lactating goats	Fattening goats
Wheat (%)	14	ni	ni
Barley (%)	18	25	20
Oats (%)	ni	35	40
Soybean meal (%)	5	10	10
Rapeseed meal (%)	10	10	10
Sunflower meal (%)	5	ni	ni
Beans (%)[(b)]	10	ni	ni
Maize gluten feed (%)	ni	ni	ni
Wheat feed (%)[(a)]	15	10	10
Oat feed (%)[(a)]	ni	ni	ni
Sugar beet pulp (%)[(b)]	14	1	1
Molasses (%)[(b)]	4	4	4
Vegetable oils (%)[(b)]	5	5	5
Minerals, vitamins etc. (%)[(b)]	ni	ni	ni
% of non-forage feeds in the diet	50	75	40

ni: not included in the diet formulations.
(a): By-products of processing these grains See Commission Regulation (EU) No 575/2011 of June 2011 for full description.[14]
(b): No data for the sum of fumonisins concentration were available, and therefore no contribution from these feeds has been assumed.

Concentrations calculated by using the mean concentrations of fumonisins reported for the individual feeds in Appendix Table C.6.

Concentrations calculated by using the 95th percentile concentrations of the sum of fumonisins and its hidden forms reported for the individual feeds in Appendix Table C.6.

C.2.2. Pigs and poultry

Sufficient data for species-specific compound feeds for pigs, and for most categories of poultry (fattening chickens, ducks and turkeys, and for laying hens), were provided (Table C.2) and these were used to estimate exposure to the sum of fumonisins and FBs hidden forms.

[14] Commission Regulation (EU) No 575/2011 of 16 June 2011 on the Catalogue of feed materials. OJ L 159, 17.6.2011, p. 25–65.

C.2.3. Rabbits

Rabbits are usually fed a pelleted diet (in the form of complete feedingstuffs) consisting of dried forages, cereals and vegetable proteins supplemented with minerals, vitamins and trace elements. Lebas and Renouf (2009) reviewed diet formulations used in experimental studies: in 58 diets, cereals and cereal by-products (mostly wheat bran) accounted for up to 40% of all ingredients. In these studies, maize was a major cereal grain and was included in more than one-third of all diets. In northern Europe, however, maize may be replaced by barley and wheat. In this opinion, the feed ingredients used in a typical French commercial rabbit compound, as provided by T. Gidenne, (Personal communication, 2011) have been used, details of which are given in Table C.5.

C.2.4. Farmed fish (salmonids and carp)

Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fishmeal and fish oils, and although alternative sources of oil and protein (e.g. soybean meals and vegetable oils) are increasingly being used fish-derived feeds still remain the major ingredients.

For many fish species, digestion of complex carbohydrates and the metabolic utilisation of the absorbed glucose is low, reflecting the scarcity of carbohydrates in the aquatic environment (Guillaume et al., 2001). Instead, fish obtain much of their energy from protein in the diet. Where carbohydrates are used, they generally require some form of pre-treatment (e.g. cooking, flaking or toasting).

Berntssen et al. (2010) provided details of the composition of a diet for growing salmonids, and the CONTAM Panel used this feed formulation to estimate the exposures (Table C.5).

In contrast, studies with the common carp (*Cyprinus cardio*) have demonstrated greater intestinal amylase activity than in carnivorous fish, which accounts for the better utilisation of carbohydrates by these fish. The optimum level of carbohydrates appears to be 30–40% (Food and Agriculture Organization of the United Nations (FAO), Aquaculture Feed and Fertiliser Resources Information System[15]), which allows for higher levels of cereals than in diets for salmonids. The CONTAM Panel used the ingredients of commercial compound feeds for carp reported by Schultz et al. (2012) to estimate exposure to the sum of FBs and FBs hidden forms.

C.2.5. Farmed mink

Mink are carnivorous animals and are fed high protein diets consisting mainly of meat and meat by-products. Commercially manufactured mink feed consists largely of fish and land animal by-products, with lesser amounts of cereals and cereal by-products, and supplemented with mineral/vitamin premixtures. Mink are fed diets high in protein, although their nutritional requirements vary according to the animal's physiological stage (e.g. gestating, lactating and growing) and climatic conditions, particularly temperature. The proportions of cereal grains, their products and by-products used in estimating the exposure are given in Table C.5.

