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SCIENTIFIC OPINION

Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

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. Modified (often called "masked") mycotoxins are metabolites of the parent mycotoxin formed in the plant or fungus, e.g. by conjugation with polar compounds. Fumonisins, which are difficult to extract from the plant matrix, are also termed modified mycotoxins. The CONTAM Panel considered it appropriate to assess human exposure to modified forms of the various toxins in addition to the parent compounds, because many modified forms are hydrolysed into the parent compounds or released from the matrix during digestion. For modified forms of zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins, 100 %, 30 %, 10 % and 60 % were added, respectively based on reports on the relative contribution of modified forms. The same factors were used for animal exposure from feed. In the absence of specific toxicity data, toxicity equal to the parent compounds was assumed for modified mycotoxins. Risk characterization was done by comparing exposure scenarios with reference doses of the parent compounds. In humans, all lower bound (LB) and upper bound (UB) mean and 95th percentile exposures to the sum of modified and parent toxins were below the respective provisional maximum tolerable daily intakes (PMTDIs) and tolerable daily intakes (TDIs), with two exceptions: for zearalenone and modified zearalenone the UB 95th percentile exposure was up to 2.2-fold the TDI. For fumonisins and modified fumonisins the exposure of toddlers and other children exceeded the PMTDI at both the LB and the UB estimates, which could be of concern. For farm animal species and pets the exposure to the sum of modified and parent toxins was in general not of concern. The risk in fish could not be addressed. The CONTAM Panel identified several uncertainties and data gaps for modified mycotoxins.

KEY WORDS

modified mycotoxins, masked mycotoxins, zearalenone, nivalenol, T-2 and HT-2 toxin, fumonisins, human and animal health

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[1] On request from the European Commission, Question No EFSA-Q-2013-00720, adopted on 25 November 2014.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the risk to human and animal health related to the presence of metabolites and the masked or bound forms of fumonisins, zearalenone, T-2 and HT-2 toxins and nivalenol in food and feed. Modified forms of deoxynivalenol are covered by a separate request. The scientific opinion should comprise the evaluation of the toxicity of these metabolites and masked or bound forms of these mycotoxins for animals and humans compared with the toxicity of the parent mycotoxins. Furthermore, it should contain an assessment of the co-occurrence of the metabolites and masked or bound forms of these mycotoxins in food and feed and an estimation of the human dietary exposure and the animal feed exposure compared with the dietary exposure to the parent mycotoxins. Finally, an assessment of the human and animal health risks should be performed.

The Panel has applied the term modified mycotoxins for masked and bound mycotoxins and mycotoxin metabolites. Modified mycotoxins have up to recently only occasionally been detected when analysing the mycotoxins in their original form, and therefore they have commonly been termed as "masked". In this opinion, the structurally altered forms of mycotoxins covered by the terms of reference are referred to as "modified mycotoxins". The mycotoxins in their unchanged forms are referred to as "parent compounds".

Modified mycotoxins are found in plants resulting from plant defence reactions after fungal infection or are produced by the fungus itself. Modified mycotoxins are metabolites of the parent mycotoxin formed in the plant or fungus, e.g. by conjugation with polar compounds. The most common conjugations are with glucose and modified glucose but also with other groups such as sulphate and glutathione. For fumonisins there is evidence that there are forms that are covalently or non-covalently bound to the matrix. Therefore the term modified fumonisins includes both covalently and non-covalently (i.e. physically entrapped) bound forms for the purpose of this opinion.

Protocols for modified mycotoxin analysis are mainly based on water/acetonitrile extraction followed by analysis with liquid chromatography–tandem mass spectrometry (LC-MS/MS). Both targeted and untargeted methods are used. Immunochemical methods for mycotoxins may not detect modified forms. Concerning fumonisins and their entrapped forms, variability due to different extraction strategies is high. There is a lack of properly validated routine methods owing to the lack of commercially available calibrants and reference materials.

Modified mycotoxins can be released, hydrolysed, biotransformed and absorbed in the gastrointestinal tract, primarily as the parent compound. Although the toxicity of conjugated mycotoxins is not addressed in the present opinion, data in the literature shows that conjugates of xenobiotics can be of toxicological significance. Based on this the CONTAM Panel assumed, as a pragmatic approach, that all modified forms have the same toxicity as their parent compounds.

Bran- and fiber-enriched products are more prone to contamination with mycotoxins, including their modified forms. The EFSA occurrence database contains no data on modified mycotoxins covered by the present opinion. Therefore, occurrence is based on limited information reported in the literature. Literature data shows that modified forms of mycotoxins may add substantially to the overall mycotoxin levels, in particular for zearalenone and fumonisins. For fumonisins, the major contribution comes from physically entrapped parent compounds, whereas for the other compounds, metabolites are the main contributors to modified forms. In order to assess occurrence and exposure, the CONTAM Panel added 100 %, 30 %, 10 % and 60 % to the levels of the parent compounds to account for the modified forms of zearalenone, nivalenol, the sum of T-2 and HT-2 toxins and fumonisins, respectively.

Occurrence data about modified mycotoxins in animal products (i.e. milk and dairy, meat, eggs) are not available, but occurrence in animal products is expected to be very low, since carry-over of *Fusarium* toxins from feed is considered insignificant for human exposure based on currently available data.

The risk characterization for humans is performed by comparing the estimated combined exposure to parent and modified forms of mycotoxins with the established health based guidance values (HBGVs) for the respective parent compounds.

Using the lower bound (LB) approach, no consumers with mean or 95th percentile exposure have combined exposure to zearalenone and modified zearalenone above the tolerable daily intake (TDI) set for zearalenone, indicating no concern. However, if the concentration of modified and parent zearalenone in cereals would be closer to the upper bound (UB) level, high consumers (95th percentile) may have an exposure up to 2.2-fold the TDI for zearalenone of 0.25 µg/kg body weight (b.w.) per day, which would be of concern.

Exposure to the sum of nivalenol and modified nivalenol is not of concern, as the highest 95th percentile UB exposure across studies is less than 20 % of the TDI for nivalenol of 1.2 µg/kg b.w. per day.

Exposure to the sum of T-2 and HT-2 toxins and their modified forms is considered not to be of concern, since all the LB and UB exposures across studies were lower than the TDI of 0.1 µg/kg b.w. per day for the sum of T-2 and HT-2 toxins, with the exception of the highest UB exposure derived for toddlers' (≥ 1 year to < 3 years) exposure, which was similar to the TDI.

The exposure to fumonisins and their modified forms could be of concern, especially in children's age groups. In toddlers, 0.2-14 % (LB) or 43-66 % (UB) and in other children 0-38 % (LB) to 11-59 % (UB) across studies could exceed the provisional maximum TDI (PMTDI) for fumonisins of 2 µg/kg b.w. per day. At high (95th percentile) exposure, the maximal exceedance was 2.5-fold (LB) to 3-fold (UB) the PMTDI.

In order to assess occurrence in feed and exposure of animals, the CONTAM Panel added equal factors for modified mycotoxins in feed as in food.

Levels in feed have been compared with guidance values for feed, if established, and estimated exposure in animals is evaluated in relation to established NOAELs/LOAELs for different animals, when available. Furthermore, known differences in sensitivities to mycotoxins between different species were taken into account when NOAELs/LOAELs were lacking. For fumonisins, contaminated feed has been used in the studies used for derivation of NOAELs/LOAELs, with the exception of poultry, for which the pure compound has been added to feed. For the other *Fusarium* toxins, experiments were carried out with application of pure compounds.

For zearalenone and its modified forms, the mean exposure in pigs in general and the 95th percentile exposure in piglets is not of concern. Occurrence data were inadequate to conclude on risks for fattening pigs and sows receiving feed with a higher than average contamination level. Owing to the lack of either occurrence data or NOAELs/LOAELs, the risk for cattle, goats, rabbits and fish cannot be characterised fully. Estimated exposure in sheep, horses, poultry, cats and dogs is not of concern.

For nivalenol and its modified forms, exposure in pigs, ruminants, poultry, horses, rabbits, cats and dogs is not of concern. Risk could not be assessed in fish because of the lack of occurrence data in feed.

For T-2 and HT-2 toxins and their modified forms, the estimated exposure in pigs, ruminants, horses, poultry, and dogs is not of concern. Risk could not be assessed in fish because of the lack of occurrence data in feed. There is no NOAEL/LOAEL for cats, which are known to be sensitive to T-2 and HT-2 toxins. A concern cannot be excluded in cats but is unlikely based on low exposure.

For fumonisins and their modified forms, exposure in pigs, ruminants, poultry, horses, rabbits, cats and dogs is not of concern. Risk could not be assessed in fish because of the lack of occurrence data in feed.

The CONTAM Panel notes that there is a need for more information on the chemical structures of modified mycotoxins and for further work to identify modified mycotoxins not yet characterised. The

nomenclature, including abbreviations, should be standardised for mycotoxins and their modified forms. Furthermore, there is a need for properly validated and sensitive routine analytical methods. The fate of modified forms upon food and feed processing needs further investigation. More occurrence data on mycotoxins and their modified forms in food and feed need to be generated, in particular for fish and pet food. There is also a need for toxicological data on modified mycotoxins. Re-assessments of the animal health effects of zearalenone and fumonisins are needed in order to set NOAELs/LOAELs for these compounds.

TABLE OF CONTENTS

Abstract 1
Key words 1
Summary 2
Background as provided by the European Commission 7
Terms of reference as provided by the European Commission 8
Assessment 9
1. Introduction 9
2. Legislation 9
3. Previous evaluations considered for the assessment of modified mycotoxins 10
3.1. Human health risk assessments 10
3.1.1. Zearalenone 11
3.1.2. Nivalenol 11
3.1.3. T-2 and HT-2 toxins 12
3.1.4. Fumonisins 12
3.1.5. Summary table of HBGVs derived in previous risk assessments 13
3.2. Previous animal health risk assessments of parent compounds 14
3.2.1. Zearalenone 14
3.2.2. Nivalenol 14
3.2.3. T-2 and HT-2 toxin 15
3.2.4. Fumonisins 16
3.2.5. Summary table of NOAELs/LOAELs derived for animals in previous assessments 16
4. Chemistry of modified forms of certain mycotoxins 17
4.1. Terminology, abbreviations and synonyms 18
4.2. Zearalenone and other resorcylic acid lactones (RALs) 20
4.3. Trichothecenes (nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, diacetylscirpenol, neosolaniol) 22
4.4. Fumonisins 23
4.5. Modified forms of other mycotoxins 24
5. Natural occurrence and environmental fate 24
6. Methods of analysis 28
6.1. Sampling and storage 29
6.2. Extraction, sample clean-up and concentration 29
6.3. Modified forms of zearalenone 29
6.4. Modified forms of trichothecenes 29
6.5. Modified forms of fumonisins 30
6.6. Chromatographic methods 31
6.7. Targeted methods for modified forms of zearalenone and trichothecenes 31
6.8. Targeted methods for modified forms of fumonisins 34
6.9. Immunochemical methods 36
6.10. Conclusions on methods of analysis 36
7. Hazard identification and characterisation 36
7.1. Bioavailability of modified mycotoxins 36
7.2. Toxicity of modified forms of certain mycotoxins in experimental animals 38
8. Occurrence of modified forms of certain mycotoxins in food and feed 40
8.1. Modified forms of zearalenone 41
8.2. Modified forms of trichothecenes 42
8.3. Modified forms of fumonisins 43

8.4. Impact of food and feed processing 44
8.4.1. Effect of sorting and milling 44
8.4.2. Impact food and feed production including thermal treatment 44
8.5. Carry-over 46
8.6. Conclusions on occurrence 46
9. Exposure assessment in animals and humans 47
9.1. Previously reported exposure assessments to modified mycotoxins in humans 47
9.2. Human exposure to modified forms of certain mycotoxins in comparison with exposure to their parent compounds 47
9.2.1. Exposure to zearalenone and its modified forms 47
9.2.2. Exposure to nivalenol and its modified forms 49
9.2.3. Exposure to T-2 and HT-2 toxin and their modified forms 51
9.2.4. Exposure to fumonisins and their modified forms 52
9.3. Animal exposure to modified forms of certain mycotoxins in comparison with exposure to their parent compounds 54
9.3.1. Exposure to zearalenone and its modified forms 55
9.3.2. Exposure to nivalenol and its modified forms 57
9.3.3. Exposure to the sum of T-2 toxin and HT-2 toxin and their modified forms 59
9.3.4. Exposure to fumonisins and their modified forms 61
10. Risk characterisation 63
10.1. Human health risk characterisation 63
10.1.1. Zearalenone 64
10.1.2. Nivalenol 64
10.1.3. T-2 and HT-2 toxin 64
10.1.4. Fumonisins 64
10.2. Animal health risk characterization 65
10.2.1. Zearalenone and its modified forms 65
10.2.2. Nivalenol and its modified forms 67
10.2.3. T-2 and HT-2 toxin and their modified forms 67
10.2.4. Fumonisins and their modified forms 69
10.3. Uncertainty analysis 70
10.3.1. Assessment objectives 70
10.3.2. Exposure scenario/model/input parameters 70
10.3.3. Other uncertainties 71
10.3.4. Summary of uncertainties 71
Conclusions and recommendations 72
Conclusions 72
Recommendations 78
References 79
Appendices 89
Appendix A. Occurrence data for fumonisins 89
Appendix B. Food consumption data for fumonisins 92
Appendix C. Exposure assessment for fumonisins 93
Appendix D. Data management and validation of occurrence data on zearalenone and fumonisins in feed 99
Appendix E. Descriptive statistics of of occurrence data on zearalenone and fumonisins in feed 100
Glossary and abbreviations 105
Glossary 105
Abbreviations 106

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Scientific Committee on Food (SCF) has adopted a scientific opinion

- on 2 December 1999 on deoxynivalenol (DON)[4].

- on 17 October 2000 on fumonisin B1 in food[5], updated on 4 April 2003 as regards fumonisin B2 and fumonisin B3[6]

The Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) adopted an opinion on a request from the Commission

- on 2 June 2004 on deoxynivalenol as undesirable substance in animal feed[7]

- on 28 July 2004 on zearalenone as undesirable substance in animal feed[8]

- on 22 June 2005 on fumonisins as undesirable substance in animal feed[9]

- on 31 May 2011 on the risks for public health related to the presence of zearalenone in food[10]

- on 30 November 2011 on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed[11]

- on 17 May 2013 the risks for animal and public health related to the presence of nivalenol in food and feed[12].

Plants, as living organisms, can alter the chemical structure of mycotoxins as part of their defence against xenobiotics. The extractable conjugated or non-extractable bound mycotoxins formed remain present in the plant tissue but are currently neither routinely screened for in food nor regulated by legislation, thus they may be considered masked. *Fusarium* mycotoxins (deoxynivalenol, zearalenone, fumonisins, nivalenol, T-2 toxin, HT-2 toxin) are prone to metabolisation or binding by plants.

[4] Opinion of the Scientific Committee on Food on Fusarium-toxins Part 1: Deoxynivalenol (DON) , (expressed on 2 December 1999) http://ec.europa.eu/food/fs/sc/scf/out44_en.pdf

[5] Opinion of the Scientific Committee on Food on Fusarium-toxins Part 3: Fumonisin B_1 (FB_1) (expressed on 17 October 2000) http://ec.europa.eu/food/fs/sc/scf/out73_en.pdf

[6] Updated opinion of the Scientific Committee on Food on Fumonisin B1, B2 and B3 (expressed on 4 April 2003) http://ec.europa.eu/food/fs/sc/scf/out185_en.pdf

[7] Opinion of the Scientific Panel on contaminants in the Food Chain of the European Food Safety Authority (EFSA) on a request from the Commission related to deoxynivalenol as undesirable substance in animal feed, adopted on 2 June 2004.

[8] Opinion of the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) on a request from the Commission related to zearalenone as undesirable substance in animal feed, adopted on 28 July 2004.

[9] Opinion of the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) on a request from the Commission related to fumonisins as undesirable substance in animal feed, adopted on 22 June 2005.

[10] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for public health related to the presence of zearalenone in food. EFSA Journal 2011;9(6):2197. [124 pp.] doi:10.2903/j.efsa.2011.2197

[11] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on risks for animal and public health related to the presence of T2 and HT2T2 toxin in food and feed. EFSA Journal 2011; 9(12):2481. [187 pp.] doi:10.2903/j.efsa.2011.2481.

[12] EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2013. Scientific Opinion on risks for animal and public health related to the presence of nivalenol in food and feed. EFSA Journal 2013;11(6):3262.

In the above mentioned scientific assessments on *Fusarium* toxins, the toxicity, animal and human exposure and the risks for animal and public health for the parent mycotoxin are assessed and no or limited reference is made to the metabolites and the masked or bound form of these *Fusarium* toxins.

The Commission has recently requested to EFSA to assess the risks to animal and human health related to the presence of deoxynivalenol, metabolites of deoxynivalenol and masked deoxynivalenol in feed and food and therefore the metabolites and masked or bound forms of deoxynivalenol have not to be addressed in this opinion.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risks to animal and human health related to the presence of metabolites and the masked or bound forms of fumonisins, zearalenone, T-2 and HT-2 toxin and nivalenol in food and feed.

The scientific opinion should, *inter alia,* comprise the:

a) evaluation of the toxicity of the metabolites and masked or bound forms of these mycotoxins for animals and humans and this compared to the toxicity parent mycotoxins

b) assessment of the co-occurrence of the metabolites and masked or bound forms of these mycotoxins in food and feed.

c) estimation of the dietary exposure of the EU population to the metabolites and masked or bound forms of these mycotoxins including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc) and this compared to the dietary exposure to the parent mycotoxins.

d) estimation of the exposure of the different animal species to the metabolites and the masked or bound forms of these mycotoxins from feed and this compared to exposure to the parent mycotoxins.

e) assessment of the human health risks for the EU population including for specific (vulnerable) groups of the population as the consequence of the estimated dietary exposure to the metabolites and masked or bound forms of these mycotoxins and the parent mycotoxins.

f) assessment of the animal health risks for the different animal species as the consequence of the estimated exposure from animal feed to the metabolites and masked or bound forms of these mycotoxins and the parent mycotoxins.

ASSESSMENT

1. Introduction

Food- and feed-related mycotoxins are produced by fungi living on edible plants. The two main defence reactions of plants against mycotoxins are chemical modification and compartmentation. Both processes can alter the chemical structure of these toxins rendering them (at least in part) extractable conjugated and/or non-extractable bound mycotoxins or mycotoxin metabolites. Since these altered mycotoxins are usually not detected when analysing the mycotoxins they originated from, they are commonly termed "masked".

The term "masked mycotoxins" was originally introduced in the early 1990's (Gareis et al., 1990) and, according to the recent literature (Berthiller et al., 2013), refers exclusively to plant metabolites. However, mycotoxins can be modified by living organisms other than plants (i.e. fungi, bacteria, mammals), and by further processing of the edible plants. All these compounds are not covered by the term "masked mycotoxins". In this opinion, according to Rychlik et al. (2014), the structurally altered forms of mycotoxins covered by the terms of reference are referred to as "modified mycotoxins".

For fumonisins there is evidence that there are forms that are covalently or not covalently bound to the matrix. Therefore the term modified fumonisins includes both covalently (i.e. NDF-FB) and not covalently (i.e. physically entrapped) bound forms for the purpose of this opinion.

Most mycotoxins and their modified forms tend to be mainly concentrated in the bran fractions or outer layers of the grains so that other parts of the cereal structure that produce fractions, such as white flour or maize grits, are usually contaminated with lower concentrations of mycotoxin than the fractions or outer layers that are present in the original whole grain.

In accordance with the terms of reference in the present opinion, the risks for public and animal health related to modified forms of zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins are evaluated. This evaluation is done in comparison with the mycotoxins in their unchanged forms, which are referred to as "parent compounds" throughout the text. In the present opinion modified mycotoxins occurring in edible plants (including forms arising from both plant and fungal metabolism), resulting from food and feed processing and from carry-over from contaminated feed to livestock animals are considered. Evaluation of modified mycotoxins resulting from mammalian metabolism is outside the scope of this mandate.

2. Legislation

Article 2 of Council Regulation (EEC) No 315/98[13] stipulates that food containing a contaminant in an amount unacceptable for public health shall not be placed on the market, that contaminant levels should be kept as low as can reasonably be achieved and that, if necessary, the EC may establish maximum levels for specific contaminants. These maximum levels are laid down in the Annex of Commission Regulation (EC) No 1881/2006[14] and may include limits for the same contaminants in different foods, analytical detection limits and reference to the sampling and analysis methods to be used. Modified forms of the *Fusarium* toxins covered by the present mandate are not considered in the Regulation. However, maximum levels (MLs) are listed for the parent compounds zearalenone, T-2 and HT-2 toxins and fumonisins. Nivalenol is not included in the Annex.

[13] Council Regulation (EEC) No 315/93 of February 1993 laying down Community procedures for contaminants in food . OJ L 37, 13.2.1993, p. 1-5.

[14] Regulation (EC) No 1881/2006 of the European Parliament and the Council of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5-24.

Council Directive 2002/32/EC[15] regulates undesirable substances in feed and the Annex I to it contains a list with MLs for certain feed contaminants. Neither the modified forms of the mycotoxins covered by the present mandate nor any of their parent compounds are considered in this Directive. However, Commission Recommendation 2006/576/EC[16] that stipulates data gathering for certain mycotoxins provides guidance values for zearalenone and fumonisin B_1 (FB_1) and FB_2 in animal feed. Furthermore, indicative levels for T-2 and HT-2 toxins in cereals used for feed are provided in Commission Recommendations 2013/165/EU[17] and 2013/637/EU[18].

For the parent compounds of the modified mycotoxins considered in this opinion there are no MLs in feed. Instead, the Commission decided to set guidance values as presented in Commission Recommendation 2006/576/EC (deoxynivalenol, zearalenone, the sum of fumonisins B_1 and B_2, and more recently for the sum of T-2 and HT-2 toxin). These guidance values are merely set to provide orientation to Member States to judge the acceptability of cereals, cereal by-products and compound feed for animal feeding. It is unclear what the relationship is to adverse effects observed in animals. However, it is also mentioned in Commission Recommendation 2006/576/EC that the guidance values for cereals and cereal by-products have been determined for the most tolerant species and should be regarded as upper guidance values.

For zearalenone, guidance values (Commission Recommendation 2006/576/EC) are 0.1 mg/kg for compound feed for piglets and gilts, 0.25 mg/kg for sows and fattening pigs and 0.5 mg/kg for dairy cattle, sheep (including lambs) and goats (including kids). For cereals and cereal products, a guidance value of 2 mg/kg was set, with the exception of maize by-products for which a value of 3 mg/kg was set.

No guidance values have been set for nivalenol.

For the sum of T-2 and HT-2 toxins, the guidance value (Commission Recommendation 2013/165/EU) is 250 µg/kg, except for feed for cats for which a value of 50 µg/kg (Commission Recommendation 2013/637/EU) was established because they are more sensitive. For oat milling products a value of 2 000 µg/kg was set and for other cereal products 500 µg/kg. There is also a range of guidance values for products for human consumption.

For the sum of fumonisins B_1 and B_2, guidance values are 5 mg/kg for compound feed for pigs, horses rabbits and pet animals, 20 mg/kg for poultry, calves (< 4 months), lambs and kids, 50 mg/kg for adult ruminants (> 4 months) and mink. For maize and maize by-products, a value of 60 mg/kg was set.

3. Previous evaluations considered for the assessment of modified mycotoxins

3.1. Human health risk assessments

No comprehensive risk assessments or health-based guidance values (HBGV) are currently available for modified forms of the *Fusarium* mycotoxins addressed in the present opinion. The terms of reference request that the toxicity of the modified forms be compared with the parent compounds. Risk assessments and HBGVs for their respective parent compounds (zearalenone, nivalenol, T-2 toxin, HT-2 toxin and fumonisins) have been presented by EFSA and the Joint FAO/WHO Expert Committee on

[15] Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed. OJ L140, 30.5.2002, p. 10 – 21.

[16] Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T2 and HT2 and fumonisins in products intended for animal feeding. OJ L 229, 23.8.2006, p. 7-9.

[17] Commission Recommendation 2013/165/EU of of 27 March 2013 on the presence of T2 and HT2T2 toxin in cereals and cereal products. OJ L 91, 4.4.2013, p. 12 – 15.

[18] Commission Recommendation 2013/637/EU of of 4 November 2013 amending Recommendation 2006/576/EC as regards T2 and HT2 toxin in compound feed for cats. OJ L 294, 6.11.2013, p. 44.

Food Additives (JECFA) recently. The HBGVs and exposure assessments established in these previous opinions will be used for the present assessment. For fumonisins recent EFSA exposure assessments are not available. An exposure assessment was carried out therefore for the present opinion. Since no recent EFSA HBGV is available for fumonisins, the one established in a recent JECFA evaluation (FAO/WHO, 2012) has been used for risk assessment.

3.1.1. Zearalenone

The CONTAM Panel has developed a scientific opinion assessing the risks for public health related to the presence of zearalenone in food (EFSA CONTAM Panel, 2011a). The Panel concluded that limited evidence is available for the carcinogenicity of zearalenone, which acts as an *in vivo* clastogen. Zearalenone exerts oestrogenic activity in mammals, the most sensitive species being the pig. An tolerable daily intake (TDI) of 0.25 µg/kg body weight (b.w.) per day was derived from a no observed effect level (NOEL) of 10 µg/kg b.w. per day for oestrogenic effects observed in pigs (increased number of piglets with swollen and reddened vulva and cervix, increased weight of uteri), applying an uncertainty factor (UF) of 40 (4 for inter- and 10 for intra-species differences). An UF of 40 was considered to be appropriate by the CONTAM Panel, since it is likely that the human female would not be more sensitive to oestrogens in general, or zearalenone or its metabolites in particular, than the female pig. Therefore it was considered not necessary to include an additional UF of 2.5 for toxicodynamic differences between pigs and humans.