C.2.6. Companion animals (dogs and cats)

Most small companion animals derive their nutritional needs from processed food, and in 2010 EU annual sales of pet food products was approximately 8.3 million tonnes.[16] Although a wide range of ingredients is used in commercial diets, most dog and cat diets contain at least some animal protein. Other ingredients include cereals (predominantly wheat, rice or maize), cereal by-products, vegetable proteins and by-products of human food production. The ingredients will vary depending both on the availability of feed materials and the nutrient requirements of the animals.

The European Pet Food Industry Federation (FEDIAF) has provided information on typical inclusion levels of cereals, cereal by-products and other feed materials in dry cat and dog food.[17] In the absence of sufficient data on species-specific manufactured complete feedingstuffs, the CONTAM Panel has used example diets based on information provided by FEDIAF[16] (details given in Appendix C, Table C.5).

[15] http://www.fao.org/fishery/affris/affris-home/en/
[16] Available online: www.Fediaf.org
[17] The European Pet Food Industry Federation (FEDIAF), Personal communication by email, May 2016.

Table C.5: Assumed diet composition (%) for farmed fish (salmonids and carp), farmed rabbits, farmed mink and companion animals (cats and dogs), and the calculated mean lower bound and upper bound levels of FBs and FBs + hidden forms in these diets

Feed materials	Farmed fish		Farmed rabbits	Farmed mink[b]	Companion animals	
	Salmonids	Carp			Cats	Dogs
Wheat (%)	13.2	24	ni	6	10	10
Barley (%)	ni		ni	1	ni	ni
Maize (%)	ni	10	17.6	6	5	6
Oats (%)	ni	ni	ni	ni	1	0.5
Soybean meal (%)	12.3	32.4	ni	ni	8	4
Rapeseed meal (%)	ni	12.5	ni	ni	ni	ni
Maize gluten meal (%)	11.5	ni	ni	ni	17	15
Sunflower meal (%)[a]	ni	ni	20.0	ni	ni	ni
Lucerne meal (%)[a]	ni	ni	19.1	ni	ni	ni
Beans (%)[a]	ni	ni	10.4	ni	1	2
Peas (%)	ni	ni	ni	ni	ni	ni
Wheat feed (%)	ni	ni	18.3	ni	12	20
Sugar beet pulp (%)	ni	ni	11.9	ni	ni	ni
Fishmeal (%)[a]	30.5	6.7	ni	ni	6	0.5
Meat meal (%)[a]	ni	ni	ni	40	38	40
Molasses (%)[a]	ni	ni	ni	ni	ni	ni
Fish and vegetable oils (%)[a]	31.9	2.3	ni	8	ni	ni
Other feeds (unspecified) (%)[a]	ni	1	ni	ni	ni	ni
Minerals, vitamins etc. (%)[a]	0.6	3.6	2.7	3	2.0	2.0

ni: not included in the diet formulations.
(a): No data for FBs or FBs or its hidden forms concentration were available, and therefore no contribution from these feeds has been assumed.
(b): Diet formulation based on data provided by the Finnish Fur Breeders Association in 2015 and translated from Finnish to English, www.profur.fi

Concentrations calculated by using the mean concentrations of the sum of FBs reported for the individual feeds in Table C.6.

Concentrations calculated by using the 95th percentile concentrations of the sum of FBs and its hidden forms reported for the individual feeds in Table C.6.

Table C.6: Levels of fumonisins and the sum of fumonisins and its hidden forms (μg/kg DM) in species-specific compound/complementary feeds and feed materials used to estimate exposure by farmed livestock and companion animals

Compound/ complementary feeds	Fumonisins				Fumonisins + hidden forms			
	P95		Mean		P95		Mean	
	LB	UB	LB	UB	LB	UB	LB	UB
Dairy cows: high yielding	89	208	275	682	143	333	440	1,091
Beef cattle: fattening	274	434	1,436	1,436	434	694	2,298	2,298
Horses	13	337	0.0	333	21	539	0.0	533
Pig: starter	154	413	770	943	246	661	1,232	1,509
Pig: finisher	164	321	568	756	262	514	909	1,209
Pig: breeding	23	70.2	125	178	37	112	200	286
Feed materials								
Wheat	162	343	148	341	260	548	236	545
Barley	25	223	77	341	40.9	357	123	545
Oats	26	217	110	341	43	347	176	545
Maize (corn)	804	1,022	3,854	3,939	1,287	1,635	6,167	6,303