A total of 13 075 analytical results obtained from food samples and 9 877 results on unprocessed grains sampled in 19 European countries between 2005 and 2010 were used as basis for the exposure assessment. Zearalenone was reported at quantifiable levels in 15 % of the samples, with highest concentrations in wheat bran, corn and products thereof. Grains and grain-based foods, in particular grains and grain milling products, bread and fine bakery wares, made the largest contribution to the zearalenone exposure in all age classes. Total chronic dietary exposures for adults (≥ 18 years to < 65 years old) (minimum lower bound (LB) to maximum upper bound (UB)) ranged from 2.4 to 29 ng/kg b.w. per day for average consumers and from 4.7 to 54 ng/kg b.w. for high consumers (95^{th} percentile consumption in total population).The highest exposure estimates were for toddlers (aged ≥ 12 months to < 36 months), at 9.3 to 100 ng/kg b.w. per day for average consumers, and 23 to 277 ng/kg b.w. per day for high consumers.

Overall, estimates of chronic dietary exposure to zearalenone based on the available occurrence data were below or in the region of the TDI of 0.25 µg/kg b.w. per day (250 ng/kg b.w. per day) for all age groups.

3.1.2. Nivalenol

In 2013, the CONTAM Panel published a scientific opinion aimed at identifying animal and public health risks related to the presence of nivalenol in food and feed (EFSA CONTAM Panel, 2013). The Panel concluded that nivalenol is unlikely to be genotoxic and that based on the available data conclusions could not be drawn on its carcinogenicity. Critical effects in mammals are immunotoxicity and haematotoxicity. A TDI of 1.2 µg/kg b.w. per day was established based on a lower 95 % confidence limit for a benchmark response of 5 % extra risk ($BMDL_{05}$) of 350 µg/kg b.w. per day derived from reduced white blood cell counts observed in a 90-day rat study and by applying an UF of 300 to account for inter- and intra-species differences.

A total of 3 846 data on food and 7 611 data on unprocessed grains reported from 2001 to November 2011 were used for exposure assessments. A high proportion (90 %) of results was below the limit of detection (LOD) or limit of quantification (LOQ) in food. The vast majority of data were on grains and grain-based foods. The highest mean concentrations for nivalenol in food and unprocessed grains were observed in oats, maize, barley and wheat and products thereof. Higher concentrations were observed in unprocessed grains than in grains for human consumption. Total chronic dietary exposures in the adult

population (minimum LB to maximum UB) ranged from 0.4 to 75 ng/kg b.w. per day for average consumers, to 1.1 to 224 ng/kg b.w. per day for high (95th percentile) consumers. In elderly (≥ 65 years to < 75 years old) and very elderly (≥ 75 years old) populations, the chronic dietary exposure to nivalenol was slightly lower than in other adults. The highest chronic exposure was estimated for toddlers (≥ 1 year to < 3 years old) ranging from 4.3 to 202 ng/kg b.w. per day for average consumers, to 12 to 484 ng/kg b.w. per day for high consumers. Grains and grain-based foods made the largest contribution to nivalenol exposure. Important contributors were bread and rolls, grain milling products, pasta, fine bakery wares and breakfast cereals.

The CONTAM Panel concluded that residues of nivalenol in products of animal origin could only marginally contribute to human exposure.

All chronic dietary exposures to nivalenol estimated, based on the available occurrence data in food, were below the TDI of 1.2 µg/kg b.w. per day, and are therefore not of health concern.

3.1.3. T-2 and HT-2 toxins

In 2011, the CONTAM Panel adopted a scientific opinion aimed at assessing the risks incurred with the presence of T-2 and HT-2 toxin in food and feed (EFSA CONTAM Panel, 2011b). The Panel concluded that T2 and HT2 impair protein and DNA synthesis and induce haematotoxicity and myelotoxicity. In view of the rapid metabolism of T-2 to HT-2 toxin a group TDI of 0.1µg/kg b.w. per day was established covering both compounds based on a $BMDL_{05}$ of 10 µg/kg toxin/kg b.w. per day for T-2 toxin for immunological effects observed in pigs (decreased anti-horse globulin titre, lymphocyte stimulation with A-HG/PHA/ConA, leucocytes and t-lymphocytes and histological changes in thymus, spleen and lymph nodes) and applying an UF of 100.

A total of 17 683 analytical results for T-2, 16 536 for HT-2 toxin and 20 519 for the sum of T-2 and HT-2 toxin in food, feed and unprocessed grains, collected between 2005 and 2010 from 22 European countries were used as a basis for the exposure assessment. HT-2 toxin concentration represents about two thirds of the sum of T-2 and HT-2 toxin concentration. Highest mean concentrations for the sum of T2 and HT2 were observed in grains and grain milling products. For adults (≥ 18 years to < 65 years old), the minimum LB to maximum UB exposure was 3.4 to 18 ng/kg b.w. per day for average consumers, and 7.2 to 39 ng/kg b.w. for high consumers (95th percentile consumption in total population). The highest chronic dietary exposure estimates are for toddlers (≥ 1 year to < 3 years old), at 12 to 43 ng/kg b.w. per day for average consumers, and 23 to 91 ng/kg b.w. for 95th percentile consumers. Grains and grain-based foods made the largest contribution to the exposure to the sum of T-2 and HT-2 toxin.

Overall, estimates of chronic dietary exposure for populations of all age groups to the sum of T-2 and HT-2 toxin based on the available occurrence data are below this group TDI of 100 ng/kg b.w. per day, and therefore considered of no health concern.

3.1.4. Fumonisins

The most recent comprehensive risk assessment on health effects related to the presence of fumonisins in food has been presented by JECFA (FAO/WHO, 2012) and is based mainly on studies carried out with FB_1. Kidney and liver are the organs most sensitive to fumonisin-mediated toxicity in rats and mice. JECFA concluded that fumonisin B_1 is not mutagenic but capable of inducing reactive oxygen species that could lead to DNA damage and induced kidney and liver tumours in rodents. Based on the structural similarities of the different fumonisin derivatives a group provisional maximum TDI (PMTDI) for fumonisins B_1, B_2 and B_3 of 2 µg/kg b.w. per day based on a $BMDL_{10}$ of 165 µg/kg b.w. per day for fumonisins B_1 for increased incidence of megalocytic hepatocytes observed in a chronic study with mice was derived, adding an UF of 100 for inter-species and intra-species differences.

Dietary exposure levels were assessed based on occurrence data made available to the JECFA and considering the Global Environment Monitoring System (GEMS)/Food consumption cluster diets, which provide mean per capita consumption values based on Food and Agriculture Organization of the United Nations (FAO) food balance sheet data for raw commodities and some semi-processed commodities for 13 clusters of countries. Mean exposure levels estimated for the four clusters covering several European countries ranged from 0.5 to 1.5 µg/kg b.w. per day (minimum LB to maximum UB).

In 2014, EFSA performed an evaluation of the increase in risk for public health related to a possible temporary derogation from the maximum level of deoxynivalenol, zearalenone and fumonisins for maize and maize products (EFSA, 2014). An exposure assessment was performed considering the EFSA Comprehensive European Food Consumption Database. The occurrence data were corresponding to the European data that were considered by JECFA in its risk assessment, plus an additional dataset on maize and maize products.

Exposure estimated in these two assessments are not directly comparable to exposure estimated in the EFSA assessments available for the other mycotoxins. The consumption data considered by JECFA are not the same as the ones considered in the EFSA assessments. The EFSA evaluation performed in 2014 was considering the exceptional situation of high contamination of maize and maize based products. For the sake of comparability with the exposure levels estimated for the other mycotoxins, an exposure assessment to total fumonisins was performed (Appendices A – C).

The dataset used for the exposure assessment of fumonisins contained 3 654 analytical results obtained on food samples that were collected between 2000 and 2010 and reported by 11 European countries (the European data that were considered by the JECFA in its risk assessment (FAO/WHO, 2012). Around 44 % of the analytical results corresponded to the sum of fumonisins B_1, B_2 and B_3 whereas the other results corresponded to the sum of fumonisins B_1 and B_2 only. As done in the JECFA evaluation, the results corresponding to the sum of fumonisins B_1 and B_2 were used together with the ones corresponding to the sum of fumonisins B_1, B_2 and B_3, without any adjustment. In the children age groups, the mean exposure levels range between 0.17 and 1.33 µg/kg b.w. per day at the LB and 0.47 and 1.69 µg/kg b.w. per day at the UB. The 95th percentile of exposure was between 0.49 and 3.29 µg/kg b.w. per day at the LB and between 1.53 and 4.21 µg/kg b.w. per day at the UB. In the surveys covering adult age groups (including adolescents), the mean exposure levels are below the HBGV, being between 0.05 and 0.75 µg/kg b.w. per day at the LB and 0.33 and 1.13 µg/kg b.w. per day at the UB. However, the 95th percentile of exposure is in the region of the HBGV for some surveys, being between 0.12 and 1.76 µg/kg b.w. per day at the LB and 0.56 and 2.27 µg/kg b.w. per day at the UB. Overall, the mean exposure levels estimated are below the PMTDI, but the 95th percentile estimates are in the region of the PMTDI for some surveys.

3.1.5. Summary table of HBGVs derived in previous risk assessments

In Table 1 a summary of the health-based guidance values derived for zearalenone, nivalenol, T-2 and HT-2 toxin and fumonisins is presented. The TDIs are all based on experiments in which pure compound was administered.

Table 1: Health-based guidance values (HBGVs) for zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins

Compound	Health-based guidance values	Reference
Zearalenone	**TDI: 0.25 µg/kg b.w. per day** based on a NOEL of 10 µg/kg b.w. per day for oestrogenic effects in pigs, UF 40	EFSA CONTAM Panel (2011a)
Nivalenol	**TDI: 1.2 µg/kg b.w. per day** based on a $BMDL_{05}$ of 350 µg/kg b.w. per day for reduced white blood cell counts in a 90-day rat study, UF 300	EFSA CONTAM Panel (2013)
T-2 and HT-2 toxins	**Group TDI: 0.1µg/kg b.w. per day** based on a $BMDL_{05}$ of 10 µg//kg b.w. per day for T-2 toxin for immunological/haematological effects seen in pigs, UF 100	EFSA CONTAM Panel (2011b)
Fumonisins (FB_1, FB_2, FB_3)	**Group PMTDI**: **2 µg/kg b.w. per day** based on a $BMDL_{10}$ of 165 µg/kg b.w. per day for megalocytic hepatocytes in mice, UF 100	FAO/WHO (2012)

$BMDL_{05}$: 95 % lower confidence limit for the benchmark dose response of 5 % extra effect; $BMDL_{10}$: 95 % lower confidence limit for the benchmark dose response of 10 % extra effect; b.w.: body weight; TDI: Tolerable Daily Intake; PMTDI; Provisional Maximum Tolerable Daily Intake; NOEL: No Observed Effect Level; UF: Uncertainty Factor.

3.2. Previous animal health risk assessments of parent compounds

As for human health, animal health risk assessments of modified forms of zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins are lacking. Such evaluations have been made available, however, for the parent compounds and will be considered for the present assessment, as presented briefly below.

3.2.1. Zearalenone

The CONTAM Panel issued an opinion related to zearalenone as an undesirable substance in animal feed (EFSA, 2004). The Panel concluded that in mammals the most prominent effects of zearalenone result from its interaction with oestrogen receptors leading to hyperoestrogenism including reduced fertility. Female pigs are the most sensitive species. Similarly, sheep are susceptible to zearalenone-induced fertility effects while poultry and ruminants show a lower sensitivity to zearalenone mediated effects. However, the Panel concluded that it was not possible to derive NOAELs/LOAELs , since the data available were inadequate.

Zearalenone occurs frequently in cereals and grains in particular in maize and maize by-products. Contamination of cereals with zearalenone varies considerably. Concentrations ranging from less than 0.05 mg/kg to a few mg/kg feeding stuff are reported. The Panel concluded in 2004, that due to the great variability in diet composition for the major farm animal species a calculation of actual exposure levels based on occurrence data of zearalenone in individual feed materials could not be carried out. For the current opinion, new data were evaluated showing 1980 acceptable entries (see Appendix E) and these were used for a new exposure assessment. In the more recent opinion on zearalenone in food (EFSA CONTAM Panel, 2011a), a TDI for human exposure was established based on adverse effects in pigs with a NOEL of 10 µg/kg b.w. In addition, also the guidance values set by the EU will be used for evaluating the potential contribution of modified forms to the zearalenone level. However, it should be stressed that the guidance values are not directly linked to animal health, although they take into consideration species differences in sensitivity.

3.2.2. Nivalenol

In their scientific opinion on animal and public health risks related to the presence of nivalenol in food and feed (EFSA CONTAM Panel, 2013), the CONTAM Panel established a lowest observed adverse effect level (LOAEL) of 100 µg/kg b.w. per day for pigs based on observations of pathological changes in the gastrointestinal tract, kidneys and spleen and increased immunoglobulin A levels in plasma. No relevant data were available for ruminants, rabbits, fish, horses, dogs and cats. LOAELs of 360 µg/kg

b.w. per day were derived from erosion and reduced weights of gizzards and of 53 µg/kg b.w. per day from pale and fragile livers and pale kidneys in broiler chicken and laying hens, respectively.

Animal exposure to nivalenol is primarily from consuming cereal grains and cereal by-products. For dairy cows and beef cattle, the estimated LB and UB exposures to nivalenol were between 0.077 and 0.69 µg/kg b.w. per day. Exceptions to this were dairy cows and beef cattle fed on diets consisting predominantly of maize silage. The estimated LB and UB exposures to nivalenol for these were between 1.9 and 4.6 µg/kg b.w. per day. For small ruminants, the estimated LB and UB exposures to nivalenol were between 0.14 and 0.95 µg/kg b.w. per day. The estimated LB and UB exposures for pigs were between 0.24 and 1.6 µg/kg b.w. per day, for poultry 0.24 and 1.9 µg/kg b.w. per day, for rabbits 0.20 and 0.77 µg/kg b.w. per day, and for horses 0.090 and 0.35 µg/kg b.w. per day, respectively. LB and UB exposure estimates of 0.054 and 0.21 µg/kg b.w. per day were calculated for farmed fish. For companion animals, estimated LB and UB exposure for dogs (0.10 and 0.40 µg/kg b.w. per day, respectively) were marginally higher than for cats (0.091 and 0.35 µg/kg b.w. per day, respectively).

Overall, the exposure estimates based on occurrence data in feed indicated that the risk of adverse health effects of feed containing nivalenol was low for pigs and poultry. A health risk assessment for ruminants, rabbits, fish, horses, dogs and cats could not be carried out because of a lack of toxicological data. The Panel noted, however, that the susceptibility of ruminants to nivalenol-mediated toxicity is likely to be low, based on the observed detoxification of the compound by de-epoxidation by rumen microorganisms.

3.2.3. T-2 and HT-2 toxin

In their opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed (EFSA CONTAM Panel, 2011b) the CONTAM Panel derived a LOAEL of 29 µg/kg b.w. per day for pigs on the basis of immunological effects seen with T-2 toxin. Gastrointestinal lesions, altered serum proteins and haematological lesions were seen in ruminants (lambs or calves) at 300 µg/kg b.w. per day of T-2 toxin, which could be considered as a LOAEL according to the Panel. A NOAEL could not be established for poultry but LOAELs of 40 µg T-2 toxin/kg b.w. per day were set each for broiler chickens, turkeys (lesions in the oral cavity) and ducks (reduction in body weight gain) and for laying hens at 120 µg T-2 toxin/kg b.w. per day (infertility and reduced number of eggs). For rabbits a NOAEL of 100 µg/kg b.w. per day was identified for T-2 based on moderate signs of haematological and hormonal effects at 200 – 500 µg/kg b.w. per day. A NOAEL of 13 µg T-2/kg b.w. per day for fish was derived from reduced feed intake, growth and haematocrit and increased mortality at higher doses. Based on the data available, no NOAELs/LOAELs could be established for horses, dogs and cats.

The occurrence data used for the exposure assessment of animals to T-2 and HT-2 toxin are already described in Section 3.1.5.

Animal exposure to the sum of T-2 and HT-2 toxin is primarily from consuming cereal grains and cereal by-products; levels in forages and oilseed meals are generally low. The animals considered were dairy cows, beef cattle, sheep and goats, pigs and piglets, hens, broiler chickens, turkeys, ducks, rabbits, fish, dogs, cats and horses. The highest UB exposure based on the available occurrence data in feed was for milking goats at 3.3 µg/kg b.w. per day and the lowest was for farmed fish at 0.19 µg/kg b.w. per day. Based on estimates of feed intake and the available occurrence data on feeding stuffs, the exposures to the sum of T-2 and HT-2 toxin for ruminants are substantially lower than the LOAELs identified, and are therefore considered unlikely to be a health concern. For pigs and poultry, comparison of the estimates of exposure based on the reported levels of the sum of T-2 and HT-2 toxin in feeds to the $BMDL_{05}$ for pigs indicate that the risk of adverse health effects of feed containing T-2 and HT-2 toxin is low for these species.

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

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

3.2.4. Fumonisins

The CONTAM Panel evaluated toxicity of fumonisins in feed for different animal species of relevance (EFSA, 2005). Fumonisin B_1 was identified as the most toxic fumonisin derivative. Pigs and horses have been identified as the species most sensitive to FB_1 -mediated toxicity. A LOAEL of 200 µg/kg b.w. per day was derived for pigs based on increased sphinganine/sphingosine ratios in serum and for horses based on equine leukoencephalomalacias (a condition also associated with increased sphinganine/sphingosine ratios). For adult ruminants, a LOAEL was set at 2400 µg/kg b.w. per day derived from observations indicative of liver damage in steers, while in calves a NOAEL of 600 µg/kg b.w. per day was observed, based on liver changes and impaired blast genesis at higher dose. For poultry, a LOAEL of 2 000 µg/kg b.w. per day was derived from increased liver sphinganine and sphinganine/sphingosine ratios. In fish (carp) a LOAEL of 10 mg/kg feed was reported based on pathological alterations in pancreas, kidney, heart and brain.

In general, fumonisins are thus far almost exclusively found in maize with levels ranging from 0.02 mg/kg to sometimes tens of mg/kg. In most samples, fumonisin B_1 is the most prevalent toxin which co-occurs with fumonisins B_2 and FB_3. Maize fractions destined for animal feed can be expected to contain higher levels of fumonisins than raw materials. Fumonisins in maize components of animal feed averaged 24 mg/kg, 8.1 mg/kg, 5.7 mg/kg and 1.1 mg/kg in maize screenings, maize meal, maize germ and maize germ bran respectively. It was assumed in the opinion that maize derived feedingstuffs for ruminants and monogastric animals would usually account for less than 20 % of the dry matter intake. As regards young pigs, assuming that they would feed exclusively on maize, a maximum intake of 0.1 mg/kg b.w. per day of fumonisins was estimated.

3.2.5. Summary table of NOAELs/LOAELs derived for animals in previous assessments

In Table 2 a summary of the NOAELs/LOAELs derived for zearalenone, nivalenol, T-2 and HT-2 toxin and fumonisins is presented. For fumonisins in the studies used for derivation of NOAELs/LOAELs, contaminated feed has been used with the exception of poultry for which pure compound has been added to feed. For the other *Fusarium* toxins, experiments were carried out with application of pure compound.

Table 2: NOAELs/LOAELs in animals for zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins

Compound	NOAELs/LOAELs	Reference
Zearalenone	No NOAELs/LOAELs set because of inadequate data. **Pigs** and **sheep** identified as most susceptible species. **Pigs – NOEL: 10 µg/kg b.w. per day** (oestrogenic effects) This NOEL was used to derive the human TDI. Guidance values feed (2006/576/EC): 0.1 mg/kg for compound feed for piglets and gilts, 0.25 mg/kg for sows and fattening pigs and 0.5 mg/kg for dairy cattle, sheep (including lamb) and goats (including kids).	EFSA (2004) EFSA CONTAM Panel (2011a)
Nivalenol	**Pigs – LOAEL: 100 µg/kg b.w. per day** (gastro intestinal-, kidney-, and spleen pathology, ↑ IgA plasma) **Poultry – LOAEL: 53 µg/kg b.w. per day** (liver and kidney effects) No NOAELs/LOAELs set for ruminants, rabbits, fish and companion animals because of lack of data. No guidance values set.	EFSA CONTAM Panel (2013)
T-2 and HT-2 toxins (based on T-2 toxin)	**Pigs – LOAEL: 29 µg/kg b.w. per day** (immunological/ haematological effects) **Ruminants – LOAEL: 300 µg/kg b.w. per day** (gastro intestinal and haematological lesions, altered serum protein) **Poultry – LOAEL: 40 µg/kg b.w. per day** (oral cavity lesions) **Rabbits – NOAEL: 100 µg/ kg b.w. per day** (haematological/hormonal effects) **Fish – NOAEL: 13 µg/kg b.w. per day** (↓ feed intake, ↓ growth, haematocrit, ↑ mortality) No NOAELs/LOAELs derived for companion animals. Indicative value for feed (2013/165/EU) is 250 µg/kg, and the guidance value (2013/637/EU) is 50 µg/kg for cats (2006/576/EC, as amended).	EFSA CONTAM Panel (2011b)
Fumonisins (based on fumonisin B_1)	**Pigs - LOAEL: 200 µg/kg b.w. per day** (↑ sphinganine/ sphingosine ratios in serum) **Ruminants – NOAEL: 600 µg/kg b.w. per day** (liver changes, impaired blastogenesis) **Poultry – LOAEL: 2000 µg/kg b.w. per day** (↑ sphinganine and sphinganine/ sphingosine ratios in liver) **Fish – LOAEL: 10 mg/kg feed** (organ pathology) **Horses – LOAEL: 200 µg/kg b.w. per day** (equine leukoencephalomalacias which are associated with with ↑ sphinganine/sphingosine ratios) Guidance values feed (2006/576/EC): 5 mg/kg for compound feed for pigs, horses (Equidae), rabbits and pet animals, 20 mg/kg for poultry, calves (< 4 months), lambs and kids, 50 mg/kg for adult ruminants (> 4 months) and mink.	EFSA (2005)

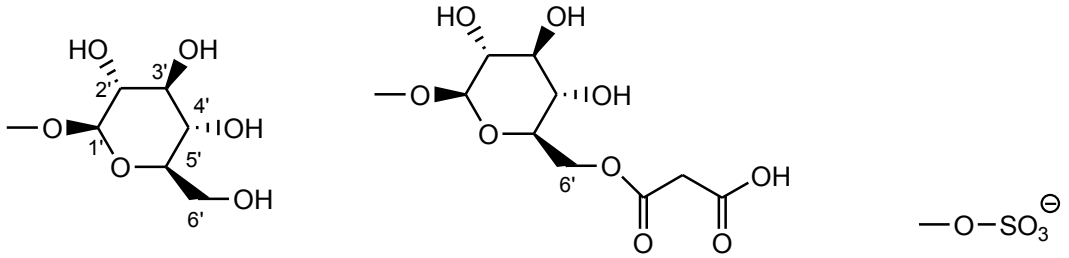
HBGV: Health-Based Guidance Value; b.w.: body weight; TDI: Tolerable Daily Intake; NOAEL: No Observed Adverse Effect Level; NOEL: No Observed Effect Level; LOAEL: Lowest Observed Adverse Effect Level; UF: Uncertainty Factor; ↑: increased; ↓ decreased.

4. Chemistry of modified forms of certain mycotoxins

As will be discussed in more detail in Section 5, modified mycotoxins are usually formed via conjugation of the parent fungal metabolite (mycotoxin) in plants. The predominant pathway is glycosylation, sometimes followed by subsequent metabolic steps to facilitate compartmentation. These pathways are not unique for mycotoxins but are also used by plants to metabolize other xenobiotics, e.g. pesticides, or secondary plant metabolites, e.g. flavonoids.

In contrast to the chemistry of the parent mycotoxins, which has been thoroughly studied in most cases, very little is known about the chemistry of modified forms of mycotoxins to date. In many cases, the evidence for the formation of modified mycotoxins is limited to their detection by high-performance liquid chromatography–mass spectrometry (HPLC-MS), and structural identification is preliminary and mostly based on data obtained by MS and tandem MS (MS/MS). These methods do not provide information on the exact structure of the attached mono- or disaccharide group and the regio- and stereoisomerism. Only in rare cases have glycosides of mycotoxins been chemically synthesized and rigorously identified by methods such as nuclear magnetic resonance (NMR) spectroscopy, e.g. deoxynivalenol-3-glucoside and zearalenone-14-glucoside. Therefore, only limited information is available at present on the chemistry of the modified forms of most mycotoxins covered in this opinion, and it will be given together with the chemical information on the parent (unconjugated) toxins. In addition to the chemical composition, data on the ultraviolet (UV) absorbance and, in some cases, fluorescence of the toxins is included (Sydenham et al., 1996). UV absorbance of the parent mycotoxin is, in general, not affected by conjugation and therefore useful for the analysis of both the parent compounds and the modified forms, e.g. by HPLC with UV detection.

For most monoglycosylated mycotoxins, it is assumed that a ß-D-glucopyranoside group (Figure 1) is attached to a hydroxyl group of the parent mycotoxin. In some cases, e.g. for zearalenone, the formation of a malonylglucoside and a sulphate (Figure 1) has been demonstrated. However, it should be noted that the exact structures of the malonylglucoside has not been proven for modified mycotoxins but is inferred from modified pesticides and modified phytochemicals, e.g. flavonoids. In rare cases, e.g. with type B trichothecenes, glutathione and cysteine have been identified as conjugate groups.



ß-D-Glucopyranoside (ß-D-Glc*p*)

6'-Malonyl-ß-D-glucopyranoside (6'-Malonyl-ß-D-Glc*p*)

Sulphate (Sulf)

Figure 1: Carbohydrate and sulphate groups attached to mycotoxins to yield the modified forms

4.1. Terminology, abbreviations and synonyms

Most mycotoxins have complex names. It is common to use abbreviations even for the parent compounds. The names of the modified mycotoxins become inevitably more unwieldy due to the addition of the conjugate moieties. At present, the abbreviations used in the scientific literature for several parent mycotoxins and their modified forms are not consistent. For example, zearalenone is abbreviated by some authors and journals as ZEA, while others use ZON or ZEN (Metzler, 2011). Glucosides are commonly designated by attaching the abbreviation Glc, Gluc or G. However, the last two are sometimes also used for glucuronides. Moreover, different numbering systems are still in place for some mycotoxins, which complicates the unambiguous designation of the chemical structures of modified mycotoxins, i.e. at which position the conjugate moiety or other chemical groups are attached to the parent toxin. An example is again zearalenone with its conjugated and hydroxylated metabolites (Metzler, 2011).

An unequivocal system of abbreviations and numbering is proposed below. This system would avoid confusion and facilitate the reading of publications from different institutions, and it is expandable to meet future demands.

Table 3 provides a summary of the abbreviations used for parent mycotoxins and their conjugate groups. Only mycotoxins dealt with in this opinion are included, and the abbreviations are divided into those used in this opinion and others used synonymously in the scientific literature. The abbreviations for the parent mycotoxins in this opinion were selected based on their unambuiguity and wide-spread use. For conjugates with carbohydrates, a nomenclature is proposed for this opinion which is used in carbohydrate chemistry and which clearly designates the specific carbohydrate (e.g. Glc for glucose, Man for mannose, Xyl for xylose etc) and its oxidation state (e.g. Glc for glucose, GlcA for glucuronic acid). Although at present the number of different carbohydrates found in modified mycotoxins is very limited, research on conjugates of secondary plant metabolites (e.g. flavonoids) and pesticides in plants leaves little doubt that the diversity of carbohydrates found in conjugated mycotoxins will increase in the future. Moreover, it is to be expected that mycotoxins bind covalently to plant cell walls or other cellular macromolecules via carbohydrate conjugate groups. The chemical structures of such bound mycotoxins can be easily described by the proposed nomenclature, which is suitable to abbreviate even very complex polysaccharides. In cases where the specific carbohydrate is unknown, no abbreviation should be used and the name should indicate the class of carbohydrate (e.g. hexoside or pentoside).

In addition to the parent mycotoxin and its conjugate group, the sites of the covalent bonding on both moieties and, in the case of carbohydrates, the type of ring (e.g. pyranose or furanose) should be given for a complete designation of the modified mycotoxin. So far, only glycosidic bonding, i.e. bonding at the C-1 of the carbohydrate molecule, of D-configurated carbohydrates has been described for modified mycotoxins. In many cases, however, the stereochemistry of the glycosidic bond (α or ß), the type of the carbohydrate ring (pyranose or furanose), and/or the site of bonding to the mycotoxin are not known. The proposed nomenclature can deal with all situations, e.g. ZEN-Glc (glucose conjugate of ZEN with unknown site and stereochemistry of bonding and type of ring), ZEN-14-*O*-Glc (short form ZEN14Glc, glucose conjugate with unknown ring type bonded to the 14-hydroxyl group of ZEN), or ZEN-14-*O*-ß-D-Glc*p* (short form ZEN14ßDGlc, D-glucopyranose with a ß-glycosidic bond to the 14-hydroxyl group of ZEN). Even non-glycosidic bonding can be indicated by using the style common in carbohydrate chemistry, making this abbreviation system highly versatile for future developments in the field of modified mycotoxins.

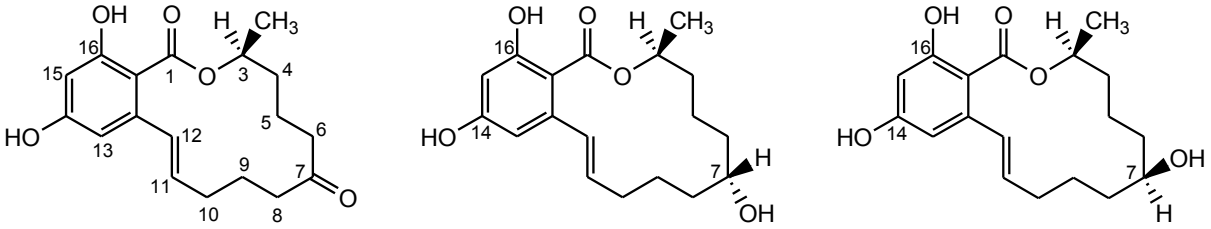
Table 3: Abbreviations and synonyms for parent mycotoxins and their conjugate groups

Mycotoxin	Proposed abbreviation	Synonym
Zearalenone	ZEN	ZEA, ZON, Z
Zearalenol	ZEL	ZOL
Zearalanone	ZAN	
Zearalanol	ZAL	
Nivalenol	NIV	
Fusarenon-X	FUS-X	FusX
T-2 toxin	T2	
HT-2 toxin	HT2	
Deoxynivalenol	DON	D
Acetyldeoxynivalenol	Ac-DON	A-DON, ADON
3-Acetyldeoxynivalenol	3Ac-DON	
15-Acetyldeoxynivalenol	15Ac-DON	
Neosolaniol	NEO	NeoSol
Diacetoxyscirpenol	DAS	
Ochratoxin A	OTA	OA
Ochratoxin B	OTB	OB
Ochratoxin α	OTα	
Fumonisin B	FB	FumB, FUMB
Hydrolyzed fumonisin B	HFB	
Partially hydrolysed fumonisin B	PHFB	
N-deoxyfructos-1-yl fumonisin B	NDF-FB	
N-carboxymethyl fumonisin B	NCM-FB	
Patulin	PAT	PT
Glucoside	Glc	G
Glucuronide	GlcA	GA, G
Sulphate	Sulf	S

4.2. Zearalenone and other resorcylic acid lactones (RALs)

Zearalenone was the first mycotoxin for which transformation to the ß-D-glucopyranoside was demonstrated in wheat and maize cell cultures (Engelhardt et al., 1988) and the term "masked mycotoxin" was coined (Gareis et al., 1990).

Zearalenone (ZEN, Figure 2) is the trivial name for 3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione (Chemical Abstracts Service (CAS) No. 17924-92-4, $C_{18}H_{22}O_5$, molecular weight (MW) 318). ZEN is a macrocyclic ß-resorcylic acid lactone (RAL) produced by *Fusarium* spp. (reviewed in Chełkowski, 1998). Besides ZEN there are several other closely related RALs formed in fungal cultures, e.g α-zearalenol (α-ZEL, Figure 2) and β-zearalenol (ß-ZEL, Figure 2) (Pfeiffer et al., 2010).



ZEN α-ZEL ß-ZEL

Figure 2: Zearalenone (ZEN) and the α- and ß-form of zearalenol (ZEL)

The following UV absorption maxima and molar extinction coefficients were measured by Sydenham et al. (1996) for ZEN in methanol: λ_{max} 235 nm (29 200), 274 (13 040), 316 (5 870); for ß-ZEL in methanol: λ_{max} 236 nm (26 030), 274 (11 130), 316 (4 620). Fluorescence of ZEN (in methanol): Excitation at 315 nm, emission at 460 nm; ß-ZEL (in methanol): Excitation at 315 nm, emission at 460 nm.

As ZEN has two hydroxyl groups, i.e. at positions 14 and 16, two regioisomeric monoglucosides can theoretically be formed. ZEN-14-*O*-ß-D-glucopyranoside (ZEN14ßDGlc*p*, $C_{24}H_{32}O_{10}$, MW 480) was first identified and characterized with ^{1}H- and ^{13}C-NMR spectroscopy as a transformation product of ZEN by *Rhizopus spp*. (Kamimura, 1986). Chemical synthesis by Zill et al. (1990) and Grabley et al. (1992) from ZEN yielded ZEN14ßDGlc*p* (which was then termed 4-glucoside because a different numbering system was used for ZEN) with high yield and purity, and the structure of the *Rhizopus* biotransformation product was confirmed by identical ^{1}H-NMR spectra. The occurrence of ZEN14ßDGlc*p* has since been repeatedly demonstrated both in plant cell cultures and other *in vitro* systems as well as a contaminant in moldy cereal products (see Section 8 on Occurrence of modified forms of certain mycotoxins in food and feed).

The regioisomeric ZEN16ßDGlc*p* ($C_{24}H_{32}O_{10}$, MW 480) has only very recently been detected in an *in vitro* system, i.e. yeast cells expressing a barley UDP-glucosyltransferase, and the structure confirmed by ^{1}H- and ^{13}C-NMR spectroscopy (Kovalsky Paris et al., 2014).

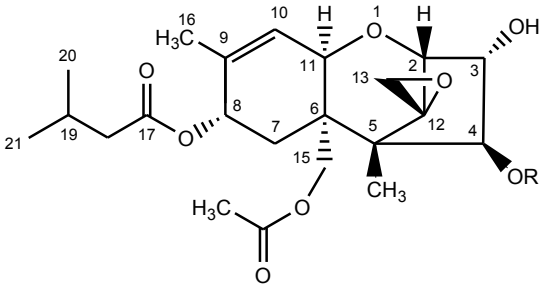
The transformation of ZEN to α-ZEL and ß-ZEL (Figure 2) and the subsequent glycosylation of the three mycotoxins was observed in maize cell suspension cultures (Engelhardt et al.,1999) and in the model plant *Arabidopsis thaliana* based on HPLC-MS/MS analysis (Berthiller et al., 2006). Using an engineered yeast system expressing an *Arabidopsis thaliana* UDP glucosyltransferase, Berthiller et al. (2009) obtained the pure monoglucosides of α-ZEL and ß-ZEL in sufficient amounts for ^{1}H- and ^{13}C-NMR spectroscopy and established their chemical structures as 14-O-ß-D-glucopyranosides ($C_{24}H_{34}O_{10}$, MW 482). Chemical reduction with sodium borohydride of the keto group at position 7 of ZEN14ßDGlc*p* gave rise to a 1:1 mixture of the 14-O-ß-D-glucopyranosides of α-ZEL and ß-ZEL, which were identical in HPLC-MS/MS with the plant metabolites (Berthiller et al., 2009). More recently, Mikula et al. (2013) reported on the chemical synthesis of the 14-O-ß-D-glucopyranosides of α-ZEL and ß-ZEL by regioselective Königs-Knorr glucuronidation of ZEN followed by reduction of the 7-keto group with sodium borohydride, which also led to a partial reduction of the protected glucuronic acid group.

In the study of Berthiller et al. (2006) in *Arabidopsis thaliana*, more than 10 additional conjugated metabolites of ZEN, α-ZEL and ß-ZEL were detected by HPLC-MS/MS, including malonylglucosides, dihexosides (presumably diglucosides) and pentosylhexosides (presumably xylosylglucosides). However, the exact chemical structures of these conjugates still have to be elucidated.

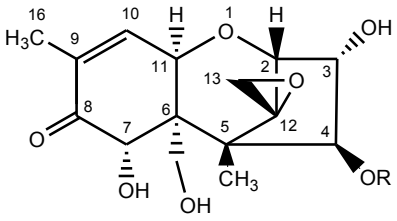
A sulphate conjugate of ZEN was isolated from a culture of *Fusarium graminearum* by Plasencia and Mirocha (1991). On the basis of fast-atom-bombardment mass spectrometry, ^{1}H NMR and UV spectroscopy, and chemical and enzymatic reactions, the structure of ZEN14Sulf ($C_{18}H_{21}O_{8}S^{-}$, MW of the anion 397) was determined (termed ZEN4Sulf according to the old numbering system). The conjugate is soluble in water, methanol, ethanol, and ethyl actetate, slightly soluble in chloroform, and insoluble in hexane, benzene and petroleum ether. ZEN14Sulf was also formed in cultures of other *Fusarium* strains (Plasencia and Mirocha, 1991), and of *Rhizopus arrhizus* (El-Sharkawy et al., 1991). ZEN14Sulf appears to be a frequent contaminant of cereal-based food and feed (see Section 5). Recently, ZEN14Sulf has also been detected by LC-MS/MS in liquid cultures of two *Aspergillus oryzae* strains and seven *Rhizopus species* (Brodehl et al., 2014). These fungi are used for food processing, e.g. the fermentation of soy products. As a further modified mycotoxin, the sulphate conjugate of α-ZEL ($C_{18}H_{23}O_{8}S^{-}$, MW of the anion 399) has been demonstrated in eight out of nine of these cultures by LC-MS/MS, but the exact position of the sulphate group (C-7, C-14, or C-16) is not known.

4.3. Trichothecenes (nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, diacetylscirpenol, neosolaniol)

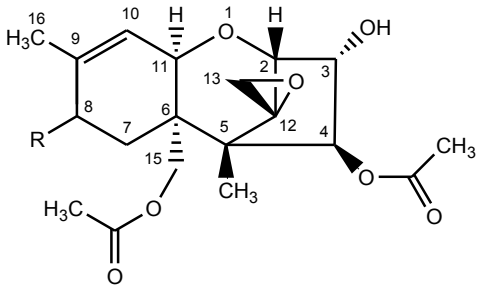
Trichothecenes comprise a large group of mycotoxins, which are produced by *Fusarium* species. Approximately 180 congeners have been discovered so far. The chemical structures of the trichothecenes discussed in this opinion and of DON are depicted in Figure 3.



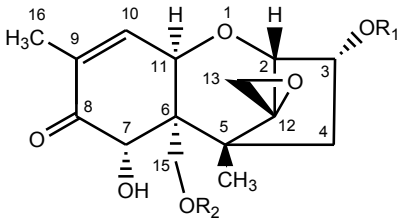
T2: R = $COCH_3$
HT2: R = H



NIV: R = H
FUS-X: R = $COCH_3$



DAS: R = H
NEO: R = OH



DON: $R_1 = R_2 = H$
3Ac-DON: $R_1 = COCH_3$; $R_2 = H$
15Ac-DON: $R_1 = H$; $R_2 = COCH_3$

Figure 3: Chemical structures of the type A trichothecenes T-2 toxin (T2), HT-2 toxin (HT2), 4ß,15-diacetoxyscirpenol (DAS), neosolaniol (NEO), and the type B trichothecenes nivalenol (NIV), fusarenon-X (FUS-X), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3Ac-DON) and 15-acetyldeoxynivalenol (15Ac-DON)

Trichothecenes share a tetracyclic sesquiterpenoid 12,13-epoxy-trichothec-9-ene ring system and are divided into four groups (A-D) according to different functional groups. Whereas type A and B trichothecenes are frequently found as contaminants in cereals and other commodities, type C and D trichothecenes occur only rarely in food and feed. Typical type A trichothecenes, which all have either no substituent or a functional group other than a ketone at the C-8 position, are T-2 toxin, HT-2 toxin, neosolaniol, and 4ß,15-diacetoxyscirpenol (Figure 3). Type B trichothecenes all have a keto group at the C-8 position and include nivalenol, fusarenon-X, deoxynivalenol, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol (Figure 3).

Nivalenol (NIV, Figure 3) is the trivial name for 3α,4β,7α,15-tetrahydroxy-12,13-epoxy-trichothec-9-en-8-one (CAS No. 23282-20-4, $C_{15}H_{20}O_7$, MW 312). Nivalenol is a white crystalline powder with a melting point of 222-223 °C and its specific rotation has been determined as $[\alpha]^{24}_D = +21.54°$ (c = 1.3 mmol/L in ethanol) (Tatsuno et al., 1968). It is soluble in organic solvents of medium to high polarity, such as acetonitrile, methanol, ethanol, ethyl acetate and chloroform, and slightly soluble in water (Budavari, 1989). In methanol, nivalenol has a maximum UV absorption at 221 nm and a molar

absorption coefficient (ε) of 6470 L/mol; after excitation at 240 nm, its fluorescence light is emitted at 480 nm (Sydenham et al., 1996).

Fusarenon-X (FUS-X, Figure 3) has the chemical name 3α,7α,15-trihydroxy-4ß-acetoxy-12,13-epoxy-trichothec-9-en-8-one (CAS No. 23255-69-8, $C_{17}H_{22}O_8$, MW 354) is a biosynthetic precursor of NIV containing an acetyl group at the 4-hydroxy position of NIV. In methanol, FUS-X has a maximum UV absorption at 229 nm and a molar absorption coefficient (ε) of 6 920 L/mol; after excitation at 238 nm, its fluorescence light is emitted at 480 nm (Sydenham et al., 1996).

In HPLC-MS/MS studies of wheat grain artificially infected with *Fusarium* fungi, two compounds were detected which were tentatively identified as 3-*O*-monoglucosides of NIV and FUS-X on the basis of accurate mass measurements, characteristic ions and MS/MS fragmentation patterns (Nakagawa et al., 2011). The elemental formulas and molecular weights are $C_{21}H_{30}O_{12}$ and 474 for the putative NIV3Glc, and $C_{23}H_{32}O_{13}$ and 516 for FUS-X3Glc, respectively.

T-2 toxin (T2, Figure 3) is the trivial name for 3α-hydroxy-4β,15-diacetoxy-8α-(3-methylbutoxy)-12,13-epoxy-trichothec-9-ene (CAS No. 21259-20-1, $C_{24}H_{34}O_9$, MW 466). T2 forms white needles with a melting point of 151-152 °C (Bamburg et al., 1968) and has a specific rotation of [α]26 D = +15° (c = 2.58 in 95 % ethanol) (Pohland et al., 1982). The structure of **HT-2 toxin** (HT2, Figure 3) differs from that of T2 toxin only by the loss of the acetyl group at the C4-position. Accordingly, HT2 has the chemical name 3α,4ß-dihydroxy-15-acetoxy-8α-(3-methylbutoxy)-12,13-epoxy-trichothec-9-ene (CAS No 26934-87-2, $C_{22}H_{32}O_8$, MW 424). The melting point of HT2 is similar to that of T2, at 151-152 °C. At room temperature HT2 toxin forms white crystals. The solubility of both T2 and HT2 is poor in water but good in most organic solvents including methanol, ethanol, acetone, chloroform, ethyl acetate, diethyl ether and acetonitrile (Yates et al., 1968). T2 and HT2 have no UV absorption at wavelengths above 220 nm (Sydenham et al., 1996).

Monoglucosides of T2 ($C_{30}H_{44}O_{14}$, MW 628) and HT2 ($C_{28}H_{42}O_{13}$, MW 586) were tentatively identified in solid and liquid cultures of *Fusarium sporotrichioides* recently, using HPLC-MS/MS methodology (Busman et al., 2011). Based on the availability of hydroxyl groups and in analogy to other trichothecenes, the authors proposed that glycosylation has occurred at the 3-hydroxyl group. Production of the two glucosides was also observed in kernels from wheat and oat inoculated with *F. sporotrichioides* in the same study and in isolates of *Fusarium langsethiae* from wheat in Italy (Lattanzio et al., 2013).

Diacetylscirpenol (DAS, Figure 3) is 3α-hydroxy-4ß,15-diacetoxy-12,13-epoxy-trichothec-9-ene (CAS No. 2270-40-8, $C_{19}H_{26}O_7$, MW 366).

Neosolaniol (Figure 3) is 3α,8α-dihydroxy-4ß,15-diacetoxy-12,13-epoxy-trichothec-9-ene (CAS No. 36519-25-2, $C_{19}H_{26}O_8$, MW 382) and thus the 8α-hydroxylated derivative of DAS. Like other type A trichothecenes, DAS and NEO are soluble in moderately polar solvents, such as chloroform, diethyl ether, ethyl acetate, and acetone. DAS and NEO have no UV absorption at wavelengths above 220 nm (Sydenham et al., 1996).

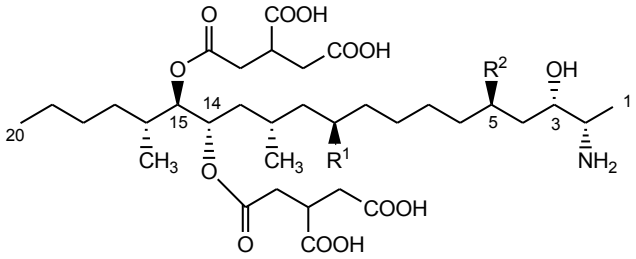
Monoglucosides of DAS ($C_{25}H_{36}O_{12}$, MW 528) and NEO ($C_{25}H_{36}O_{13}$, MW 544) were identified by HPLC-MS/MS in isolates of *F. langsethiae* from wheat in Italy (Lattanzio et al., 2013).

Deoxynivalenol (DON) and the isomeric monoacetylated **3-acetyl-DON** (3Ac-DON) and **15-acetyl-DON** (15Ac-DON) are type B trichothecences. Their chemical formulas are depicted in Figure 3. However, these compounds will not be discussed here since they are covered by a separate request.

4.4. Fumonisins

Fumonisins are a group of mycotoxins produced by various *Fusarium* fungi and are frequently found in stored maize worldwide. They are long-chained aliphatic amines carrying methyl and hydroxyl groups

at various positions of the aliphatic chain. Two of the hydroxyl groups are esterified with tricarboxylic acids. The dominant fumonisins found as food contaminants belong to the B group and comprise FB_1 to FB_4 (Figure 4). FB_1 is chemically described as 1,2,3-propanetricarboxylic acid, 1,1'-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl]ester (CAS No. 116355-83-0, $C_{34}H_{59}NO_{15}$, MW 721). Alkaline treatment leads to partially or fully hydrolyzed fumonisins, in which one or both of the tricarboxylic acid moieties are cleaved off. Fumonisins have no UV absorption at wavelengths above 200 nm (Sydenham et al., 1996).



FB_1: $R^1 = OH$; $R^2 = OH$
FB_2: $R^1 = H$; $R^2 = OH$
FB_3: $R^1 = OH$; $R^2 = H$
FB_4: $R^1 = H$; $R^2 = H$

Figure 4: Fumonisins of the B group

Multiple forms of fumonisins occur in food. In addition to free aglycones, the parent compounds are physically entrapped into the structure of macromolecular components such as starch or proteins and only extractable after hydrolysis of the macromolecules (Dall'Asta et al., 2010). Depending on the conditions of the hydrolysis, partially or fully hydrolyzed fumonisins are released. Owing to the presence of an amino group (at C2, see Figure 4) and the carboxyl groups, fumonisins can undergo various chemical reactions, e.g. Maillard-type reactions with carbohydrates or ester/amide formation with proteins or carbohydrates. Such reactions occur during thermal processing of food (Humpf and Voss, 2004) and give rise to a multitude of products, which causes a major analytical problem.

The occurrence of fumonisins esterified with fatty acids has only recently been reported (see Section 5). The structures of 3-*O*- and 5-*O*-linoleoyl-FB_1 ($C_{52}H_{89}NO_{16}$, MW 983), 3-*O*- and 5-*O*-oleoyl-FB_1 ($C_{52}H_{91}NO_{16}$, MW 985), and 3-*O*- and 5-*O*-palmitoyl-FB_1 ($C_{50}H_{89}NO_{16}$, MW 959) were proposed for six esterified FB_1 isomers detected in small amounts in a *Fusarium verticillioides*-inoculated rice culture (Bartók et al., 2013). Even lower amounts of the respective *N*-acyl-FB_1 isomers were found in the same culture.

4.5. Modified forms of other mycotoxins

There is evidence for the formation of modified forms for a few other mycotoxins, i.e. ochratoxin A (Ruhland et al., 1996a, b), destruxin B (Pedras et al., 2001), patulin (Fliege and Metzler, 2000; Baert et al., 2007) and the *Alternaria* toxins alternariol and alternariol-9-*O*-methylether (Hildebrand et al., in press), however data are very limited. As these toxins are not included in the Terms of Reference, they will not be discussed in this opinion.

5. Natural occurrence and environmental fate

The term "masked mycotoxins" was first introduced by Gareis et al. (1990) to define a mycotoxin derivative that may be cleaved during digestion in mammals to release its parent form. After more than a decade, soluble and insoluble conjugates of mycotoxins that can be formed upon chemical or biochemical reaction fall under the umbrella of this definition. A tentative comprehensive classification

is reported in Figure 5, mainly based on the mechanism of masking (modified from Rychlik et al., 2014). Compounds deriving from chemical reactions occurring upon processing, as described in Section 8.4, are also reported in the figure.