Compound/ complementary feeds	Fumonisins				Fumonisins + hidden forms			
	P95		Mean		P95		Mean	
	LB	UB	LB	UB	LB	UB	LB	UB
Soybean meal	2.0	363	0.1	796	3.1	580	0.1	1,273
Rapeseed meal	6.5	344	0.0	342	10	551	0.0	547
Sunflower meal	0.6	257	0.0	341	1.0	411	0.0	545
Peas	0.0	336	0.0	351	0.0	538	0.0	561
Maize gluten feed	1,802	1,915	8,318	8,409	2,884	3,065	13,309	13,454
Wheat feed	12.4	211	34	341	20	338	55	545
Oat feed	0.0	34.1	0.0	34.1	0.0	54.5	0.0	54.5
Sugar beet pulp	1.5	356	0.0	346	2.4	569	0.0	554
Maize silage	160	208	804	804	256	333	1,286	1,286
Grass hay	30	69	43	114	48	110	69	182
Cereal straw	0.0	114	0.0	113	603	1,377	2,909	3,473

LB: lower bound; DM: dry matter; UB: upper bound.

Appendix D – Derivation of the additional factor for hidden fumonisins

The additional factor accounting for hidden fumonisins has been calculated based on raw data obtained on maize and products thereof and reported in the following studies:

- Bryła M, Jędrzejczak R, Roszko M, Szymczyk K, Obiedziński MW, Sekul J and Rzepkowska M, 2013. Application of molecularly imprinted polymers to determine B_1, B_2, and B_3 fumonisins in cereal products. Journal of Separation Science, 36, 578–584.
- Bryła M, Roszko M, Szymczyk K, Jędrzejczak R, Słowik E and Obiedziński MW, 2014. Effect of baking on reduction of free and hidden fumonisins in gluten-free bread. Journal of Agricultural and Food Chemistry, 62, 10341–10347.
- Bryła M, Szymczyk K, Jędrzejczak R and Obiedziński MW, 2015. Free and hidden fumonisins in various fractions of maize dry milled under model conditions. LWT-Food Science and Technology, 64, 171–176.
- Dall'Asta C, Falavigna C, Galaverna G, Dossena A and Marchelli R, 2010. *In vitro* digestion assay for determination of hidden fumonisins in maize. Journal of Agricultural and Food Chemistry, 58, 12042–12047.
- Dall'Asta C, Falavigna C, Galaverna G and Battilani P, 2012. Role of maize hybrids and their chemical composition in Fusarium infection and fumonisin production. Journal of Agricultural and Food Chemistry, 60, 3800–3808.
- Oliveira MS, Diel ACL, Rauber RH, Fontoura FP, Mallmann A, Dilkin P and Mallmann CA, 2015. Free and hidden fumonisins in Brazilian raw maize samples. Food Control, 53, 217–221.

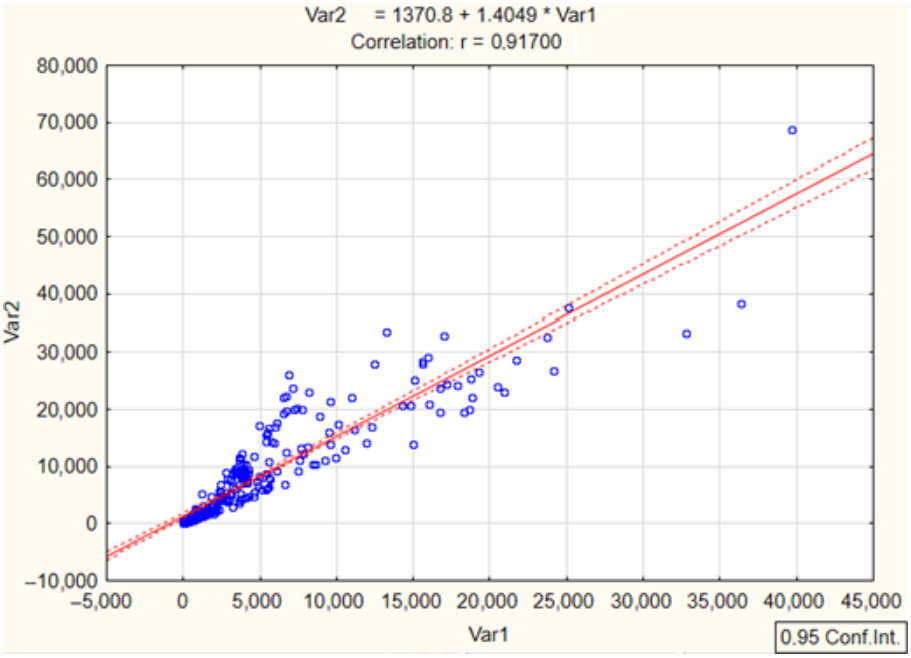
Data were given as the sum of $FB_1 + FB_2 + FB_3$, for a total of n = 316 samples, collected over 6 years (2009–2015) with a wide geographical distribution (Italy, Poland, Brazil).