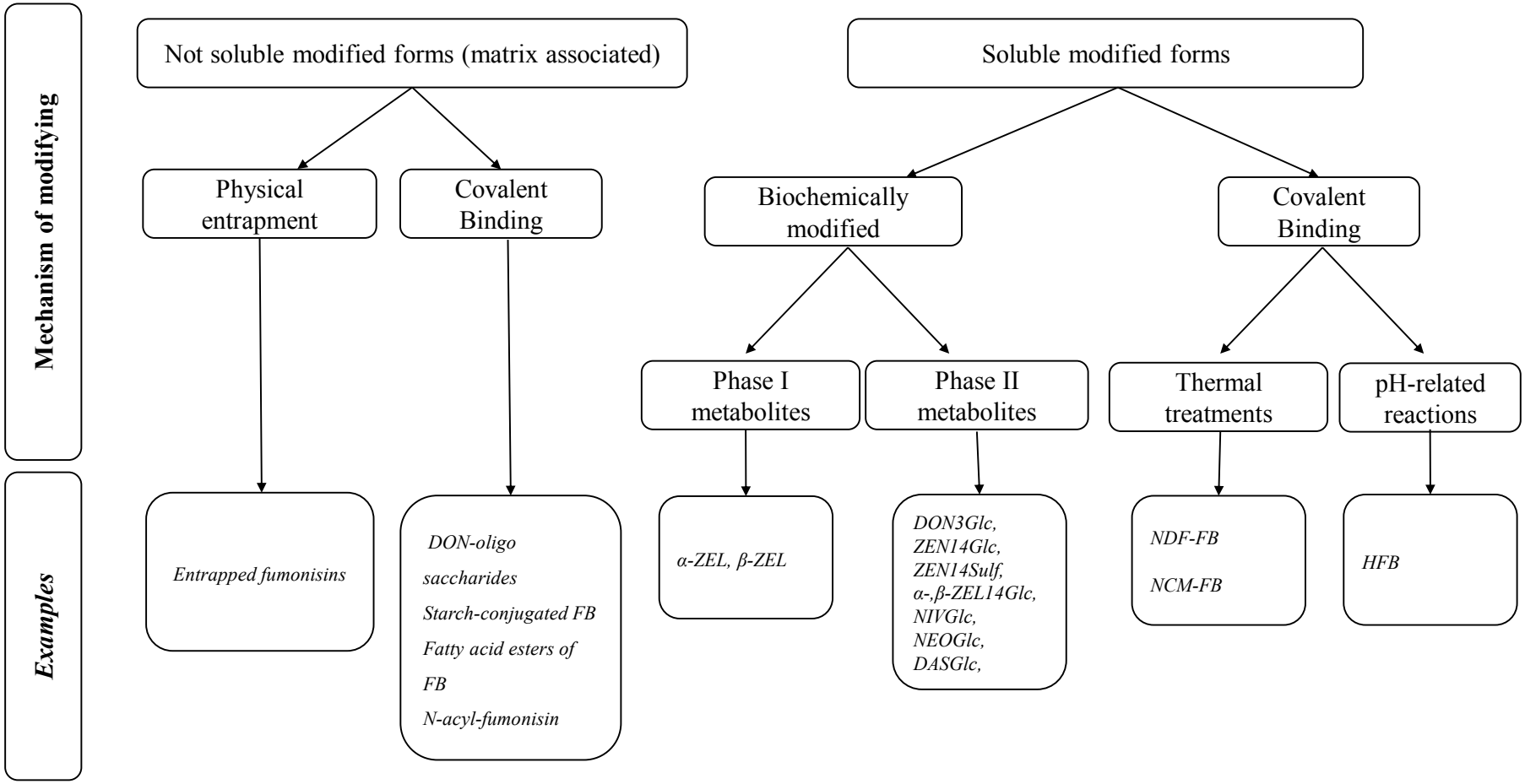


Figure 5: Summary table with main modified mycotoxin species and respective pathways

Modified mycotoxins are mainly produced by plants via enzymatic transformations related to detoxification processes (phase II metabolites) and have been related to a resistance mechanism exerted by plants to counteract pathogen invasion (Lemmens et al., 2005; Berthiller et al., 2007, 2013).

Mycotoxin accumulation in plants mainly occurs in the field, upon fungal infection during the growing season. These toxic compounds are able to interact with vital cell functions, thus being a target of the plant's metabolic detoxification processes. Two main detoxification systems are reported for plants: chemical modification and compartmentation.

Chemical modification in plants is commonly exerted via conjugation reactions, involving residues such as glucose, malonic acid and glutathione (GSH, -glutamyl-cysteinyl-glycine). Commonly, glycosyl residues are added to hydroxyl, thiol, amino and carboxy groups on the parent compound, while malonyl residues are added to hydroxyl and amino groups. Electrophilic sites on the parent compounds are more prone to conjugation to GSH residues. These phase II reactions, commonly catalyzed by glucosyl-, malonyl- and glutathione-*S*-transferases, generate more water-soluble compounds, which can be better eliminated from the cytosol via membrane-bound transporters into the vacuolar or apoplastic space. (Marrs, 1996; Berthiller et al., 2013). In general, these conjugates are assumed to be less toxic for the generating organism (i.e. plants) than the parent compounds.

Among detoxification enzymes, the activity of UDP-glucosyl-transferases (UGT) and glutathione-*S*-transferases (GSTs) to chemicals have been mainly studied in plants (Dixon et al., 1998; Brazier et al., 2002).

Since plants are not able to exert active elimination, compartmentation plays a very important role in the detoxification system. Through the sequestration of conjugates into the vacuole or their irreversible binding to cell walls, detoxification products are permanently stored in the plant tissue rather than excreted. Compounds that are sufficiently polar, can undergo compartmentation in their parent form.

It has been proven that, upon fungal infection, an intense plant-pathogen cross-talk takes place and regulates both fungal infection and mycotoxin accumulation. In response to microbial attack, plants activate a complex series of responses that lead to the local and systemic induction of a broad spectrum of antimicrobial defenses (Berthiller et al., 2013). At the same time, the pathogen activates similar systems, to alter host defense responses, and thus to promote pathogen virulence and disease production. Both plant and pathogen signaling systems may involve different pathways, among those lipid-derived compounds (i.e. jasmonates and oxylipins) play a crucial role (Gao et al., 2007, 2009; Tsitsigiannis and Keller, 2007; Brodhagen et al., 2008).

A number of ongoing studies are addressing the role played by the plant-pathogen cross-talk and by the plant's ability to express UGTs and GSTs in the mycotoxin accumulation and masking, but conclusive results are still lacking.

As an example, significant correlations between specific quantitative trait loci (QTLs) in field resistance against *Fusarium* Head Blight disease and ability to convert DON into DON3G have been pointed out in soft wheat (Lemmens et al., 2005). Similar studies have also preliminarily addressed the formation of DON3G in barley and durum wheat (Gardiner et al., 2010; Cirlini et al., 2014).

Although, among *Fusarium* mycotoxins, the conversion of ZEN, DON, T2 and HT2, FUS-X, NIV, DAS and NEO to their modified forms has been reported so far (Schneweis et al., 2002; Berthiller et al., 2005; Nakagawa et al., 2011, 2013; Lattanzio et al., 2013), the occurrence of modified forms in naturally infected cereals and products thereof have only been described for DON and ZEN (Berthiller et al., 2013; De Boevre et al., 2013). In addition, one recent study reports the occurrence of fatty acid esters of fumonisins in naturally infected maize (Falavigna et al., 2013).

Zearalenone-14-glucoside (ZEN14Glc) was the first modified mycotoxin to be discovered and characterized: its formation was indeed demonstrated in maize cell cultures (Engelhardt et al., 1988),

and then its fate after ingestion in pigs was described (Gareis et al., 1990). Its occurrence was later described in a wide range of grains (Schneweis et al., 2002; De Boevre et al., 2013). Deoxynivalenol-3-glucoside (DON3Glc) (Berthiller et al., 2013; De Boevre et al., 2013) is, however, the most reported modified mycotoxin, along with the two acetylated derivatives of DON (15-acetyldeoxynivalenol, 15Ac-DON and 3-acetyldeoxynivalenol, 3Ac-DON), being proven to frequently occur in naturally infected cereals such as wheat, barley and maize. Besides them, zearalenone-14-sulphate (ZEN14Sulf) was also described in cereal-based products (Vendl et al., 2010). In addition, polyglycosylated forms and glutathione- or cysteyl- conjugates forms have been described mainly for type B trichothecenes (Lattanzio et al., 2013; Kluger et al., 2013).

There is also strong evidence for the compartmentation of fumonisins in plants; occurrence of these physically entrapped forms has been proven in raw maize as well as in derived food, although the nature of the masking mechanism has not been completely clarified (Dall'Asta et al., 2009a, 2010). Several studies demonstrated a good correlation between associative forms of fumonisins (so-called "hidden fumonisins") and the fatty acid profile in maize grown in the field under natural conditions (Dall'Asta et al., 2012). Since, fatty acids are strongly implied in the plant-pathogen interaction, such complexation may occur as a consequence of the cross-talk with an elicitation of the oxylipin pathways in maize (Scala et al., 2013). Very recently, the occurrence of FB_1 fatty acid esters in naturally infected corn kernels has been described (Falavigna et al., 2013). Although already reported by Bartok et al. (2010) in artificially inoculated rice, these compounds have never been described before in corn. In particular, the derivatives formed by FB_1 esterification with oleic and linoleic acids have been detected. These strongly hydrophobic compounds seem to accumulate in at least highly infected kernels, but it is still unclear if they are formed by the fungus or by the plant (Falavigna et al., 2012, 2013).

Similarly, Bartok et al. (2014) reported the occurrence of *N*-palmitoyl-, *N*-oleoyl- and *N*-linoleoyl-FB_1 derivatives in rice cultures of F. *verticillioides*. However, no data on natural occurrence in maize or other cereals are available so far.

6. Methods of analysis

Parent mycotoxins in food- and feedingstuffs are commonly analysed by chromatographic methods, including thin layer chromatography (TLC), gas-chromatography (GC) or liquid chromatography (LC), and immunochemical methods such as enzyme-linked immunosorbent assay (ELISA).

Commonly two different approaches are reported in the literature to detect modified mycotoxins, defined as "direct" and "indirect" methods.

Direct methods are targeted to determine the conjugated forms as they are. This approach usually is based on MS analysis and requires standard calibrants as well as reference materials for validation. Besides DON3Glc, direct methods have been developed mainly for ZEN derivatives, since calibrants can be rather easily synthesized. Although very reliable, only a few validated direct methods have been reported in the literature so far, owing to the common lack of analytical standards. Most of them have been recently reviewed by Cirlini et al. (2012) and Berthiller et al. (2013).

Indirect methods are commonly based on chemical and/or enzymatic treatments to cleave the conjugated forms and release the parent compound. In such a way, the sum of "parent and modified" forms will be simultaneously detected in the sample. The indirect approach can be potentially applied to all modified forms, it works with all the most common chromatographic or immunochemical techniques and it does not requires any specific calibrant, besides the parent compound. In addition, insoluble modified forms, such as DON bound to cell walls or matrix-entrapped fumonisins, can be detected only by indirect methods. The main drawback is actually due to the efficacy of the cleavage reaction in different matrices. Indirect methods are available for fumonisins as well as modified forms of trichothecenes and zearalenone.

6.1. Sampling and storage

Concerning the sampling of modified mycotoxins, the strategies commonly used for their parent compounds are also suitable for conjugates (Van Egmond et al., 2007). Sampling methods are thus in agreement with the current sampling legislation (Commission Regulation (EC) No 401/2006[19]). It must be underlined here that the sampling step is a key step for the accurate and reliable determination of parent and modified mycotoxins in food and feed.

Modified mycotoxins are rather stable under laboratory conditions, but no specific study has been performed so far to assess their stability during storage (Berthiller et al., 2009).

6.2. Extraction, sample clean-up and concentration

Usually, the extraction procedure of modified mycotoxins requires the use of a more polar solvent mixture than for their parent compounds; the mostly used solvent mixtures are based on water, methanol and/or acetonitrile, in different percentages. It has to be considered, however, that conjugates are usually more polar than that used their parents, and that conjugated and parent forms generally co-occur in food. The extraction procedure, thus, should allow the simultaneous extraction of both forms with comparable recoveries.

6.3. Modified forms of zearalenone

Extraction mixtures for ZEN, αZEL and βZEL and their naturally-occurring modified forms are based on acetonitrile and water in different ratios (Cirlini et al., 2012; Berthiller et al., 2013), eventually acidified with acetic acid (Sulyok et al., 2006). The pH of the media seems to play a crucial role: since the glycosylated conjugates are more polar than their parent compounds, Vendl et al. (2009) studied the efficiency of different extraction mixtures in neutral, acidic, neutral polar and acidic polar conditions, using maize samples spiked with multi-mycotoxin standard solutions containing ZEN, ZEN14Glc and ZEN14Sulf. Results showed that the use of a high water content in the extraction solvent causes lower recoveries for ZEN and its derivatives, than in neutral or acid organic mixtures.

De Boevre and coworkers used a mixture of acetonitrile/water in combination with a hexane defatting step, for simultaneous extraction of DON, ZEN, T-2 and 10 metabolites thereof (De Boevre et al., 2012, 2013). It was reported that neutral and acidic extraction gave similar results for DON3Glc, 3Ac-DON, ZEN14Glc, αZELGlc and βZELGlc (Vendl et al., 2009). Using a higher proportion of water reduced the recovery of non-polar toxins. Acidic conditions slightly improved the recovery of DON and significantly improved the yield of ZEN.

After sample extraction, the clean up step is usually skipped to avoid further losses, and the extract is directly analysed by LC-MS/MS, using the so-called “dilute and shoot” approach (Sulyok et al., 2006).

Concerning indirect methods, ß-glucosidase, cellulase and cellobiase have been used to cleave ZEN14Glc, obtaining an almost quantitative release of ZEN (Beloglazova et al., 2013).

6.4. Modified forms of trichothecenes

As reported for ZEN derivatives, the best recovery is obtained also for trichothecenes and their modified forms when acetonitrile and water in different ratios are used as the extraction mixture (Lattanzio et al., 2012; Berthiller et al., 2013), eventually acidified with acetic or formic acid (Sulyok et al., 2006; Nakagawa et al., 2013). Since bran and whole grain products are complex matrices, most of the

[19] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels ofmycotoxins in foodstuffs. OJ L 70, 9.3.2006, p. 12.

intereferences affecting the further steps of the analysis were avoided by using a preliminary defatting step with hexane. Final recovery was consequently increased (De Boevre et al., 2012, 2013).

After sample extraction, the extracts are filtered and analysed with or without prior clean-up, which is generally based on the use of multifunctional column, immunoaffinity column (IAC), solid-phase extraction (SPE) etc., and is usually aimed at enhancing signal to noise ratio for extracts obtained by complex matrices with heavy matrix effects (Malachova et al., 2011; Lattanzio et al., 2013).

Commercially available clean-up columns for mycotoxins, including SPE and IACs, are often less suitable for conjugated derivatives, since these forms are more polar than their parents. On account of the very good sensitivity of mass spectrometry equipment available nowadays, extracts obtained from the acetonitrile/water/acetic acid mixture were suitable for analysis without further clean-up, using the so-called "dilute and shoot" approach (Sulyok et al., 2006). De Boevre tested four SPE cartridges and showed that no acceptable recoveries were obtained for modified mycotoxins (De Boevre et al., 2012).

Flow-through cartridges were also checked for their applicability to multianalyte purification in a certain polarity range (Lattanzio et al., 2013). Results showed that these systems, in the vast majority of cases, did not permit efficient recovery of modified mycotoxins.

Recently, the application of a QuEChERS-like (Quick, Easy, Cheap, Effective, Rugged and Safe) approach was reported by Zachariasova et al. (2010) for parent and modified forms of trichothecenes in wheat. Although Zachariasova et al. (2010) did not report satisfactory recovery for DON3Glc owing to its higher polarity than DON, a different result was obtained by Dall'Asta et al. (2013), by lowering the polarity of the solvent mixture. Indirect methods are not often applied to analysis of modified forms of trichothecenes, since the enzymatic cleavage has been proven to lead to a lower yield than those obtained for ZEN derivatives (Berthiller et al., 2011). This is mainly due to the nature of the glucosidic bond that involves, in this case, an alcoholic and not a phenolic group.

Enzyme treatments with amylolytic (α-amylase, amyloglucosidase), proteolytic (papain) or cell wall degrading (cellulase, xylanase) enzymes have been proposed for releasing DON from bran (Zhou et al., 2008); although never tested so far, a similar approach could be followed for other modified mycotoxins. This is in agreement with the higher content of modified mycotoxins (DON3Glc) released upon malting in the brewing process (Lancova et al., 2008; Vendl et al., 2009), as described in Section 8.4.3.

6.5. Modified forms of fumonisins

Fumonisins will lose their conjugated side chains upon treatment with strong bases. 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). In particular, it has been observed that performing alkaline hydrolysis of contaminated corn products leads to an often higher amount of released hydrolysed fumonisins than that stoichiometrically derived by the conversion of the fumonisins detectable by routine analytical methods.

The general approach to the evaluation of modified fumonisin forms is based on an alkali treatment of the sample that induces the loss of the tricarballylic side chains of fumonisins, releasing the hydrolysed fumonisins (HFBs), which can be easily quantified by LC–MS. Indeed, comparing the results obtained after the hydrolysis step (amount of total fumonisins) with the amount of fumonisins determined by the normal approach (parent fumonisins), it is possible to evaluate the amount of bound or physically entrapped forms. For the extraction of total fumonisins, the sample undergoes a hydrolysis step with 2 N KOH for 60 minutes at room temperature. The aqueous phase is then extracted with acetonitrile, dried under a nitrogen stream and redissolved in water/methanol before LC-MS/MS analysis. The estimated recovery for HFB_1, HFB_2 and HFB_3 ranged from 92 % to 98 % with a low quantification limit (70 µg/kg for HFB_1, HFB_2 and HFB_3).

A QuEChERS-like approach was also reported for determination of physically entrapped fumonisins (Dall'Asta et al., 2010). This approach, originally developed for pesticides, is based on the use of a mixture of salts in combination with aqueous-organic solvents allowing for protein precipitation and matrix disaggregation. When primary amines are avoided, both parent and physically entrapped forms of fumonisins are efficiently recovered from the samples.

Among the conjugated forms of fumonisins, NDF-FB_1 and NCM-FB_1 have been described in processed food (Castelo et al., 2001; Seefelder et al., 2001; Voss et al., 2001). These forms 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.

Fatty acid esters of FB_1 have been recently reported in rice and maize (Bartok et al., 2010; 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.

6.6. Chromatographic methods

Mass spectrometry based techniques are the method of choice for masked mycotoxin analysis. The selectivity of this detection approach allows avoiding clean-up steps, being particularly useful for the simultaneous determination of multiple mycotoxins and multiple metabolites.

Those methods aimed at detecting a specific target analyte (or a group of analytes) are defined targeted methods and are mainly based on LC-MS/MS. These protocols should be validated and usually allow a reliable and accurate quantification of the target analytes. Quantification mainly involve multiple reaction monitoring (MRM) analysis, while identification and characterization of metabolites are commonly based on enhanced product ion (MS/MS) and multiple MS (MS3) modalities.

Most of the LC-MS/MS methods published so far for masked mycotoxins have been reviewed by Cirlini et al. (2012) and Berthiller et al. (2013).

The method of choice for the identification of unknown masked mycotoxins in processed or unprocessed food is high-resolution MS, mainly used as untargeted analysis. Unknown mycotoxin conjugates, indeed, occur often in naturally contaminated samples at low amounts. Isolation and characterization by means of traditional spectroscopic techniques, such as NMR, is thus very challenging and high resolution MS can act as a good alternative, since additional information such as the structure of the parent compound and its general fragmentation pathways are usually available.

The most used MS methods are those based on soft ionization modes such as electrospray (ESI) or atmospheric pressure chemical ionization (APCI). In particular, collision-induced dissociation (CID) experiments with MS/MS can be used for analysing the dissociation of the pseudo-molecular ions preferentially generated by the soft ionization methods. The equipment mainly used for structure elucidation are triple-quadrupoles and ion traps, together with hybrid instruments such as combinations of quadrupole and linear ion trap, quadrupole and time-of-flight (Q-TOF), linear ion trap and Fourier transform ion-cyclotron resonance (FT-ICR).

For the screening of unknown compounds, full scans over a wide mass range are often used. TOF and Fourier transform (e.g. Orbitrap) instruments are superior to quadrupole mass spectrometers in terms of full-scan sensitivity, mass accuracy and resolving power. Besides the acquisition of accurate masses of analytes, other additional post-data acquisition mathematical tools can be applied to help the molecular identification.

6.7. Targeted methods for modified forms of zearalenone and trichothecenes

Most of the methods reported in the literature so far involve a separation on C18 column using gradient elution with a mixture of water/methanol or water/acetonitrile in different proportions as eluent (Cirlini

et al., 2012; Berthiller et al., 2013; De Boevre et al., 2013). Detection is mainly performed by ESI source, utilizing the MRM mode in both positive and negative polarity according to the analyte. Eluents are commonly modified upon addition of formic or acetic acid or by addition of cationisation agents such as ammonium acetate. The use of slightly acidic conditions allows control of the pH of the eluent, as this is often a critical point, as some mycotoxins such as fumonisins may otherwise show peak tailing and low efficiency in chromatographic separations.

The majority of the methods reported in the literature so far are focused on the simultaneous detection of ZEN and its modified forms as well as DON and its modified forms (Sulyok et al., 2006; Berthiller et al., 2007; Vendl et al., 2009; De Boevre et al., 2012). Often other minor trichothecenes can be also detected. This multitoxin approach is often based on a compromise in terms of recovery and accuracy. Specific conditions are reported in Table 4.

Although conjugated forms of T2 and HT2, as well as NIV and DAS glucosides, have been reported in the literature, there is a lack of validated protocols for these compounds. Data are based, indeed, on a rough comparison of the analyte peak area with the one obtained for the parent compound. Furthermore, the extraction strategy is very generic, without any evaluation of the recovery (Lattanzio et al., 2013).

Table 4: Overview of analytical procedures reported in the literature for ZEN and its modified forms

Target analyte	Matrix	Extraction mixture	Clean up	Separation	Detection	Recovery	References
ZEN, ZEN14Glc, ZEN14Sulf	*Arabidopsis thaliana*	ACN/H_2O (75/25)	None	HPLC (RP-C18): MeOH + 5 mM aqueous ammonium acetate	QTrap-MS/M: ESI source, MRM mode	100 %	Berthiller et al. (2006)
DON, 3Ac-DON, 15Ac-DON, DON3Glc, ZEN, ZEN14Glc, ZEN14Sulf, HFB_1	Cereals	ACN/H_2O/AcOH	None	HPLC (RP-C18): ACN/H_2O/AcOH + 5 mM aqueous ammonium acetate	QTrap-MS/M: ESI source, MRM mode	Not reported	Sulyok et al. (2006); Berthiller et al. (2007); Dall'Asta et al. (2009a)
DON, ZEN, DON3Glc, 3Ac-DON, ZEN14Glc, ZEN14Sulf	Cereal-based food	ACN/H_2O/AcOH	None			Not specified	Vendl et al. (2010)
DON3Glc, α-, βZEL14Glc, ßZEL14Glc, ZEN14Glc, ZEN14Sulf	Maize/wheat/oats /bread/cornflakes	ACN/H_2O/AcOH 79:20:1	Hexane defatting				De Boevre et al. (2012)

ACN: acetonitrile; AcOH: acetic acid; MeOH: methanol.

6.8. Targeted methods for modified forms of fumonisins

For the separation and detection of physically entrapped fumonisins, a very useful approach is to convert the molecules into their hydrolysed counterparts by chemical or enzymatic hydrolysis before the chromatographic analysis. This approach allows for the detection of the sum of parent and bound fumonisins (often referred as "total fumonisins") as hydrolysed forms. The main drawback is that where the concentration of parent compound is needed, two different determination should be performed to obtain data for parent and total fumonisins, thus increasing the method uncertainty.

Kim et al. (2003) separated HFB_1 and HFB_2 on a C18 column, in an LC–MS system, using 0.1 % aqueous formic acid and methanol/acetonitrile as mobile phase and positive ESI as detection mode. More recently, a method for the detection of physically entrapped fumonisins after alkaline hydrolysis in the forms of HFB_1, HFB_2 and HFB_3 was validated by Dall'Asta et al. (2008). A LC–MS/MS system was used, where LC was coupled with a triple quadrupole mass spectrometer equipped with an ESI interface (positive ion mode). The chromatographic conditions used water and methanol as eluents, both added with 0.1 % of formic acid. Detection of the analytes was achieved by multiple reaction monitoring (MRM) mode. The method allowed to determine parent and modified fumonisins without any clean up step.

N-deoxy-fructosyl fumonisin B1 (NDF-FB_1) and N-carboxymethyl-fumonisin B_1 (NCM-FB_1) are usually analysed by LC-MS/MS using the same extraction procedures commonly applied for the parent compounds (Seefelder et al., 2003)

Firstly identified by Bartok et al. (2010), fatty acid esters of FB_1 have been also reported in corn by Falavigna et al. (2013). Bartok et al. (2010) developed two methods, based on RP-HPLC/ESI-ITMS and RP-HPLC/ESI-TOFMS, for the identification of palmitoyl-, oleoyl- and linoleoyl-*O*-derivatives of FB_1 in fungal cultures. Starting from these methods, Falavigna et al. (2013) proposed a LC-ESI-MS/MS method for the determination of these compounds in corn kernels, involving a solid-liquid extraction followed by a dilute and shoot approach.

Very recently, a LC-ESI-MS/MS based method for N-acyl fatty acid derivatives of FB_1 has been proposed by Bartok et al. (2013). The method was developed for fungal cultures of *F. verticillioides* and involved a SPE purification step before chromatographic analysis.

Similar compounds were found in fried tortillas and corn-based food, using thermal treatments (Park et al., 2013). The authors suggested an apolar extraction followed by SPE purification. Afterwards, the eluate was further hydrolysed under alkaline conditions to get hydrolysed fumonisins. After another SPE-C18 purification step, HFBs were detected by LC-FLD with derivatisation with ortho-phthalaldehyde (OPA).

The specific conditions of analysis are reported in Table 5.

Table 5: Overview of analytical procedures reported in the literature for fumonisins and their modified forms

Target analyte	Matrix	Extraction mixture	Clean up	Separation	Detection	Recovery	References
HFB_1, $PHFB_1$, $PHFB_2$	Corn flakes	Ethyl acetate after a hydrolysis step	C18 and FumoniTest™ column	HPLC (RP-C18): 1 % HOOH/ACN/MeOH	Fluorescence detection/ESI-MS/MS : positive ionization, SIM mode	63–86 %	Kim et al. (2003)
HFB_1, HFB_2, HFB_3	Corn and corn-based food	ACN after a hydrolysis step	None	HPLC (RP-C18): 1 % HOOH//MeOH	ESI-MS/MS: positive ionization, MRM mode	92–98 %	Dall'Asta et al. (2009b, 2010, 2012)
Fatty acid esters of fumonisins	Corn and rice	H_2O/MeOH 25/75	None	HPLC (RP-C18): 1 % HOOH//MeOH	ESI-MS/MS: positive ionization, MRM mode	Not reported	Falavigna et al. (2012, 2013)
NDF- FB_1	Corn-based products	H_2O/MeOH/ACN 50/25/25	None	LC-ESI-MS/MS	ESI-MS/MS: positive ionization, MRM mode	Not reported	Voss et al. (2001); Seefelder et al. (2003)
Matrix bound fumonisins	Corn grits or extruded products	H_2O/MeOH/ACN 50/25/25 followed by KOH hydrolysis	OASIS™ HLB column	HPLC (RP-C18): 1 % HOOH//MeOH	Fluorescence detection	Not reported	Bullerman et al. (2008); Jackson et al. (2011)
N-acyl fatty acid derivatives of FB_1	Fungal cultures	H_2O/ACN 1/1	None	HPLC (RP-C18): 1 % HOOH//MeOH	ESI-MS/MS: positive ionization, MRM mode	Not reported	Bartok et al. (2013)
N-acyl fatty acid derivatives of FB_1	Retail fried corn foods	Hexane/Chloroform extraction, KOH hydrolysis	Bond Elut silica SPE, OASIS™ HLB			72 – 85 %	Park et al. (2013)

ACN: acetonitrile; AcOH: acetic acid; MeOH: methanol. For the purposes of this opinion the term modified fumonisins includes both covalently (i.e. NDF-FB) and not covalently (i.e. physically entrapped) bound forms.

6.9. Immunochemical methods

ELISA methods continue to be widely used for fast screening of commodities and foods for mycotoxins owing to their relatively low cost and easy application (Berthiller et al., 2013). However, the application of antibody-driven systems to modified mycotoxin detection is still poor. Antibodies developed against the parent mycotoxin can potentially cross-react with modified forms if the epitope is not sterically hindered by the metabolism (Köppen et al., 2010). Only a few studies on the evaluation of ELISA for the recognition of modified mycotoxins have been performed, probably on account of the unavailability of analytical standards from the market (Dzuman et al., 2014). To date, only two attempts at developing antibodies for modified forms have been reported, one being focused on ZEN14Glc determination (Beloglazova et al., 2013) and the other one on T2 glucoside detection (Maragos et al., 2013).

For accurate data interpretation, a deep knowledge on cross-reactivity of ELISA kits is crucial. However, owing to the lack of commercially available calibrants for modified mycotoxins, cross-reactivity studies are lacking, with the exception of DON3Glc (Tangni et al., 2010; Veršilovskis et al., 2011; Dzuman et al., 2014).

6.10. Conclusions on methods of analysis

Protocols for modified mycotoxin analysis are mainly based on water/acetonitrile extraction followed by a dilute and shoot analysis by LC-MS/MS. There is, however, a general lack of analytical standards and reference materials. Since the development of fit-for-purpose validated methods is based on the use of proper calibrants and naturally incurred reference materials, a very low number of properly validated methods for modified mycotoxins are available. Modified mycotoxins such as ZEN14Glc, ZEN14Sulf or T2Glc are in-house chemically or enzymatically synthesized for research purposes, or isolated from naturally contaminated samples (McCormick et al., 2012; Mikula et al., 2013) and are not commercially available so far. Also biosynthetic methods for glucosylation are known (Berthiller et al., 2009). In this case, the storage of in-house standards should carefully consider the stability of the compound under storage conditions. Soluble conjugated mycotoxins are prone to hydrolysis to their parent toxins in aqueous or methanolic solutions (i.e. ZEN14Sulf to ZEN). The use of aprotic solvents (i.e. acetonitrile) is thus strongly recommended (Berthiller et al., 2013).

Concerning physically entrapped fumonisins, recovery is strongly affected by the efficiency of the analytical protocol, the critical steps being the sample preparation (i.e. particle size) and extraction. An optimal recovery could be obtained when a suitable protocol, involving proper grinding and using extracting mixtures able to disrupt physical complexation, is implemented.

7. Hazard identification and characterisation

7.1. Bioavailability of modified mycotoxins

Little is known about bioavailability of masked forms of mycotoxins other than DON and to some extent ZEN. Therefore, below studies on DON are also presented to exemplify the principles for the bioavailability of modified mycotoxins.

A rather important discussion with respect to modified mycotoxins, is whether they can be hydrolysed and absorbed in the gastrointestinal (GI) tract. In one of the first studies on modified mycotoxins, Gareis et al. (1990) treated a young pig (27 kg) for 14 days with about 600 µg/day of the 14-glucoside[20] of ZEN spiked to the feed. Both ZEN and its oestrogenic metabolite α-ZEL were detected in urine and faeces in varying daily amounts. Highest amounts were observed on day 6 with an estimated excretion in urine around 70 and 90 µg/day for ZEN and α-ZEL, as compared with 80 and 40 µg/day in the

[20] Termed 4-glucoside in original paper.

faeces. This study implies that substantial amounts of the glucoside are hydrolysed and subsequently absorbed, thus contributing to the overall exposure to this mycotoxin.

This observation was supported by a recent paper by Veršilovskis et al. (2012), who exposed rats by oral gavage to a mixture of the ZEN14Glc, DON3Glc and fully ^{13}C-labelled ZEN and DON (each at a dose of 25 µg). Rats were killed after 55 minutes and various tissues and parts of the GI tract (tissue plus content) were analysed. No parent mycotoxins, the glucosides or glucuronides were detected in liver or bladder, potentially related to the short exposure. Highest amounts of both ZEN14Glc but also parent unlabelled ZEN (one quarter to one third of the glucoside) were detected in the stomach, confirming the hydrolysis of this glucoside in the upper part of the GI tract as shown by Gareis et al. (1990). Levels in the small intestine and the colon were much lower with relatively higher levels of the parent form. Also the glucuronide was detected in the stomach (small amounts) and intestines. The situation for DON3Glc was very different with hardly any hydrolysis in the stomach and non-detectable levels in the intestines.

The relative resistance to degradation of DON3Glc is in line with results from in vitro studies by Berthiller et al. (2011), showing no hydrolysis under acidic conditions or in the presence of various digestive enzymes. However, the glucoside was substantially hydrolysed by a number of bacteria that can be present in the lower part of the GI tract, such as *Enterococcus mundtii* and *Lactobacillus plantarum*.

In a follow-up study, Nagl et al. (2012) exposed rats to 2.0 mg/kg b.w. of DON or 3.1 mg/kg b.w. of DON3Glc. In the case of DON, about equal amounts of DON plus known metabolites (15 and 13 %) were recovered in urine and faeces, in the urine primarily as the glucuronide and in the faeces as the de-epoxide, DOM-1. In the case of the glucoside, most of the compound was recovered in the faeces (17 %) as DON and DOM-1, and a small amount of the glucoside. The urine contained much lower amounts (4 %) and indicates that the glucoside is probably hydrolysed at the lower part of the GI tract, resulting in a much lower but possibly not negligible bioavailability of the parent mycotoxin. Overall recovery of the administered compounds was rather low, being 28 % for the parent compound and 21 % for the glucoside.

Using an *in vitro* digestion model, De Nijs et al. (2012) showed similar results to Berthiller et al. (2011), i.e. that DON3Glc is not hydrolysed under conditions typical of the upper parts of the human GI tract. Furthermore, DON3Glc was not absorbed or metabolized by CaCo-2 intestinal cells, an *in vitro* model to study intestinal absorption.

Nagl et al. (2014) also treated piglets orally with DON or DON3Glc. It was shown that the major part of the applied DON (85 %) was excreted in the urine over 24 hours, primarily within the first 8 hours. Most of the DON was excreted as the parent compound (51 %) but also the 3-glucuronide (19 %) and 15-glucuronide (15 %) were detected at substantial amounts. In the case of DON3Glc, less than half of the dose was recovered (42 %), most of it in the urine (40 %) and in this case most of it in the urine collected between 8 and 24 hours, suggesting a delayed absorption. Since no urine was collected after 24 hours, a major part of the dose might have been missed. Only a minor amount in the urine was present as DON3Glc and most of it was DON and to some extent the two glucuronides. When DON3Glc was injected in the blood of piglets, almost all of the dose was excreted within 8 hours in the urine, almost completely as DON3Glc. This study shows that in pigs DON can be released from DON3Glc, probably in the distal part of the GI tract. At least half of the dose should as such be taken into account, but based on the delayed absorption, this fraction might be even higher.

Dall'Erta et al. (2013) also used a human *in vitro* model mimicking different steps of the digestive process to study the fate of DON3Glc, ZEN14Glc, ZEN14Sulf and the parent compounds. The applied model consists of a sequence of three incubation steps, reproducing salivary juice (pH 6.8-7, 5 minutes; main enzyme: α-amylase), gastric juice (pH 2 - 3, 2 hours; main enzyme: pepsine), and duodenal juice and bile (pH 6.5 - 7, 2 hours; main enzymes: pancreatine, lipase) (Versantvoort et al., 2005).

While the findings of Dall'Erta et al. (2013) on DON are in line with previous studies, for the ZEN14Glc this seems contradictory with the results of Veršilovskis et al. (2012). When the glucosides were incubated with human intestinal bacteria, both the glucoside and sulphate of ZEN were completely degraded within 30 minutes, partly resulting in the parent mycotoxin (61 % after 30 minutes, 40 % after 24 hours) and a large number of unknown metabolites. In the case of DON3Glc, the degradation was much slower but complete after 24 hours with DON as the major metabolite (90 %). Only traces of DOM-1 were detected. Results in complete agreement were described by Gratz et al. (2013) for DON and DON3Glc. The authors incubated the modified compound with a fecal suspension obtained by volunteers. While DON3Glc was efficiently cleaved to DON in four to six hours in all the samples, only one out of five volunteers was able to convert DON into DOM-1. Besides the expected inter-individual variability, the possible microbiome-specificity of this detoxification mechanism was thus pointed out.

The same *in vitro* digestion model was used to investigate the nature of modified mycotoxins of fumonisins, being either covalently bound to food components or hidden owing to entrapment in the food matrix. The presence of such modified fumonisins has been shown after alkaline treatment of corn and corn products, resulting in much higher levels than after normal extraction (Dall'Asta et al., 2010; Falavigna et al., 2012). Alkaline hydrolysis results in the formation of hydrolysed FBs (HFBs) and cannot reveal whether the mycotoxins are bound or physically entrapped. The same authors showed that incubation of corn and corn-based products in the digestion model resulted in higher levels of parent fumonisins than normally extracted (on average about two-fold). This strongly indicates that the modified fumonisins in corn were physically entrapped rather than bound, but also that they can be released in the GI tract of humans and thus contribute to the exposure. Furthermore, Dall'Asta et al. (2010) showed that an extraction according to the QuEChERS - technique results in fumonisin levels similar to those obtained after alkaline hydrolysis or incubation in the *in vitro* digestion system.

Concerning modified fumonisins, Cirlini et al. (2014) showed that NDF-FB_1 can be partially cleaved under digestive conditions, while it is rather stable when incubated with human intestinal bacteria. An opposite behaviour was reported for HFB_1 that is partially degraded to unknown compounds by gut microbiota. These results are in agreement with data reported by Hahn et al. (in press) about the toxicokinetic and the toxicity in rats of orally administered FB_1, HFB_1 and NDF-FB_1.

In particular, the study performed by Hahn et al. (in press) demonstrated that the administration of HFB_1 and NDF-FB_1 does not increase the Sa/So ratio in rats, thus indicating a poor absorption or a lack of toxicological activity compared with the parent compound.

Overall these data show that there is limited information on the bioavailability of modified mycotoxins, with varying results for different toxins. However, release and absorption of the parent mycotoxin is certainly possible and, as such, modified mycotoxins may contribute to the overall exposure.

7.2. Toxicity of modified forms of certain mycotoxins in experimental animals

Unlike their parent compounds for which health HBGVs have been derived both for humans and animals on the basis of in most cases relatively abundant information on their toxic properties, no toxicological data are available to date for the modified forms of ZEN, NIV, T2, HT2 and fumonisins.

Metabolic conjugation with polar molecules such as carbohydrates and sulphate, also known as phase II metabolism, is commonly considered as an inactivation reaction, because the aglycone usually loses its biological activity and is eliminated from its site of action. However, there are exceptions to this general rule, and conjugates may still be of toxicological significance through various mechanisms, e.g. (a) by retaining or even acquiring biological activity after conjugation, (b) through subsequent hydrolysis of the conjugate and release of the parent aglycone, or (c) by improving the absorption of the bioactive agent. For mycotoxins, studies on the toxicity of the masked forms are not available. Therefore, the mechanisms outlined above will be briefly illustrated with conjugates of other xenobiotics.

Although glucuronidation of numerous drugs or phytochemicals is associated with the loss of biological activity, a recent study by Ruotolo et al. (2014) with the major mammalian metabolites of the weak phytoestrogen quercetin (QUER, Figure 6) showed that QUER-3-O-glucuronide, but not QUER-3'-*O*-sulphate, exhibited about the same oestrogenic activity as parent QUER in yeast and in human breast cancer cells *in vitro*. Using the same cell systems, trans-resveratrol (RES, Figure 6), its major plant metabolite RES-3-*O*-glucoside, and the main mammalian metabolites RES-3-*O*-sulphate, RES-3-*O*-glucuronide, and RES-4'-*O*-glucuronide were assayed for oestrogenic and anti-oestrogenic activity. Whereas none of the compounds was oestrogenic, a fairly strong anti-estrogenic activity was observed for RES-3-*O*-sulphate but not for the other substances (Ruotolo et al., 2013).

In a study with daidzein (DAI, Figure 6) and genistein (GEN, Figure 6), which are the major phytoestrogens present in many soy food items, Zhang et al. (1999) found that DAI-7-*O*-glucuronide and GEN-7-*O*-glucuronide still had a significant affinity for oestrogen receptors present in mouse uterine cytosol, albeit by a factor of about 10 and 50 less than the unconjugated DAI and GEN, respectively. Moreover, DAI-7-*O*-glucuronide and GEN-7-*O*-glucuronide were shown to activate human natural killer cells *in vitro* at nutritionally relevant concentrations, with both glucuronides being more potent than unconjugated GEN (Zhang et al. 1999).

GEN and DAI were also used by Pugazhendhi et al. (2008) to study the effect of conjugation with sulphate on their oestrogenicity in cultured MCF-7 human breast cancer cells. Competition with 17ß-estradiol for binding to the oestrogen receptors, induction of a stably transfected oestrogen-responsive reporter gene, and stimulation of cell growth were used as endpoints. In no case did sulphation abolish oestrogenic activity. For the 4'-*O*-sulphates of GEN and DAI, a modest reduction of oestrogenicity was observed, whereas opposing effects were disclosed for the 7-*O*-sulphates: the oestrogenic activity was substantially reduced for GEN-7-*O*-sulphate but increased for DAI-7-*O*-sulphate in comparison with the unconjugated phytoestrogens. Thus, conjugation may even enhance the biological activity, and inverse effects may occur between structurally related compounds.

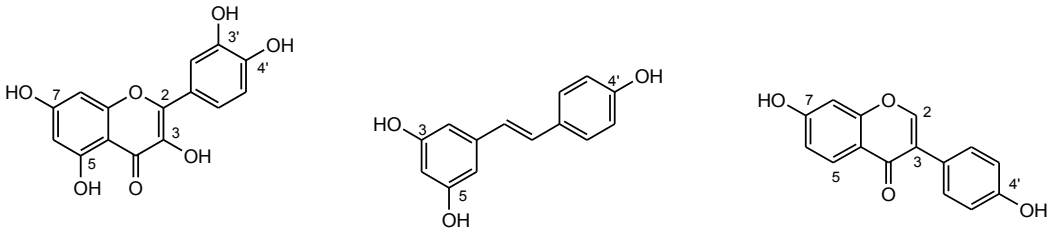


Figure 6: Examples of aglycones forming conjugates with different biological properties

In some instances, the formation of glucuronides and sulphates can render a non-reactive aglycone chemically reactive. Examples for the "metabolic activation" via glucuronidation are the so-called acyl glucuronides of carboxylic acids, amply known from non-steroidal anti-inflammatory agents such as ketoprofen (Figure 6). Such acyl glucuronides are electrophilic and can rearrange by intramolecular acyl migration, hydrolyze to the parent carboxylic acid, or alkylate proteins (Bailey and Dickinson, 2003).

Basically the same behaviour might be expected from acyl glucuronides and acyl glucosides of mycotoxins bearing carboxyl groups, such as ochratoxin A and citrinin, but acyl conjugates of mycotoxins have not been reported to date. Formation of sulphates is a well-known metabolic activation pathway of allylic alcohols (such as 1'-hydroxy-safrole, Figure 6), because the sulphate ion is a good leaving group and favors formation of electrophilic carbocations (Rietjens et al., 2005).

Although most glucosides, glucuronides and sulphates are chemically stable, they are prone to enzymatic hydrolysis by glucosidases, glucuronidases and sulphatases, respectively. Such enzymes are present in various compartments of the mammalian organism and also in the gut microflora. For example, most dietary flavonoids, which exist in planta predominantly as ß-glycoside conjugates, are hydrolyzed in the small intestine by the enzyme lactase phloridzin hydrolase in the brush-border of the epithelial cells, followed by passive diffusion into these cells (Manach and Donovan, 2004). An alternative hydrolytic step is mediated by a cytosolic ß-glucosidase within the epithelial cells after uptake of the polar glucosides via the active sodium-dependent glucose transporter SGLT1; conjugated flavonoids reaching the distal regions of the small intestine are hydrolyzed by bacterial glucosidases (Manach and Donovan, 2004). Likewise, glucuronidated metabolites of the flavonoid aglycones excreted via the bile are hydrolyzed in the intestine and undergo enterohepatic circulation.

It is generally assumed that the free aglycone has a higher bioavailability than the conjugate form owing to its higher lipophilicity and ability for passive diffusion through cell membranes. However, there are reported cases where the opposite is true. When equivalent doses of the soy isoflavone DAI were orally administered to male volunteers either as free aglycone or as DAI-7-*O*-ß-D-glucoside, the systemic bioavailability, maximum plasma concentration and cumulative recovery of DAI metabolites in urine were three to six times higher after the ingestion of the glucoside than after ingestion of free DAI (Rüfer et al., 2008). The greater bioavailability of the conjugate form is believed to be mostly due to its higher water solubility, which increases its diffusion through the aqueous boundary layer as well as the mucous layer of the intestine as compared with the more lipophilic aglycone.

These few examples illustrate the complex mechanisms by which conjugates may exert toxic effects. At least four different molecular mechanisms may render the masked form of a mycotoxin toxicologically active: (1) the conjugation may not abolish the activity of the parent compound; (2) conjugation may infer activity to the parent compound which is not present before; (3) the modified form may facilitate absorption of the mycotoxin; and (4) the modified form may be hydrolysed in the gastrointestinal tract and the released parent mycotoxin may be absorbed. The last mechanism is probably the most frequent one. However, as there are virtually no data on the toxicokinetics and toxicity of modified mycotoxins, the contribution of the modified form to the effects of the parent mycotoxin cannot be quantified at present.

Based on the few data currently available, and following a pragmatic approach, the modified forms of a mycotoxin are assumed to exert the same toxicity as the parent compound.

8. Occurrence of modified forms of certain mycotoxins in food and feed

Masked forms have been identified so far for DON, NIV, FUS-X, T2, HT2, ZEN, NEO, and OTA. Moreover, there is some evidence for the compartmentation of fumonisins in plants.

Up to now, the occurrence of masked forms in naturally infected cereals and products thereof have been described only for DON and ZEN (Berthiller et al., 2013; De Boevre et al., 2013). In addition, one recent study reports the occurrence of fatty acid esters of fumonisins in naturally infected maize (Falavigna et al., 2013).

Occurrence data for the masked forms are commonly expressed as relative percentage with respect to the parent compounds. Following data are given accordingly.

8.1. Modified forms of zearalenone

The masked forms of ZEN and its derivatives α- and β-ZELs have been studied in food and feed. The identification of ZEN-conjugated forms has been performed using *A. thaliana* or cell cultures of cereals, demonstrating the formation of a wide number of extractable compounds such as glucosides, malonylglucosides, dihexosides and pentosylhexosides (Berthiller et al., 2006).

A survey of 10 wheat grain samples revealed the relative proportion of ZEN14Glc to ZEN to be up to 30 % (Schneweis et al., 2002), as reported in Table 6. Low amounts of ZEN14Sulf were found in different commodities (wheat flour, whole-meal wheat bread, maize meal, biscuits, wheat flakes, bran flakes, muesli, crackers, cereal snack bars and polenta), with the highest concentration being 6.1 µg/kg in bran flakes, reaching a concentration up to 20 % in respect of the parent compound (Vendl et al., 2009). However, in a survey of extractable conjugated *Fusarium* mycotoxins in cereal-based raw materials and finished products, none of the 84 cereal-based products analysed contained ZEN14Glc, α- or β-ZEL and their glucosides αZEL14Glc and βZEL14Glc (Vendl et al., 2010). The method used for these analyses showed a good sensitivity with LOD in the range 1 – 10 µg/kg for ZEN and its derivatives.

Streit et al. (2013) analysed 83 samples of feed and feed raw materials for the co-occurrence of mycotoxins. A multi-mycotoxin approach was followed, also including the determination of DON3Glc, ZEN14Glc and ZEN14Sulf forms. All of them were found to contain 7 to 69 mycotoxins and their masked forms. Although ZEN14Glc was not detected, DON3Glc and ZEN14Sulf were frequently found with 75 % and 49 % positive samples, respectively. ZEN14Sulf accounted for up to 30 % of its precursor.

To date, the most comprehensive survey about ZEN and its conjugated forms has been performed by DeBoevre et al. (2012, 2013). In this study, cereal-based food products (n = 174) were analysed for the occurrence of DON, ZEN, T2 and HT2 together with their conjugated forms. The applied method was fully validated according to the EU requirements, obtaining a LOQ between 10 and 26 µg/kg for all the analysed matrices (maize, wheat, oats, breakfast cereals, bread).

Among the products analysed within the study, all purchased from the Belgian market in 2011, fibre-enriched bread samples (44 %) were significantly contaminated with ZEN, seven of them being above the EU maximum limit of 50 µg/kg. ZEN occurred also in the bran-enriched samples (39 %) and in more than half of the breakfast cereal samples (52 %), but the highest incidence (62 %) was observed for oatmeal. Together with ZEN, α- and β-ZELs were also detected in most of the samples.

The incidence of ZEN in food and feed matrices was 80 %. α-ZEL and β-ZEL occurred in 53 and 63 % of the samples, respectively. Data are reported in Table 6. ZEN14Glc, ZEN14Sulf, αZEL14Glc and βZEL14Glc occurred on average in 29 %, 8 %, 10 % and 19 %, respectively, of the fibre-enriched bread samples. When the total sum of the masked forms is considered, concentration up to 100 % of the parent compound is obtained. In the bran-enriched bread samples ZEN14Sulf occurred in 6 % of the samples, while the glucosylated forms were found in 6 % (ZEN14Glc), 3 % (αZEL14Glc) and 6 % (βZEL14Glc). In general, ZEN derivatives occurred at an overall concetration up to 74 % of the parent compound. Concerning the breakfast cereal samples, αZEL14Glc, βZEL14Glc and ZEN14Sulf occurred at the same level of incidence (26 %, 29 % and 27 %), while ZEN14Glc was observed in 40 % of the samples. ZEN14Glc appeared in 38 % of the oatmeal samples (De Boevre et al., 2012, 2013). Furthermore, in the case of oatmeal and breakfast cereals, the overall concentration of masked forms exceed the concentration of ZEN.

Table 6: Occurrence of ZEN conjugates in raw cereals and cereal-based products

Matrix		Conjugates	n (pos)	% with respect to ZEN	Reference
Raw cereals	Maize	ZEN14Sulf	41	Up to 30 %	Streit et al. (2013)
	Wheat	ZEN14Glc	10	Up to 30 %	Schneweis et al. (2002)
Food	Wheat flour	ZEN14Sulf	3	Up to 20 %	Vendl et al. (2010)
	Crackers	ZEN14Sulf	3	Up to 20 %	Vendl et al. (2010)
	Breakfast cereals – bran flakes	ZEN14Sulf	3	Up to 15 %	Vendl et al. (2010)
	Fibre enriched bread	ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc	52	As the sum, up to 100 %	De Boevre et al. (2012, 2013)
	Bran enriched bread	ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc	36	As the sum, up to 74 %	De Boevre et al. (2012, 2013)
	Breakfast cereals	ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc	62	As the sum, up to 110 %	De Boevre et al. (2012,2013)
	Popcorn	ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc	12	As the sum, up to 22 %	De Boevre et al. (2012,2013)
	Oatmeal	ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc	13	As the sum, up to 100 %	De Boevre et al. (2012,2013)

8.2. Modified forms of trichothecenes

Most of the studies carried out so far on modified mycotoxins deal with DON3Glc natural occurrence, while other modified trichothecenes are currently still not addressed quantitatively. The reason can be found mainly in the lack of analytical standards and reference material, but it must also be considered that DON3Glc was discovered almost a decade ago, while other modified compounds have been more recently identified. Since DON and modified DON are covered by a separate request, data concerning the occurrence of modified forms of DON are not considered herein.