Table D.1: Fumonisins B data by geographical distribution, years and type of data

Country	Years	Number of data	Type of data
Italy	2009–2015	195	Field studies, natural infection
Poland	2010–2012	49	Marketed products
Brazil	2011–2012	72	Field studies, natural infection

All the studies were based on the double determination of free and total fumonisins. Briefly, the sample was splitted into two subsamples. One was directly analysed for free fumonisins, the second underwent alkaline hydrolysis before detection of HFBs (total fumonisins). The stoichiometrical difference between free and total fumonisins returned the content of hidden fumonisins. Although the applied strategy was the same, analytical methods were slightly different in terms of extraction solvent composition, pH, and instrumental set up.

As first remark, free and total fumonisins were strongly correlated in the three data set as well as in the overall data set, as reported in Figure D.1.



Plot was obtained considering the full data set (n = 316).

Figure D.1: Correlation plot between total fumonisins (Var2) and free fumonisins (Var1)

Data were described using box plot (see Figure D.2), pointing out the strong variability of the Italian and Brazilian data set compared to the Polish one. Besides sample size, this can be explained considering that Polish data were obtained from marketed samples, while Italian and Brazilian samples came from open field studies. It can be noticed as well that data set from Brazil showed higher mean concentration values and a higher variability. This can be explained considering possible differences in the agronomic and environmental conditions that can be found in South America and in Europe.

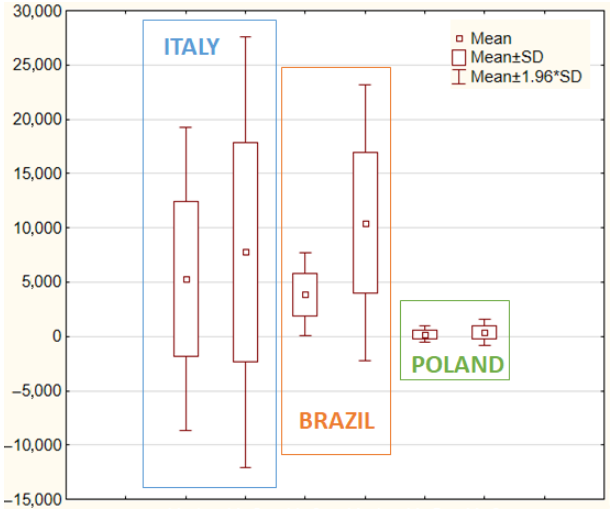


Figure D.2: Box Plot of data considered for the model set up

The overall factor obtained from the contribution of hidden fumonisins was 1.73 (see Table D.2). However, once Brazilian data are taken out, the additional factor was 1.63. Therefore, also in consideration of the previous EFSA Opinion (EFSA CONTAM Panel, 2014), the additional factor used for the exposure assessment was 1.6.

Table D.2: FBs data by geographical distribution, concentrations and derivation of factor for FBs hidden forms

Country	Mean concentration of free FBs	Mean concentration of total FBs
Italy	5,277	7,865
Brazil	3,873	10,441
Poland	202	361
	Factor	
Factor for hidden FBs (overall data set)	*1.74*	
Factor for hidden FBs (Italy+Poland)	*1.63*	

FB: fumonisin B.