Among modified trichothecenes, FUS-XGlc and NIVGlc have been reported for the first time in wheat grain that was artificially infected with *Fusarium* fungi. According to the authors, more than 15 % of FUS-X and NIV were converted into their respective glucosides (Nakagawa et al., 2011). It should be underlined that FUS-XGlc was not reported in natural infected samples so far.

Recently, the occurrence of NIVGlc was reported in wheat by Yoshinari et al. (2014), at a concentration ranging from 12 % to 27 %.

Recently, the 3-*O*-glucosides of T2 and HT2 have also been reported in wheat and oats inoculated with *Fusarium sporotrichiodes* (Busman et al., 2011). Similarly, their occurrence in naturally infected wheat and oats was described by Lattanzio et al. (2012). Levels of the HT2 and T2 parent forms were up to 85 µg/kg and 23 µg/kg, respectively, in wheat, and to 834 µg/kg and 377 µg/kg, respectively, in oats, corresponding to approximately 12 % of the parent form. Data are reported in Table 7.

Using an approach based on peak area ratio between glucoside derivatives and parent T2 and HT2, less than 1 % peak area ratio was obtained in fungal cultures tested, whereas higher glucosides values were estimated in naturally contaminated wheat and oat samples (peak area ratio up to 24 % and 2 7 % for T2Glc and HT2Glc, respectively). The latter data were similar to those reported for DON3Glc in wheat, that is up to approximately 30 % of the total DON contamination (Berthiller et al., 2005, 2009; Cirlini et al., 2013).

A very recent study demonstrates the occurrence of monoglucosides of HT2, NEO and DAS in cultures of *F. langsethiae* isolate at levels that could be roughly estimated *in vitro* to account up to 37 % of the relevant unconjugated toxin (Lattanzio et al., 2013). However, the natural occurrence in cereals of these compounds has never been reported so far.

Table 7: Occurrence of T2 and HT2 conjugates in raw cereals

Matrix		Conjugates	n (pos)	% with respect to T2/HT2	Reference
Raw cereals	Wheat	T2Glc, HT2Glc	9	Up to 12 %[(a)]	Lattanzio et al. (2012)
	Oat	T2Glc, HT2Glc	9	2 %[(a)]	Lattanzio et al. (2012)

(a): evaluated on the basis of the area ratio, owing to the lack of analytical standards

8.3. Modified forms of fumonisins

Several studies have reported the presence of bound fumonisins in food, which can be determined only after application of a hydrolysis step (Dall'Asta et al., 2008, 2009a,b). In particular, it has been observed that, after performing alkaline hydrolysis of contaminated corn products (e.g. extruded products such as cornflakes), the amount of hydrolysed fumonisins released was higher than the amount expected if all the fumonisins detected with the routine method were quantitatively hydrolysed.

This could be ascribed to a masking phenomenon, based on a probable physical entrapment of the mycotoxins into the structure of macromolecular components (such as starch) (Kim et al., 2002, 2003; Park et al., 2004). Although this phenomenon could be considered as an analytical issue, recent studies reported a strong relation with corn genotype and agronomical factors (Dall'Asta et al., 2012).

The occurrence of bound fumonisins in commercial cornflakes was first reported in 2003 (Kim et al., 2003). Samples of retail corn flakes were analysed for both free fumonisins and protein-bound fumonisins, which were extracted with sodium dodecyl sulphate and measured as hydrolyzed fumonisin B1 (FB_1) after alkaline hydrolysis. On average, a 2.6 fold higher content of FB_1 was found after hydrolysis. A similar approach was applied to 30 retail samples of heat-processed corn foods, revealing bound FB_1 in all samples at significant levels (Park et al., 2004).

The occurrence of bound fumonisins in 21 gluten-free products, at concentration levels comparable or higher than those found for the parent forms, has also been reported (Dall'Asta et al., 2008). Ninety-seven maize samples collected in Italy were analysed for free and total fumonisins just after harvesting (Dall'Asta et al., 2010). Free FBs were found in all samples at concentration levels ranging from 0.05 to 40 mg/kg, with a median value of 3.52 mg/kg after correction for moisture content. Total FBs obtained after alkaline hydrolysis were in the range 0.05 to 69 mg/kg, being significantly higher than free fumonisins in 82 of 97 samples.

Similar results were reported by Bryla et al. (2014), who found physically entrapped fumonisins in gluten-free bread up to 80 % of the parent forms. Breads were prepared exclusively with corn flour, while both yeast- and sourdough bread-making processes were considered in the study.

Among the covalent bound conjugates of fumonisins, fatty acid esters were identified in rice cultures inoculated with *F. verticillioides* – these substances are, however, most likely to have been fungal metabolites (Bartok et al., 2010). These compounds have been recently found in naturally infected corn at levels up to 5 % of the fumonisin concentration (Falavigna et al., 2013).

Table 8 below presents the occurrence of physically entrapped fumonisins and fumonisin conjugates in raw maize and corn-based products.

Table 8: Occurrence of physically entrapped fumonisins and fumonisin conjugates in raw maize and corn-based products[(a)]

Matrix		Forms	n (pos)	% with respect to $FB_1/FB_2/FB_3$	Reference
Raw maize		Physically entrapped fumonisins	31	Up to 100 %	Dall'Asta et al. (2009a)
		Physically entrapped fumonisins	97	Up to 60 %	Dall'Asta et al. (2010)
		Physically entrapped fumonisins	120	Up to 60 %	Dall'Asta et al. (2012)
		Fatty acid esters of FB_1	5	5 – 6 %	Falavigna et al. (2013)
Food	Corn flakes	Physically entrapped fumonisins	4	Up to 100 %	Dall'Asta et al. (2009a)
	Corn-based products	Physically entrapped fumonisins	6	Up to 60 %	Dall'Asta et al. (2009a)
	Gluten-free products	Physically entrapped fumonisins	21	Up to 100 %	Dall'Asta et al. (2009b)
	Gluten-free bread	Physically entrapped fumonisins	-	Up to 80 %	Bryla et al. (2014)

(a): For the purposes of this opinion the term modified fumonisins includes both covalently (i.e. NDF-FB) and not covalently (i.e. physically entrapped) bound forms.

8.4. Impact of food and feed processing

8.4.1. Effect of sorting and milling

The primary food/feed processing steps consists of selection and pre-milling. No significant thermal breakdown of mycotoxins would be expected at this stage, but moulds and mycotoxins are often concentrated in dust and broken grains which are more susceptible to fungal infection and toxin contamination. Sorting, cleaning, dehulling and debranning prior to milling may reduce the mycotoxin contamination in the grains by removing the kernels with extensive mould growth, broken kernels, fine materials and dust (Kushiro, 2008; Cheli et al., 2013). Whole grains are milled to produce flour and other fractions, then these fractions are the raw materials used to prepare the final products.

Most mycotoxins and their modified forms tend to be mainly concentrated in the bran fractions or outer layers of the grains (Edwards et al., 2011). Studies on modified forms have been addressed only for DON and DON3Glc, while no specific information has been collected for other modified forms (Blandino et al., 2014). However, owing to their comparable chemical behaviour, a similar trend is also likely for other toxins. Concerning modified mycotoxins, Kostelanska et al. (2011a) studied the fractionation of DON3Glc and DON in milling fractions, observing that their trend was similar, and white flours contained only approximately 60 % of the content of DON3Glc and DON in unprocessed wheat grains. Similar findings have been reported by Simsek et al. (2012).

8.4.2. Impact food and feed production including thermal treatment

Thermal treatment may have a significant impact in mycotoxin mitigation, but it can also lead to side reactions resulting in the formation of products with altered chemical structures (neogenesis).

This phenomenon has been mainly studied for DON and fumonisins. Hence, conversion of DON to isomeric and/or further demethylated forms is described here for illustration. Four main products called norDON A, B, C and D, together with the isomeric form isoDON, have been isolated and characterized by NMR so far (Bretz et al., 2006). Since these forms are not commonly detected in the analytical methods, their formation can result in an apparent decrease of the initial DON levels. These compounds were found in cereal-based food samples from the market at concentrations in the range 3 – 15 µg/kg.

Since the hydroxy group in position C-3 is not involved in the reaction during the formation of isomeric and demethylated products, similar rearrangements might also occur for DON3Glc, as reported by Kostelanska et al. (2011b). Nothing is known so far about possible rearrangements involving trichothecenes other than DON, but it is probably due to structural similarity.

Concerning fumonisins, the main reaction described upon thermal treatments involves reducing sugars via Maillard-type reactions and further rearrangement. The main products are *N*-carboxymethyl (NCM) fumonisin and N-deoxyfructosyl (NDF) fumonisin (see Figure 7). In addition, the hydrolysed form created by the cleavage of both carballylic moieties is commonly obtained upon alkali treatment (Humpf and Voss, 2004).

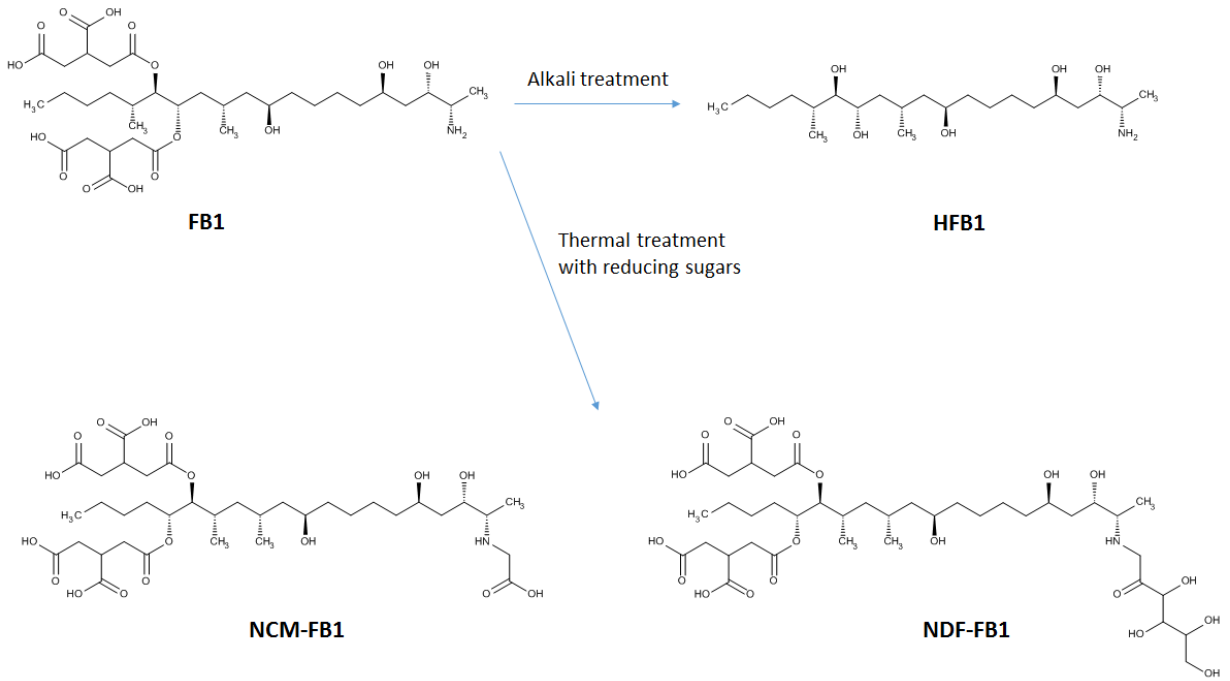


Figure 7: Formation of HFB_1, NCM-FB_1 and NDF-FB_1 from FB_1 upon processing

A study provided by De Girolamo et al. (2001) investigated the stability of fumonisins FB_1 and FB_2 during the production of corn flakes from raw corn flour by extrusion and roasting, finding approximately 60 to 70 % reduction in fumonisin content during the entire process. In this case, only 30 % of those losses were attributed to the extrusion step, where the material was subjected to 70 to 170 °C for two to five minutes.

In general, the studies available in the scientific literature indicate that the greatest reduction of fumonisins occurs at temperatures of 160 °C or more and in the presence of glucose (Humpf and Voss, 2004). Extrusion of corn grits with 10 % added glucose resulted in a 75 to 85 % decrease in FB_1 levels. Amounts of degradation products such as HFB_1 and NDF fumonisins in extruded grits depend on the type and amount of sugar added, as found by Seefelder et al. (2001) and Castelo et al. (2001). Extrusion is often associated with alkali treatment to obtain masa flour and/or tortillas. Chips obtained from masa flour contained HFB_1 at low concentrations, but almost no NCM-FB_1 and NDF-FB_1 were detected (Voss et al., 2001).

Concerning physically entrapped fumonisins, Bryla et al. (2014) reported a decrease in both parent and, to a lesser extent, entrapped forms during bread making. In particular, the baking process induced a 30 % reduction in parent forms for both yeast-fermented and sourdough breads, while the reduction for entrapped fumonisins was about 19 and 10 %, respectively.

The authors stated that entrapped fumonisins were less affected by baking owing to a matrix stabilization effect.

Among treatments for food and feed production, baking, bread making, extrusion and brewing have often been studied in recent years. Again, little information is available for other modified mycotoxins other than those originating from DON.

Baking is the most studied food process so far. Vidal et al. (2014) studied the fate of DON and DON3Glc during the bread making: DON increased from the unkneaded mix to fermented dough and decreased during baking, depending on the initial concentration in the flour. These observations were also in agreement with other previous studies, such as that performed by Wolff et al. (2004), in which DON decreased up to 25 % in the baked bread. DON3Glc content increased during fermentation as suggested by Zachariasova et al. (2012), and also during baking, most likely owing to the glycosylation of DON in the initial stages of baking before enzyme inactivation. Kostelanska et al. (2011a) noted that when so-called bread improver enzyme mixtures (i.e. xylanase and protease) were employed as a dough ingredient, a distinct increase (up to 145 %) of DON3Glc occurred in fermented dough, whereas some decrease in both DON3Glc and DON (10 % and 13 %, respectively, compared with fermented dough, and mainly in the crust) took place during baking. A similar trend was observed by Young et al. (1984) and recently in the study by Simsek et al. (2012).

Brewing and beer-making has gained great attention in recent years for the possible formation and transfer of DON and DON3Glc from barley and malt to beer (Lancova et al., 2008; Kostelanska et al., 2011b), while no specific studies have been performed for other modified mycotoxins. In particular, a very high increase of DON and DON3Glc - even up to 536 % and 210 %, respectively – was reported during the malting step and their total transfer into wort during the mashing step was described (Kostelanska et al., 2009).

8.5. Carry-over

In general the carry-over of ZEN, NIV, T2, and HT2 from feed to animal derived products is rather low (EFSA, 2005; EFSA CONTAM Panel, 2011a, b, 2013). As such it is not considered relevant for human exposure. Also the exposure from plant-derived products is much higher.

No data are available for the carry-over of modified forms of these mycotoxins but it can be assumed that the above-mentioned considerations are also applicable for the modified forms of these mycotoxins, in particular since hydrolysis in the GI tract leads to the release of the parent compounds.

8.6. Conclusions on occurrence

An increasing amount of occurrence data on modified mycotoxins is currently available from the literature. However, owing to the lack of analytical calibrants and reference materials, the majority of surveys are targeted to DON and DON3Glc, followed by ZEN and its large group of structurally related compounds. Very few data, on the contrary, are available for the modified forms of minor trichothecenes such as NEO, DAS, FUS-X, as well as for the regulated T2 and HT2.

Covalently modified forms of fumonisins have been reported in maize at low concentrations: *N*-acyl derivatives can be formed both in the field or during processing, while O-acyl forms have been recovered only in the field. Non-covalently bound fumonisins have been reported at concentrations comparable to the parent compounds.

Data about processing indicate that modified forms of DON and ZEN are mainly located in the outer part of the cereal kernels and are concentrated during bran and fiber-enriched flour production. On the other hand, there is a lack of data about possible transformation upon processing of trichothecenes other than DON and its modified forms.

Concerning fumonisins, N-acyl derivatives are formed upon extrusion in the presence of reducing sugars (i.e. cornflakes).

Occurrence data on modified mycotoxins in animal products (i.e. milk and dairy, meat, eggs) are not available, although the carry-over of *Fusarium* toxins and their structurally related compounds has never been reported so far.

According to the data reported so far, bran- and fiber-enriched products are more prone to modified mycotoxin contamination.

Modified forms of ZEN often co-occur with parent ZEN and may account for a total concentration up to 110 % of the parent compound.

Concerning NIV, its modified forms are reported in cereals amounting up to 30 % of their parent form, while modified T2 and HT2 are reported in cereals amounting to 10 % of their parent form.

Physically entrapped fumonisins have been extensively reported in maize at levels comparable to fumonisins (60 – 100 %). Covalently bound fumonisins, on the contrary, are present only at very low concentrations.

9. Exposure assessment in animals and humans

9.1. Previously reported exposure assessments to modified mycotoxins in humans

De Boevre et al. (2013) reported exposure to ZEN and its modified forms based on occurrence in cereals on the Belgian market (see Section 8.1) and consumption data from adults in the Belgian National Food Consumption Survey of 2004. In deterministic analysis, the mean exposure to the sum of ZEN (ZEN14Glc, ZEN14Sulf, αZEL, βZEL, αZEL14Glc and βZEL14Glc) LB (UB) exposure was 0.107 (0.122) µg/kg b.w. per day, whereas the 95th percentile exposure was LB (UB) 0.243 (0.277) µg/kg b.w. per day. Other data describing exposure to modified mycotoxins were not found.

9.2. Human exposure to modified forms of certain mycotoxins in comparison with exposure to their parent compounds

Human exposure to the parent mycotoxins together with their modified forms was derived by using the overall exposure levels established in recent EFSA opinions on ZEN (EFSA CONTAM Panel, 2011a), NIV (EFSA CONTAM Panel, 2013) and T2 and HT2 (EFSA CONTAM Panel, 2011b) adding a certain factor accounting for respective modified forms based on levels reported in the literature, as outlined below. For fumonisins recent EFSA exposure assessments were not available. An exposure assessment to fumonisins was carried out and is presented in detail in Appendices A - C. Also for fumonisins a factor was added to the exposures estimated for the parent compound to account for the modified forms of fumonisins.

9.2.1. Exposure to zearalenone and its modified forms

According to the latest occurrence papers (see De Boevre et al. 2013), the modified forms of ZEN (ZELs, ZEN14Glc, ZEN14Sulf, βZEL14Glc, αZEL14Glc) commonly occur together with their precursor in cereal-based food, mainly in bran- and fibre-enriched products. The percentage of occurrence with respect to ZEN ranges from 15 to 110 %, expressed as the sum of all the co-occurring modified forms in each sample (see Section 8, Table 6). At least one of these forms was present in about 50 % of the considered samples. With the exception of popcorn, the contamination was comparable in all the tested samples (n = 175) in terms of type and relative percentage of occurring forms.

Occurrence of modified forms of ZEN appear to be in the range of an addition of 100 % to the concentration of the parent compound (see Section 8.1).

Table 9 presents exposure assessments for ZEN for the different age groups (mean and 95th percentile exposures) as established in EFSA CONTAM Panel (2011a). The chronic dietary exposure to ZEN was higher in younger consumers than in adults. Also, there was a relatively high variation between the exposure estimates across European countries and dietary surveys within each age class. A similar variation is seen by the addition of 100 % (Table 10) to the parent compound.

Table 9: Summary statistics of chronic dietary exposure to ZEN (ng/kg b.w. per day) as provided in EFSA CONTAM Panel (2011a)

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants[(a)]	3.3	87	6.4	87	9.4	88
Toddlers	9.3	51	13	83	23	100
Other children	5.7	29	11	44	22	75
Adolescents	3.6	17	6.1	26	12	42
Adults	2.4	14	4.3	18	7.2	29
Elderly	2.0	13	3.4	16	6.4	26
Very elderly	2.3	12	2.9	16	7.1	29
95th percentile exposure in total population[(b)]						
Infants	33[(c)]	-[(d)]	-[(d)]	-[(d)]	-[(d)]	217[(c)]
Toddlers	24	104	31	182	50	277
Other children	9.9	59	22	80	42	124
Adolescents	7.5	38	15	53	26	76
Adults	4.7	28	9.5	35	14	54
Elderly	3.5	25	7.5	31	12	42
Very elderly	7.0	26	7.7	35	13	47

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

Table 10: Scenarios* of chronic dietary exposure to ZEN and modified ZEN, derived by adding 100 % to the exposure derived in EFSA CONTAM Panel (2011a) in order to account for modified forms of ZEN (ng/kg b.w. per day).

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants[(a)]	6.6	174	12.8	174	18.8	176
Toddlers	18.6	102	26	166	46	200
Other children	11.4	58	22	88	44	150
Adolescents	7.2	34	12.2	52	24	84
Adults	4.8	28	8.6	36	14.4	58
Elderly	4.0	26	6.8	32	12.8	52
Very elderly	4.6	24	5.8	32	14.2	58
95th percentile exposure in total population[(b)]						
Infants	66 [(c)]	-[(d)]	-[(d)]	-[(d)]	-[(d)]	**434** [(c)]
Toddlers	48	208	62	364	100	**554**
Other children	19.8	118	44	160	84	248
Adolescents	15	76	30	106	52	152
Adults	9.4	56	19	70	28	108
Elderly	7.0	50	15	62	24	84
Very elderly	14	52	15	70	26	94

*Values given in this table are rough estimates of exposures to ZEN together with modified ZEN. The values have been derived by adding 100% to the exposure values derived for ZEN (see Table 9), based on similar percentages of the ratio of contents of ZEN with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.

(a): Estimates based on only two dietary surveys.

(b): The 95th percentile estimates obtained on dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

(c): Estimates are based on only one dietary survey.

9.2.2. Exposure to nivalenol and its modified forms

The glycosylated form of NIV has only recently been identified and no occurrence data are available in the literature. However, NIV shows a structural similarity to DON, and modified forms of NIV may be expected to occur to a similar extent as that for DON. For the same reason, food processing may significantly affect the levels of NIV3Glc, i.e. thus causing the release of glycosylated forms from the cell walls as already described for DON (Kostelanska et al., 2011b; Simsek et al., 2012; Zachariasova et al., 2012). In consideration of this, an additional 30 % is considered for NIV for exposure assessment (see also Section 8.2).

Table 11 presents exposure assessments for NIV for the different age groups (mean and 95th percentile exposures), as derived in EFSA CONTAM Panel (2013). The highest chronic exposure was estimated in toddlers and was higher in younger age groups than in adults (≥ 18 years to < 65 years old). A relatively high variation between the exposure estimates across the dietary surveys within each age class was observed. There is a large difference between the LB and UB exposure estimates. The same is reflected by adding 30 % to the parent compound in order to account for exposure to modified forms of NIV (Table 12).

Table 11: Summary statistics of chronic dietary exposure to NIV (ng/kg b.w. per day) across European countries as established by EFSA CONTAM Panel (2013)

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants	2.4	136	-[(a)]	-[(a)]	4.4	140
Toddlers	4.3	81	6.3	152	8.8	202
Other children	1.3	56	5.5	97	12	132
Adolescents	1.0	45	2.1	60	6.4	80
Adults	0.4	37	1.6	56	4.8	75
Elderly	0.81	31	1.7	49	4.7	55
Very elderly	0.80	43	1.6	49	3.9	58
95th percentile dietary exposure in total population[(b)]						
Infants	16	-[(c)]	-[(c)]	-[(c)]	-[(c)]	389
Toddlers	12	203	15	317	23	484
Other children	3.0	121	12	179	22	259
Adolescents	3.0	99	6.0	124	15	147
Adults	1.1	89	4.0	112	10	224
Elderly	2.3	60	3.5	102	11	127
Very elderly	1.9	79	3.8	100	7.8	111

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

Table 12: Scenarios* of chronic dietary exposure to NIV and modified NIV (ng/kg b.w. per day) derived by adding 30 % to the exposure derived in EFSA CONTAM Panel (2013) in order to account for modified forms of NIV

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants	3.1	177	-[(a)]	-[(a)]	5.7	182
Toddlers	5.6	105	8.2	19	11	263
Other children	1.7	73	7.2	126	16	172
Adolescents	1.3	56	2.7	78.	8.3	104
Adults	0.5	48	2.1	73	6.2	98
Elderly	1.1	40	2.2	64	6.1	72
Very elderly	1.0	56	2.1	64	5.1	76
95th percentile dietary exposure in total population[(b)]						
Infants	201	-[(c)]	-[(c)]	-[(c)]	-[(c)]	506
Toddlers	16	264	20	412	30	630
Other children	3.9	157	16	233	29	337
Adolescents	3.9	129	7.8	161	20	192
Adults	1.4	116	5.2	146	13	291
Elderly	3.0	78	4.6	133	14	165
Very elderly	2.5	103	4.9	130	10	144

*The vlues given in this table are rough estimates of exposures to NIV together with modified NIV. The values have been derived by adding 30 % to the exposure values derived for NIV (see Table 11), based on similar percentages for the ratio of contents of NIV with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.
b.w.: body weight; LB: lower-bound; UB: upper-bound.
(a): Not calculated; estimates available from only two dietary surveys.

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

9.2.3. Exposure to T-2 and HT-2 toxin and their modified forms

Modified forms of T2 and HT2 have only recently been reported in the literature; thus, very few occurrence data collected under natural infection conditions have been reported so far (see Section 5).

According to Lattanzio et al. (2013), glucosylated forms of T2 and HT2 may naturally occur in both wheat and oats at concentration up to 12 % with respect to the parent compounds.

Accordingly, exposure assessment is performed considering an addition of 10 % to the exposure to the parent compounds. Due to the very low number of samples (n = 9), these data have to be considered as very preliminary.

Table 13 presents exposure assessments T2 and HT2 in the different age groups (mean and 95th percentile exposures) as derived in EFSA CONTAM Panel (2011b). The dietary exposure to the sum of T2 and HT2 is higher in younger consumers than in adults. In addition, there is a relatively high variation between the exposure estimates across the dietary surveys within each age class. The same is reflected when a 10 % additional exposure to modified forms of T2 and HT2 is added to the exposure to the parent compound (Table 14).

Table 13: Summary statistics of chronic dietary exposure to the sum of T2 and HT2 as derived in EFSA CONTAM Panel (2011b) (ng/kg b.w. per day) across European countries

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

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

Table 14: Scenarios* of chronic dietary exposure to the sum of T2 and HT2 + 10 % (ng/kg b.w. per day) across European countries

Age class	Summary statistics of exposure (ng/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
	Mean dietary exposure in total population					
Infants	6.5	12	-(a)	-(a)	6.8	18
Toddlers	13	33	18	37	31	47
Other children	11	29	15	34	18	43
Adolescents	4.8	14	8.7	21	10	26
Adults	3.7	11	6.2	15	10	20
Elderly	3.6	11	4.6	14	6.4	15
Very elderly	3.1	11	4.4	13	7.0	17
	95th percentile dietary exposure in total population(b)					
Infants	21	-(c)	-(c)	-(c)	-(c)	56
Toddlers	25	53	36	68	72	100
Other children	23	48	34	64	48	78
Adolescents	13	32	21	42	28	52
Adults	7.9	22	15	29	28	43
Elderly	7.4	23	11	25	15	29
Very elderly	5.8	19	7.7	21	13	28

*Values given in this table are rough estimates of exposures to the sum of T2 and HT2 together with the sum of modified T2 and HT2. The values have been derived by adding 10 % to the exposure values derived for the sum of T2 and HT2 (see Table 13), based on similar percentages for the ratio of contents of the sum of T2 and HT2 with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.

b.w.: body weight; LB: lower-bound; UB: upper-bound.

(a): Not calculated; estimates available only from two dietary surveys.

(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

(c): Not calculated; estimates available only from one dietary survey.

9.2.4. Exposure to fumonisins and their modified forms

According to the literature summarized in Section 8.3, the modified forms of fumonisins (both covalently and not covalently bound) commonly occur together with their precursor in corn and maize-based products. The percentage of occurrence with respect to FB_1 is ranging from 60 % up to 100 %, expressed as the sum of all the co-occurring modified forms in each sample. The presence of modified forms is very dependent on the growing season (occurrence ranging between 25 % and 100 % of the considered samples), even if general criteria are still to be defined (Dall'Asta et al., in press). However, according to the latest reports (Dall'Asta et al., 2012, in press), the ratio of modified fumonisins is higher when the overall contamination is low, while it is lower in highly contaminated samples. In consideration of this fact, the exposure assessment is performed considering an additional 60 % of modified fumonisins to the parent compound.

Table 15 presents summary statistics of the chronic dietary exposure to fumonisins across the different age groups (mean and 95th percentile exposures) as derived in this opinion (Appendix C). Higher exposure is seen in younger age groups than in adults, and maximum LB exposure is seen in surveys of "other children". The same is observed when 60 % additional exposure to modified forms of fumonisins are added to the exposure to the parent compound (Table 16).

Table 15: Summary statistics of chronic dietary exposure to fumonisins (µg/kg b.w. per day) across European countries as established in the present opinion (Appendix C)

Age class	Summary statistics of exposure (µg/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants	0.04	0.34	-(a)	-(a)	0.12	0.63
Toddlers	0.18	0.93	0.43	1.32	0.82	1.65
Other children	0.14	0.71	0.38	1.13	1.17	1.77
Adolescents	0.08	0.43	0.20	0.60	0.69	1.04
Adults	0.05	0.33	0.11	0.40	0.33	0.63
Elderly	0.05	0.31	0.07	0.35	0.28	0.53
Very elderly	0.04	0.31	0.07	0.35	0.13	0.46
95th percentile exposure in total population(b)						
Infants	0.53	1.99	-(c)	-(c)	0.53	1.99
Toddlers	0.58	2.32	1.33	2.41	1.61	3.26
Other children	0.24	1.43	0.98	2.01	3.14	4.05
Adolescents	0.14	0.87	0.59	1.29	1.58	2.19
Adults	0.09	0.59	0.29	0.81	0.83	1.25
Elderly	0.08	0.53	0.21	0.67	0.53	0.99
Very elderly	0.13	0.55	0.18	0.64	0.34	0.84

b.w.: body weight; LB: lower-bound; UB: upper-bound.
(a): Estimates based on only two dietary surveys.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.
(c): Estimates are based on one dietary survey only. Note: in order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to three figures.

Table 16: Scenarios* of chronic dietary exposure to fumonisins µg/kg b.w. per day) as established in the present opinion, derived by adding 60 % to account for modified fumonisins

Age class	Summary statistics of exposure (µg/kg b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants	0.06	0.54	-(a)	-(a)	0.19	1.01
Toddlers	0.28	1.49	0.68	2.12	1.31	2.63
Other children	0.23	1.14	0.61	1.81	1.87	2.82
Adolescents	0.12	0.69	0.32	0.96	1.11	1.66
Adults	0.08	0.53	0.17	0.64	0.54	1.01
Elderly	0.07	0.50	0.12	0.56	0.45	0.84
Very elderly	0.07	0.49	0.11	0.56	0.21	0.74
95th percentile exposure in total population(b)						
Infants	0.84	3.19	-(c)	-(c)	0.84	3.19
Toddlers	0.93	3.71	2.12	3.85	2.58	5.21
Other children	0.39	2.28	1.57	3.22	5.03	6.49
Adolescents	0.22	1.39	0.95	2.07	2.53	3.50
Adults	0.15	0.94	0.47	1.30	1.33	2.01
Elderly	0.13	0.84	0.34	1.07	0.84	1.59
Very elderly	0.21	0.89	0.28	1.03	0.54	1.34

*Values given in this table are rough estimates of exposures to fumonisins together with modified fumonisins. The values have been derived by adding 60 % to the exposure values derived for fumonisins (see Table 15), based on similar percentages for the ratio of contents of fumonisins with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.
(a): Estimates based on only two dietary surveys.

(b): The 95th percentile estimates obtained on dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.
(c): Estimates are based on one dietary survey only. Note: in order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to three figures.

9.3. Animal exposure to modified forms of certain mycotoxins in comparison with exposure to their parent compounds

Since no recent animal exposure assessments are available for ZEN and fumonisins, new assessments have been carried out in the present opinion based on the analysis of available occurrence data as presented in Appendix E to this opinion. Occurrence data on NIV and T2 and HT2 were presented by EFSA recently (EFSA CONTAM Panel, 2011b, 2013). Occurrence data of the parent compounds were used to estimate the average exposure of various animal species to ZEN (Table 17), NIV (Table 19), T2/HT2 (Table 21) and fumonisins (Table 23), and the potential increase in the exposure due to the presence of modified forms (Tables 18, 20, 22, 24). This was done using default values for feed consumption previously established and used for T2/HT2 and NIV (EFSA CONTAM Panel 2011b, 2013). The assessment is based on a similar percentage addition of modified mycotoxins in animal feed as that in human food (100 % for ZEN, 30 % for NIV, 10 % for T2 and HT2 and 60 % for fumonisins).

Where available data for compounds feeds for specific species were used. This is contrary to the method applied for NIV and T2/HT2 (EFSA CONTAM Panel 2011b, 2013), where exposure was based on data on ingredients and their contribution to compound feed, also due to the limited datasets for compound feeds. However, regarding the well-known differences in sensitivity between species, as also reflected in the guidance values provided in Commission Recommendation 2006/576/EC[21], in practice the less contaminated ingredients may be used for the more sensitive species and data on species-specific compound feeds seem to be more relevant. Where too few data were available on compound feed, estimations for pigs, poultry and rabbits were based on the levels in the category "Cereal grains and cereal by-products", using relative contributions to the feed of 74, 72 and 75 % for piglets, pigs for fattening and sows, respectively, 74, 65, 65 and 72 % for chickens for fattening, laying hens, turkeys for fattening and ducks, respectively, and 36 % for rabbits. Further details will be provided below under the sections on the different mycotoxins.

Also depending on the season, cows receive fresh grass, silage of grass, or maize and maize silage, in addition to compound feed. Consequently, the exposure differs depending on the type of feeding, the season and also the quantity of milk produced. For the present opinion the focus was on cows fed with maize and maize silage since data show that this material may in general contain the higher mycotoxin levels. A body weight of 650 kg and milk production of 40 kg/day were considered the default. The daily feed consumption and the relative contribution of different ingredients for such cows was estimated to be 15.0 kg of maize silage, 9.5 kg of maize grain and 2.8 kg of soybean meal, amounting to 27.3 kg in total (EFSA CONTAM Panel, 2011b, 2013).

For fattening beef cattle, a body weight of 400 kg was considered as the default, and for estimating the exposure to ZEN, NIV and fumonisins, a daily feed intake of 10.5 kg consisting of 9.5 kg maize silage and 1.0 kg soybean meal was applied. So again this refers to cattle fed primarily with maize silage. In the case of T2/HT2, beef cattle fed with cereals had the highest exposure, and these animals received a daily ration of 7.1 kg non-forage feed (in addition to 1.4 kg forage which normally makes no contribution to T2/HT2 intake). The non-forage feed was assumed to contain 60 % barley, 5 % rapeseed meal, 5 % sunflower meal, 10 % maize gluten feed, 4 % wheat feed, 10 % sugar beet pulp, 3 % molasses, 1 % vegetable oils and 3 % vitamins/minerals.

[21] Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T2 and HT2T2 and fumonisins in products intended for animal feeding. OJ L 229, 23.8.2006, p. 7-9.

For lactating sheep, a body weight of 80 kg was applied and a feed intake of 2.8 kg per day, of which 50 % was compound feed with 55 % cereals and cereal by-products. For lactating and fattening goats body weights of 60 and 40 kg, respectively, were applied, feed intakes of 3.4 and 1.5 kg/day and relative contributions for compound feed of 65 and 40 % with 60 and 70 % from cereals and cereal by-products.

For estimating exposure of horses, a body weight of 450 kg and feed intake of 9 kg/day was applied, of which 4.5 kg was feed with a relative composition of 40 % oats, 10 % beans, 30 % wheat feed, 12 % oat feed, 5 % molasses and 3 % minerals/vitamins. For NIV, the mean levels for cereals and cereal by-products were used for oats, wheat feed and oat feed, and for T2/HT2 toxins the levels in oat, oat middling and wheat middling were used.

For estimating the exposure of cats, a body weight of 4 kg was applied and a feed intake of 60 g/day, for dogs a body weight of 25 kg and feed intake of 360 g/day. In the absence of specific data on cat and dog feed, it was assumed that potential exposure would come from the cereals in the feed with relative contributions of 55 and 65 % for cats and dogs, respectively (EFSA CONTAM Panel, 2011a, 2013).

No occurrence data were available for fish feed, consequently no exposure assessments could be carried out.

The Panel also noted that animals may occasionally be exposed to much higher levels owing to specific contaminations of ingredients of compound feed but also silage. This is demonstrated by the data for ZEN and fumonisins in Appendix E, showing values for the P99 values that are often more than 10-fold higher than those at the P50. In particular in such cases the presence of modified forms may contribute to adverse effects on animal health. Based on the turn-over of compound feed or silage such high exposure may last for periods of weeks up to several months. However, the current database contains in general too few data to evaluate the higher end exposure of specific animal species. Where permitted by the available data, the Panel estimated the high exposure based on the P95 levels.

9.3.1. Exposure to zearalenone and its modified forms

Table 17 shows the estimated exposure of various different species based on newly retrieved occurrence data shown in Appendix E on compound feed, or in the case of cows and horses, ingredients of the rations provided to them. For lactating sheep data on compound feed were applied, taking into account that this contributes 50 % to the diet. For lactating and fattening goats data on compound feed were limited or missing, and calculations were applied as for nivalenol, i.e. based on levels in cereals and cereal-based products, using the relative contribution to compound feed and the relative contribution of compound feed to the overall diet. For dogs and cats, estimated levels and exposure were based on the levels in “Cereal grains and cereal by-products” and the relative contribution to feed. Where permitted by available data, the high exposure was also estimated based on P95 values. Table 18 shows the estimated exposure to ZEN and its modified forms based on an average contribution of 100 % of these modified forms.

Cows showed the highest exposure due to the high levels in maize silage. Cows fed on grass or grass silage are likely to have a lower exposure, although some high levels of ZEN were reported to EFSA (see Appendix E).

Table 17: Mean and P95 levels of ZEN in feed (LB/UB) and estimated exposure of various animal species to the parent compound alone

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	14	19	0.7	1.0
Pigs for fattening	100	3	18	31	0.6	0.9
Sows	200	6	72	82	2.2	2.5
Chickens for fattening	2	0.12	37	46	2.2	2.8
Laying hens	2	0.12	55	64	3.3	3.8
Turkeys for fattening	12	0.4	44	57	1.5	1.9
Ducks[a]	3	0.14	28	43	1.3	2.0
Rabbits	2	0.15	50	68	3.8	5.1
Fattening cows (corn)	400	10.5	200	218	5.3	5.7
Dairy cows	650	27.3	150	163	6.3	6.8
Sheep lactating	80	2.8	7	9	0.2	0.3
Goats lactating	60	3.4	17	20	0.9	1.1
Goats fattening	40	1.5	12	14	0.4	0.5
Horses[b]	450	9.0	4	6	0.1	0.1
Cats	4	0.06	23	28	0.4	0.4
Dogs	25	0.36	27	33	0.4	0.5
Based on P95 levels						
Piglets	20	1	48	48	2.4	2.4
Pigs for fattening	100	3	[c]	[c]	[c]	[c]
Sows	200	6	[c]	[c]	[c]	[c]
Chickens for fattening	2	0.12	128	128	7.7	7.7
Laying hens	2	0.12	183	183	11.0	11.0
Turkeys for fattening	12	0.4	[c]	[c]	[c]	[c]
Ducks[a]	3	0.14	[c]	[c]	[c]	[c]
Rabbits	2	0.15	[c]	[c]	[c]	[c]
Fattening cows (corn)	400	10.5	720	720	18.9	18.9
Dairy cows	650	27.3	555	556	23.3	23.4
Sheep lactating	80	1.4	37	37	1.3	1.3
Goats lactating	60	2.2	64	64	3.6	3.6
Goats fattening	40	0.6	46	46	1.7	1.7
Horses[b]	450	9.0	[c]	[c]	[c]	[c]
Cats	4	0.06	90	90	1.4	1.4
Dogs	25	0.36	107	107	1.5	1.5

(a): Data for feed for ducks were included in a miscellaneous group of feeds in Appendix E
(b): Because there were no data on oat middling, the values for wheat middling were used.
(c): Too few data to calculate P95 values and no exposures could be calculated.

Table 18 shows the estimated exposure to ZEN and its modified forms based on an average contribution of 100 % of these modified forms.

Table 18: Scenario of mean and P95 levels of ZEN in feed (LB/UB) and estimated exposure of various animal species including an additional 100 % contribution of modified forms[(a)]

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	29	38	1.4	1.9
Pigs for fattening	100	3	37	62	1.1	1.8
Sows	200	6	145	164	4.3	4.9
Chickens for fattening	2	0.12	73	92	4.4	5.5
Laying hens	2	0.12	110	128	6.6	7.7
Turkeys for fattening	12	0.4	89	114	3.0	3.8
Ducks	3	0.14	56	86	2.6	4.0
Rabbits	2	0.15	100	135	7.5	10.1
Fattening cows (corn)	400	10.5	401	437	10.5	11.5
Dairy cows	650	27.3	300	326	12.6	13.7
Sheep lactating	80	2.8	14	17	0.5	0.6
Goats lactating	60	3.4	33	40	1.9	2.3
Goats fattening	40	1.5	24	28	0.9	1.1
Horses	450	9.0	7	12	0.1	0.2
Cats	4	0.06	46	56	0.7	0.8
Dogs	25	0.36	55	66	0.8	1.0
Based on P95 levels						
Piglets	20	1	96	96	4.8	4.8
Pigs for fattening	100	3	(b)	(b)	(b)	(b)
Sows	200	6	(b)	(b)	(b)	(b)
Chickens for fattening	2	0.12	256	256	15.4	15.4
Laying hens	2	0.12	366	366	22.0	22.0
Turkeys for fattening	12	0.4	(b)	(b)	(b)	(b)
Ducks	3	0.14	(b)	(b)	(b)	(b)
Rabbits	2	0.15	(b)	(b)	(b)	(b)
Fattening cows (corn)	400	10.5	1439	1441	37.8	37.8
Dairy cows	650	27.3	1111	1112	46.6	46.7
Sheep lactating	80	2.8	73	73	2.6	2.6
Goats lactating	60	3.4	128	128	7.3	7.3
Goats fattening	40	1.5	92	92	3.4	3.4
Horses	450	9.0	(b)	(b)	(b)	(b)
Cats	4	0.06	180	180	2.7	2.7
Dogs	25	0.36	213	213	3.1	3.1

(a): Values given in this table are rough estimates of exposures to ZEN together with modified ZEN. The values have been derived by adding 100 % to the exposure values derived for ZEN (see Table 17), based on similar percentages of the ratio of contents of ZEN with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.

(b): Too few data were available for the parent compound to calculate P95 values and no P95 scenario exposures could be provided.

9.3.2. Exposure to nivalenol and its modified forms

In line with the recent opinion on nivalenol (EFSA CONTAM Panel, 2013), levels in feed and related exposure of the various species was based on the levels in feed ingredients, for most species only the group "Cereal grains and cereal by-products", and was done according to the contribution described under Section 9.2.2. Average and P95 levels (LB/UB) in this category were 11/43 and 66/66 µg/kg. For cows levels in maize silage, maize grain and soybean meal were used to calculate the average and P95 levels in the diet, and for horses the levels in oats, wheat feed and oat feed were used, in both cases according to the relative contribution to the diet. For sheep and goats it is assumed that the forage part does not contribute to the exposure (EFSA CONTAM Panel, 2013). Cows showed again the highest exposure owing to relatively high levels in maize silage.

Table 19: Mean and P95 levels of NIV in feed (LB/UB) and estimated exposure of different animal species to the parent compound alone

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	8.1	31.8	0.41	1.59
Pigs for fattening	100	3	7.9	31.0	0.24	0.93
Sows	200	6	8.3	32.3	0.25	0.97
Chickens for fattening	2	0.12	8.1	31.8	0.49	1.91
Laying hens	2	0.12	7.2	28.0	0.43	1.68
Turkeys for fattening	12	0.4	7.2	28.0	0.24	0.93
Ducks	3	0.14	7.9	31.0	0.37	1.44
Rabbits	2	0.15	4.0	15.5	0.30	1.16
Fattening cows (corn)	400	10.5	72.4	157.4	1.90	4.13
Dairy cows	650	27.3	47.8	110.6	2.01	4.64
Sheep lactating	80	2.8	3.0	11.8	0.11	0.41
Goats lactating	60	3.4	4.3	16.8	0.24	0.95
Goats fattening	40	1.5	3.1	12.0	0.12	0.45
Horses	450	9	4.1	16.1	0.08	0.32
Cats	4	0.06	6.1	23.7	0.09	0.35
Dogs	25	0.36	7.2	28.0	0.10	0.40
Based on P95 levels						
Piglets	20	1	48.8	48.8	2.44	2.44
Pigs for fattening	100	3	47.5	47.5	1.43	1.43
Sows	200	6	49.5	49.5	1.49	1.49
Chickens for fattening	2	0.12	48.8	48.8	2.93	2.93
Laying hens	2	0.12	42.9	42.9	2.57	2.57
Turkeys for fattening	12	0.4	42.9	42.9	1.43	1.43
Ducks	3	0.14	47.5	47.5	2.22	2.22
Rabbits	2	0.15	23.8	23.8	1.78	1.78
Fattening cows (corn)	400	10.5	368.2	368.2	9.67	9.67
Dairy cows	650	27.3	246.6	246.6	10.36	10.36
Sheep lactating	80	2.8	18.2	18.2	0.64	0.64
Goats lactating	60	3.4	25.7	25.7	1.46	1.46
Goats fattening	40	1.5	18.5	18.5	0.69	0.69
Horses	450	9	24.8	24.8	0.50	0.50
Cats	4	0.06	36.3	36.3	0.54	0.54
Dogs	25	0.36	42.9	42.9	0.62	0.62

Table 20 shows the estimated exposure to NIV and its modified forms based on an average contribution of 30 % of these modified forms.

Table 20: Scenario of mean and P95 levels of NIV in feed (LB/UB) and estimated exposure of different animal species including an additional 30 % contribution of modified forms[(a)]

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w./day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	10.6	41.4	0.53	2.07
Pigs for fattening	100	3	10.3	40.2	0.31	1.21
Sows	200	6	10.7	41.9	0.32	1.26
Chickens for fattening	2	0.12	10.6	41.4	0.63	2.48
Laying hens	2	0.12	9.3	36.3	0.56	2.18
Turkeys for fattening	12	0.4	9.3	36.3	0.31	1.21
Ducks	3	0.14	10.3	40.2	0.48	1.88
Rabbits	2	0.15	5.1	20.1	0.39	1.51
Fattening cows (corn)	400	10.5	94.1	204.7	2.47	5.37
Dairy cows	650	27.3	62.1	143.7	2.61	6.04
Sheep lactating	80	2.8	3.9	15.4	0.14	0.54
Goats lactating	60	3.4	5.6	21.8	0.32	1.24
Goats fattening	40	1.5	4.0	15.7	0.15	0.59
Horses	450	9	5.5	21.0	0.11	0.42
Cats	4	0.06	7.9	30.7	0.12	0.46
Dogs	25	0.36	9.3	36.3	0.13	0.52
Based on P95 levels						
Piglets	20	1	63.5	63.5	3.17	3.17
Pigs for fattening	100	3	61.8	61.8	1.85	1.85
Sows	200	6	64.4	64.4	1.93	1.93
Chickens for fattening	2	0.12	63.5	63.5	3.81	3.81
Laying hens	2	0.12	55.8	55.8	3.35	3.35
Turkeys for fattening	12	0.4	55.8	55.8	1.86	1.86
Ducks	3	0.14	61.8	61.8	2.88	2.88
Rabbits	2	0.15	30.9	30.9	2.32	2.32
Fattening cows (corn)	400	10.5	478.7	478.7	12.57	12.57
Dairy cows	650	27.3	320.6	320.6	13.46	13.46
Sheep lactating	80	2.8	23.6	23.6	0.83	0.83
Goats lactating	60	3.4	33.5	33.5	1.90	1.90
Goats fattening	40	1.5	24.0	24.0	0.90	0.90
Horses	450	9	32.2	32.2	0.64	0.64
Cats	4	0.06	47.2	47.2	0.71	0.71
Dogs	25	0.36	55.8	55.8	0.80	0.80

(a) Values given in this table are rough estimates of exposures to NIV together with modified NIV. The values have been derived by adding 30 % to the exposure values derived for NIV (see Table 19), based on similar percentages for the ratio of contents of NIV with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.

9.3.3. Exposure to the sum of T-2 toxin and HT-2 toxin and their modified forms

In line with the opinion on T2 and HT2 (EFSA CONTAM Panel, 2011b), levels in feed and related exposure of the various species were based on the levels in feed ingredients. This was not based on the combined group "Cereal grains and cereal by-products", but on average and P95 levels in wheat, barley, oats, maize, soybean, rapeseed and lucerne meal, wheat feed, sunflower meal, sugar beet pulp, beans and maize gluten feed and according to the respective contribution described previously (EFSA CONTAM Panel, 2011b). For sheep and lactating and fattening goats, the relative contribution of non-forage feed to the total ration was also taken into account, being 35, 75 and 40 %, respectively. For cows levels in maize silage, maize grain and soybean meal were used to calculate the average and P95 levels in the diet, and for horses the levels in oats, wheat feed and oat feed were used, in both cases according to the relative contribution to the diet.

Goats showed the highest estimated exposure owing to relatively high levels in oats that can contribute significantly to feed for goats. Rabbits also showed a relatively high exposure owing to higher levels in wheat feed.

Table 21: Mean and P95 levels of T2 and HT2 in feed (LB/UB) and estimated exposure of various animal species to the parent compound alone

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	5.4	25.3	0.27	1.27
Pigs for fattening	100	3	9.4	29.0	0.28	0.87
Sows	200	6	9.9	27.9	0.30	0.84
Chickens for fattening	2	0.12	15.8	29.2	0.95	1.75
Laying hens	2	0.12	14.1	25.8	0.85	1.55
Turkeys for fattening	12	0.4	8.1	27.6	0.27	0.92
Ducks	3	0.14	7.6	26.1	0.35	1.22
Rabbits	2	0.15	12.1	20.2	0.91	1.51
Fattening cows (corn)	400	7.1	5.5	38.8	0.10	0.69
Dairy cows	650	27.3	16.6	39.6	0.70	1.67
Sheep lactating	80	2.8	3.9	7.5	0.13	0.26
Goats lactating	60	3.4	47.4	58.9	2.68	3.34
Goats fattening	40	1.5	27.9	33.8	1.05	1.27
Horse	450	9.0	55.3	59.8	0.55	0.60
Cats	4	0.06	13.5	17.1	0.20	0.26
Dogs	25	0.36	15.9	20.2	0.23	0.29
Based on P95 levels						
Piglets	20	1	22.9	42.1	1.15	2.10
Pigs for fattening	100	3	52.6	70.9	1.58	2.13
Sows	200	6	62.8	80.2	1.88	2.41
Chickens for fattening	2	0.12	104.2	116.0	6.25	6.96
Laying hens	2	0.12	92.2	102.3	5.53	6.14
Turkeys for fattening	12	0.4	31.2	47.8	1.04	1.59
Ducks	3	0.14	42.0	59.7	1.96	2.79
Rabbits	2	0.15	73.8	78.8	5.53	5.91
Fattening cows (corn)	400	7.1	32.7	51.5	0.58	0.91
Dairy cows	650	27.3	106.6	118.3	4.48	4.97
Sheep lactating	80	2.8	22.9	25.7	0.80	0.90
Goats lactating	60	3.4	159.7	164.1	9.05	9.30
Goats fattening	40	1.5	92.9	94.8	3.48	3.55
Horse	450	9.0	167.0	167.0	1.67	1.67
Cats	4	0.06	68.3	74.9	1.03	1.12
Dogs	25	0.36	80.8	88.6	1.16	1.28

Table 22 shows the estimated exposure to T2 and HT2 and their modified forms based on an average contribution of 10 % of these modified forms.

Table 22: Scenario of mean and P95 levels of the sum of T2 and HT2 in feed (LB/UB) and estimated exposure of various animal species including an additional 10 % contribution of modified forms[(a)]

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	5.9	27.8	0.30	1.43
Pigs for fattening	100	3	10.3	31.9	0.31	0.96
Sows	200	6	10.9	30.7	0.33	0.92
Chickens for fattening	2	0.12	17.4	32.1	1.05	1.98
Laying hens	2	0.12	15.5	28.4	0.54	1.76
Turkeys for fattening	12	0.4	8.9	30.4	0.30	1.05
Ducks	3	0.14	8.3	28.7	0.39	1.32
Rabbits	2	0.15	13.3	22.2	1.00	1.67
Fattening cows (corn)	400	7.1	6.1	42.7	0.11	0.76
Dairy cows	650	27.3	18.3	43.6	0.77	1.83
Sheep lactating	80	2.8	4.2	8.2	0.15	0.29
Goats lactating	60	3.4	52.1	64.8	2.95	3.67
Goats fattening	40	1.5	30.7	37.2	1.15	1.39
Horses	450	9.0	60.8	65.8	0.61	0.66
Cats	4	0.06	14.8	18.8	0.22	0.28
Dogs	25	0.36	17.5	22.2	0.25	0.32
Based on P95 levels						
Piglets	20	1	25.2	46.3	1.26	2.31
Pigs for fattening	100	3	57.9	78.0	1.74	2.34
Sows	200	6	69.1	88.2	2.07	2.65
Chickens for fattening	2	0.12	114.6	127.6	6.88	7.66
Laying hens	2	0.12	101.4	112.5	6.09	6.75
Turkeys for fattening	12	0.4	34.4	52.6	1.15	1.75
Ducks	3	0.14	46.2	65.7	2.15	3.07
Rabbits	2	0.15	81.2	86.7	6.09	6.50
Fattening cows (corn)	400	7.1	36.0	56.7	0.64	1.01
Dairy cows	650	27.3	117.2	130.2	4.92	5.47
Sheep lactating	80	2.8	25.2	28.2	0.88	0.99
Goats lactating	60	3.4	175.7	180.5	9.96	10.23
Goats fattening	40	1.5	102.2	104.3	3.83	3.91
Horses	450	9.0	183.7	183.7	1.84	1.84
Cats	4	0.06	75.2	82.4	1.13	1.24
Dogs	25	0.36	88.8	97.4	1.28	1.40

(a): Values given in this table are rough estimates of exposures to the sum of T2 and HT2 together with the sum of modified T2 and HT2. The values have been derived by adding 10 % to the exposure values derived for the sum of T2 and HT2 (see Table 21), based on similar percentages for the ratio of contents of the sum of T2 and HT2 with respect to its modified forms in foodstuffs reported in the literature. Precise figures have been retained but should not imply spurious accuracy.

9.3.4. Exposure to fumonisins and their modified forms

Table 23 shows the estimated exposure of various different species based on newly retrieved occurrence data shown in Appendix E on compound feed in the case of pigs, or in the case of cows and horses, ingredients of the rations provided to them. For sheep and goats, the levels in cereals and cereal based products were also applied to estimate the level in compound feed and subsequently in the total diet, using the relative contributions described under Section 9.2.4. For dogs and cats, estimated levels and exposure were based on the level in "Cereal grains and cereal by-products" and the relative contribution to feed (see Section 9.3). As a similar approach was taken for chickens, turkeys and ducks for fattening, for laying hens and for rabbits since no specific data were available on compound feed for these species. Where permitted by available data, the high exposure was also estimated based on P95 values. Highest exposure was estimated for chickens and laying hens, owing to their relatively high consumption of

cereals, relative to their body weight. Dairy cows receiving maize silage were also estimated to have a relatively high exposure.

Table 23: Mean and P95 levels of fumonisins in feed (LB/UB) and estimated exposure of various animal species to the parent compound alone

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	73	205	3.7	10.3
Pigs for fattening	100	3	246	369	7.4	11.1
Sows	200	6	152	395	4.6	11.9
Chickens for fattening	2	0.12	210	306	12.6	18.3
Laying hens	2	0.12	185	268	11.1	16.1
Turkeys for fattening	12	0.4	185	268	6.2	8.9
Ducks	3	0.14	204	297	9.5	13.9
Rabbits	2	0.15	102	149	7.7	11.2
Fattening cows (corn)	400	10.5	23	313	0.6	8.2
Dairy cows	650	27.3	194	421	8.2	17.7
Sheep lactating	80	2.8	78	114	2.7	4.0
Goats lactating	60	3.4	111	161	6.3	9.1
Goats fattening	40	1.5	80	116	3.0	4.3
Horses[(a)]	450	9.0	2	52	0.0	1.0
Cats	4	0.06	156	227	2.3	3.4
Dogs	25	0.36	185	268	2.7	3.9
Based on P95 levels						
Piglets	20	1	353	449	17.6	22.5
Pigs for fattening	100	3	[(b)]	[(b)]	[(b)]	[(b)]
Sows	200	6	970	1070	29.1	32.1
Chickens for fattening	2	0.12	1117	1224	67.0	74.6
Laying hens	2	0.12	981	1093	58.9	65.6
Turkeys for fattening	12	0.4	981	1093	32.7	36.4
Ducks	3	0.14	1086	1210	50.7	56.5
Rabbits	2	0.15	543	605	40.7	45.4
Fattening cows (corn)	400	10.5	[(b)]	[(b)]	[(b)]	[(b)]
Dairy cows	650	27.3	[(b)]	[(b)]	[(b)]	[(b)]
Sheep lactating	80	2.8	415	462	14.5	16.2
Goats lactating	60	3.4	589	656	33.3	37.2
Goats fattening	40	1.5	423	471	15.8	17.7
Horses[(a)]	450	9.0	[(b)]	[(b)]	[(b)]	[(b)]
Cats	4	0.06	830	925	12.4	13.9
Dogs	25	0.36	981	1093	14.1	15.7

(a): because their were no data on oat middling, the values for wheat middling were used.
(b): too few data to calculate P95 values and no exposures could be calculated

Table 24 shows the estimated exposure to fumonisins and their modified forms based on an average contribution of 60 % of these modified forms.

Table 24: Scenarios of mean and P95 levels of fumonisins in feed (LB/UB) and estimated exposure of various animal species including an additional 60 % from modified forms[(a)]

Animal species	Live weight (kg)	Feed intake (kg per day)	Level (µg/kg)		Exposure (µg/kg b.w. per day)	
			LB	UB	LB	UB
Based on mean levels						
Piglets	20	1	117	328	5.9	16.4
Pigs for fattening	100	3	394	590	11.8	17.7
Sows	200	6	243	632	7.3	19.0
Chickens for fattening	2	0.12	336	489	20.2	29.3
Laying hens	2	0.12	295	430	17.7	25.8
Turkeys for fattening	12	0.4	295	430	9.8	14.3
Ducks	3	0.14	327	476	15.3	22.2
Rabbits	2	0.15	164	238	12.3	17.8
Fattening cows (corn)	400	10.5	38	501	1.0	13.2
Dairy cows	650	27.3	311	674	13.1	28.3
Sheep lactating	80	2.8	125	182	4.4	6.4
Goats lactating	60	3.4	177	258	10.0	14.6
Goats fattening	40	1.5	127	185	4.8	6.9
Horses	450	9.0	3	83	0.1	1.7
Cats	4	0.06	250	363	3.7	5.5
Dogs	25	0.36	295	430	4.3	6.2
Based on P95 levels						
Piglets	20	1	562	718	28.1	35.9
Pigs for fattening	100	3	(b)	(b)	(b)	(b)
Sows	200	6	1552	1712	46.6	51.4
Chickens for fattening	2	0.12	1787	1990	107.2	119.4
Laying hens	2	0.12	1569	1748	94.2	104.9
Turkeys for fattening	12	0.4	1569	1748	52.3	58.3
Ducks	3	0.14	1738	1937	81.1	90.4
Rabbits	2	0.15	869	968	65.2	72.6
Fattening cows (corn)	400	10.5	(b)	(b)	(b)	(b)
Dairy cows	650	27.3	(b)	(b)	(b)	(b)
Sheep lactating	80	2.8	664	740	23.2	25.9
Goats lactating	60	3.4	942	1049	53.4	59.4
Goats fattening	40	1.5	676	753	25.4	28.2
Horses	450	9.0	(b)	(b)	(b)	(b)
Cats	4	0.06	1328	1479	19.9	22.2
Dogs	25	0.36	1569	1748	22.6	25.2

(a): Values given in this table are rough estimates of exposures to fumonisins together with modified fumonisins. The values have been derived by adding 60 % to the exposure values derived for fumonisins (see Table 23), based on similar percentages for the ratio of contents of fumonisins with respect to its modified forms in foodstuffs reported in the literature. Acute figures have been retained but should not imply spurious accuracy.

(b): Too few data were available for the parent compound to calculate P95 values and no P95 scenario exposures could be provided

10. Risk characterisation

10.1. Human health risk characterisation

There are no toxicity data on modified forms of ZEN, NIV, T2, HT2, and fumonisins. However, there are studies indicating that glycosides of several mycotoxins and also sulphates of mycotoxins are hydrolysed in the stomach or lower in the GI tract and then absorbed. Furthermore, mycotoxins that are modified by sequestration in the plant matrix are also to a large extent released by digestion in the GI tract. Therefore, toxicity, similar to that of the parent compound can be assumed for modified mycotoxins in both animals and humans, as a pragmatic approach. The risk characterization is

performed by comparing the estimated combined exposure of parent and modified forms of mycotoxins with the established TDIs for the respective parent compounds.

Based on the few data currently available and taking a pragmatic approach, the modified forms of a mycotoxin are assumed to exert the same toxicity as the parent compound.

10.1.1. Zearalenone

The TDI for ZEN is 0.25 µg/kg b.w. per day (250 ng/kg b.w. per day). The exposure estimates with the scenario adding 100 % modified ZEN to the exposure estimates of the parent compound resulted in a mean dietary exposure ranging across age groups and surveys between 4.0 (lowest LB, in the elderly) to 200 ng/kg b.w. per day (highest UB, in toddlers). High exposure amounted to between 7.0 and 554 ng/kg b.w. per day (lowest LB to highest UB). There was, however, a relatively large difference between LB and UB exposure; the highest LB 95th percentile in toddlers was 100 ng/kg b.w. per day, which is more than five-fold lower than the UB. Again, the highest exposure was estimated for toddlers with a maximum mean exposure of 57 (LB) and 250 (UB) ng/kg b.w. per day, and a maximum high exposure of 125 to 692 ng/kg b.w. per day.

Using the LB approach even high consumers will not have a combined exposure to ZEN and modified ZEN above the TDI set for ZEN, indicating no concern. However, if the true concentration of modified and parent ZEN in cereals is closer to the UB level, high consumers may have an exposure up to 2.7-fold the TDI, which would be of concern.

10.1.2. Nivalenol

The TDI for NIV is 1.2 µg/kg b.w. per day (1 200 ng/kg b.w. per day). Estimated mean dietary exposure to NIV with the addition of 30 % modified NIV ranged between 0.5 (lowest LB) ng/kg b.w. per day to 262.6 (highest UB) ng/kg b.w. per day, whereas the highest LB and UB high 95th percentile) exposure was 29.9 and 629.2 ng/kg b.w. per day, respectively. None of these estimated exposures were close to the TDI, and estimated exposure to nivalenol and modified nivalenol is not of concern.

10.1.3. T-2 and HT-2 toxin

The group TDI for the sum of T2 and HT2 is 0.1 µg/kg b.w. per day (100 ng/kg b.w. per day). The mean dietary exposure to T2 and HT2 with the addition of 10 % modified forms ranged from 3.1 (lowest LB) to 47.3 (highest UB) ng/kg b.w. per day across age groups and surveys. The high (95th percentile) exposure was highest in toddlers, where the LB and UB exposures were 71.5 and 100.1 ng/kg b.w. per day, respectively. This high UB level is similar to the TDI, but since the UB is a conservative estimate, the exposure to the sum of T2 and HT2 with the addition of modified forms is not considered to be of concern.

10.1.4. Fumonisins

The group PMTDI for fumonisins is 2 µg/kg b.w.per day. The calculated exposure to fumonisins as parent compounds in European countries showed that mean LB and UB exposure across age class and surveys was lower than the PMTDI, whereas the 95th percentile exposure in toddlers and other children was above the PMTDI in several surveys. In other children, 0 to 18 % (LB) and up to 35 % (UB) had an exposure above the PMTDI, whereas in toddlers up to 27 % had an exposure above the PMTDI (Appendix C, Table C2). This indicates a concern for fumonisin exposure in toddlers and other children with high consumption at the present level of parent fumonisins in food, without the additional exposure from modified forms of fumonisins. However, the unexpectedly high contribution from wheat based products to the total exposure is associated with high uncertainty due to the low number of quantified results. This uncertainty also affects exposure estimates for their modified forms.

The amount of modified fumonisins that comes in addition to the parent compounds can be expected to be 60 %. With the addition of 60 % modified fumonisins, a 95th percentile LB exposure up to 5.0 µg/kg b.w. per day was seen in other children, which is 2.5-fold the PMTDI. The 95th percentile UB estimates indicated a three-fold exceedance of the PMTDI.

With the addition of 60 % modified fumonisins, 0 to 38 % (LB) of other children and 0.2 to 14 % (LB) of toddlers exceeded the PMTDI, whereas the UB estimates indicate that 11 to 59 % of other children and 43 to 66 % of toddlers exceed the PMTDI (Table 25).

Table 25: Percentage above the PMTDI across surveys in the total population with 60 % extra contribution from modified fumonisins

Age class	Percentage above the PMTDI in total population					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Infants (< 1 year old)	0.3	22	-(a)	-(a)	0.3	22
Toddlers (≥ 1 year to < 3 years old)	0.2	43	5.3	51	14	66
Other children (≥ 3 years to < 10 years old)	0.0	11	2.6	32	38	59
Adolescents (≥ 10 years to < 18 years old)	0.0	0.2	0.5	5.7	12	29
Adults (≥ 18 years to < 65 years old)	0.0	0.0	0.1	0.8	0.7	5.3
Elderly (≥ 65 years to < 75 years old)	0.0	0.0	0.0	0.3	0.0	1.3
Very elderly (≥ 75 years old)	0.0	0.0	0.0	0.0	0.2	0.9

(a): Estimates based on only two dietary surveys.

This indicates that the total fumonisin exposure including modified forms may be of concern, especially in children's age groups.

10.2. Animal health risk characterization

The animal health risk characterisation is performed as for humans, by estimating exposure to the sum of parent and modified mycotoxins in comparison with the NOAELs/LOAELs for the respective parent compounds (see Table 2). The assessment is based on a similar percentage addition of modified mycotoxins in animal feed as that in human food (100 % for ZEN, 30 % for NIV, 10 % for the sum of T2 and HT2 and 60 % for fumonisins).

Peak exposures may occur in certain feed commodities and cause an increased exposure of animals of several weeks to months. As such, the higher end of the distribution is also of interest to evaluate the risks for animals. High percentile (P95) exposures have been included when adequate feed data were available.

10.2.1. Zearalenone and its modified forms

No NOAELs/LOAELs for ZEN in animals were derived by EFSA in 2004, since the data available were considered inadequate. However, pigs and sheep were found to be the most susceptible species. In the human ZEN risk assessment from EFSA in 2011, the human TDI was based on a NOEL of 10 µg/kg b.w. per day for oestrogenic effects in pigs.

Pigs

Guidance values for ZEN in feed have been established in Commission Recommendation 2006/576/EC. These values are, in compound feed for piglets and gilts, 0.1 mg/kg feed, and for sows and fattening pigs, 0.25 mg/kg feed. These values can be used for comparison with the reported occurrence of zearalenone in compound feed (Appendix E). The reported mean and P95 values for pig feed were below the guidance values.

Exposure of pigs to ZEN is seen in relation to the NOEL of 10 µg/kg b.w. per day for oestrogenic effects in pigs in the present risk characterization. The mean estimated exposure of pigs to ZEN together with an added 100 % modified ZEN was below the above-mentioned NOEL of 10 µg/kg b.w. per day. In piglets, too, the exposure based on the 95th percentile occurrence in feed was below the NOEL.

Based on available data in feed, there is no concern for exposure to ZEN and modified ZEN in piglets. Exposure in fattening pigs and sows receiving feed with average levels is also not of concern. Occurrence data were inadequate to draw conclusions regarding fattening pigs and sows receiving feed with higher than average contamination levels.

Ruminants

The guidance value for ZEN in feed as established in Commission Recommendation (2006/576/EC) is 0.5 mg/kg feed for dairy cattle. The occurrence data for ZEN as parent compound are well below 0.5 mg/kg feed for dairy cattle. However, in maize silage, a major feed source, the P95 value exceeds the guidance value. The addition of 100 % modified ZEN to the occurrence would lead to an even higher fraction exceeding this guidance value.

EFSA has not derived a NOAEL/LOAEL for cattle, but cattle are considered to be less sensitive to ZEN than pigs (EFSA, 2004).

The estimated exposure after feeding with mixed feed (maize silage, maize grain and soy bean meal) at the 95th percentile concentration of ZEN and modified ZEN was for fattening cows 38 µg/kg b.w. per day and for dairy cows 47 µg/kg b.w. per day (i.e. being four to five-fold the NOEL derived for pigs). However, in the absence of a NOAEL/LOAEL, the risk for cattle cannot be characterised fully.

According to EFSA (2004) next to pigs sheep are rather sensitive to ZEN toxicity. Estimated exposure in sheep is lower than in pigs, and therefore not of concern.

Estimated exposure in goats is considerably lower than in cows but, owing to the lack of an effect level, the risk for goats cannot be characterised fully.

Poultry

There are neither NOAELs/LOAELs nor guidance values for poultry. The mean exposure in poultry to ZEN together with its modified forms is between 4 and 8 µg/kg b.w. per day whereas the P95 exposures are between 15 and 22 µg/kg b.w. per day. According to EFSA (2004) poultry is less susceptible to ZEN induced toxicity than pigs. The CONTAM Panel therefore consideres exposures of poultry to ZEN and its modified forms not to be of concern.

Horses

There are neither NOAELs/LOAELs nor guidance values for horses. Estimated exposure to ZEN and modified ZEN based on mean occurrence in horse feed is 0.2 µg/kg b.w. per day, thus far below the NOEL derived for pigs of 10 µg/kg b.w. per day and considered not to be of concern.

Rabbits

There are neither NOAELs/LOAELs nor guidance values for rabbits. Estimated exposure to ZEN and modified ZEN based on mean occurrence in rabbit feed is 10 µg/kg b.w. per day, thus similar to the NOEL derived for pigs. In the absence of information on susceptibility of rabbits to ZEN a concern cannot be excluded.

Fish

No occurrence data were available for fish feed, consequently no exposure assessment and risk characterisation could be carried out.

Cats and dogs

There are neither NOAELs/LOAELs nor guidance values for cats and dogs. However, estimated exposure to ZEN and modified ZEN based estimated mean and 95th percentile occurrence in grains possibly used for pet food is maximally 3 µg/kg b.w. per day and thus far below the NOEL of 10 µg/kg b.w. per day derived for pigs (sensitive to ZEN) and thus not of concern.

10.2.2. Nivalenol and its modified forms

Pigs

For NIV the CONTAM Panel established a LOAEL of 100 µg/kg b.w. per day for pigs (EFSA CONTAM Panel, 2013). The estimated exposure to NIV with the addition of 30 % modified NIV to the parent compound results in a mean exposure which is maximally 2 % (mean exposure level) to 3 % (95th percentile exposure) of the LOAEL, and is therefore not of concern.

Ruminants

No NOAELs/LOAELs have been set for NIV in ruminants. Dairy cows had the highest 95th percentile exposure of 13 µg/kg b.w. per day. In EFSA CONTAM Panel (2013) the CONTAM Panel noted that the susceptibility of ruminants to nivalenol mediated toxicity is likely to be low based on evidence of detoxification of the compound by de-epoxidation by rumen microorganisms. Based on this, exposure of ruminants to NIV and its modified forms is not of concern.

Poultry

For NIV the CONTAM Panel established a LOAEL of 53 µg/kg b.w. per day for poultry (EFSA CONTAM Panel, 2013). The highest mean and 95th percentile exposure to NIV with additional 30 % modified NIV, which was estimated for fattening chicken, was 2.5 and 3.8 µg/kg b.w. per day, respectively. This is only 5 to 7 % of the LOAEL and therefore not of concern.

Fish

As regards fish no occurrence data were available, consequently no exposure assessment and risk characterisation could be carried out.

Other livestock and companion animals

Since no NOAELs/LOAELs have been set for NIV in horses, rabbits, cats and dogs no risk characterisation can be carried out. However, 95th percentile exposures in these species ranged between 0.6 and 2.3 µg/kg b.w. per day. This is much lower than the LOAEL of 53 µg/kg b.w. per day derived for poultry, the most sensitive species for which a NOAEL/LOAEL has been derived (EFSA CONTAM Panel, 2013). Therefore exposure to NIV and its modified forms is not of concern.

10.2.3. T-2 and HT-2 toxin and their modified forms

Pigs

In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 29 µg/kg b.w. per day for pigs. The highest mean exposure was calculated for piglets and sows (about 1.0 µg/kg b.w. per day) after adding

10 % modified T2 and HT2 to the exposure of the parent compound. At the 95th percentile the exposure was estimated to be 2.3 µg/kg b.w. per day for piglets and 2.7 µg/kg b.w. per day for sows. This is up to 9 % of the LOAEL and not of concern.

Ruminants

In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 300 µg/kg b.w. per day for ruminants. In ruminants the highest 95th percentile exposure to T2 and HT2 with addition of 10 % modified forms was estimated in lactating goats at 10.4 µg/kg b.w. per day. This is 3.4 % of the LOAEL and not of concern.

Poultry

In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 40 µg/kg b.w. per day for poultry. The highest estimated exposure to T2 and HT2 toxin with addition of 10 % modified forms was in chicken for fattening with 2.0 (mean) and 7.8 (95th percentile) µg/kg b.w. per day. This is up to 20 % of the LOAEL and not of concern.

Rabbits

In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 100 µg/kg b.w. per day for rabbits. In rabbits the highest 95th percentile exposure to T2 and HT2 with addition of 10 % modified forms was estimated to be 6.5 µg/kg b.w. per day. This corresponds to 7 % of the LOAEL and thus not of concern.

Fish

In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 13 µg/kg b.w. per day for fish. No exposure data to the parent compound were available for fish. No exposure assessment to T2 and HT2 with addition of 10 % modified forms could be performed and consequently also no risk can be characterised.

Horses

No NOAELs/LOAELs have been set for horses. The estimated 95th percentile exposure in horses to T2 and HT2 with 10 % addition of modified forms was 1.8 µg/kg b.w. per day. This is lower than in sows, for which no concern was identified, indicating that exposure to the sum of T2 and HT2 with 10 % addition of modified forms is not of concern for horses.

Cats

The guidance value for cat food is 50 µg/kg feed. The mean estimated occurrence of T2 and HT2 and its modified forms in cat food is below the guidance value, however, the 95th percentile is in the range of 75 – 82 µg/kg feed exceeding the guidance value. The 95th percentile estimated exposure in cats was 0.71 µg/kg b.w. per day. No NOAELs/LOAEL is available for cats, but it is known that this species is sensitive toward T2 and HT2 induced toxicity. A concern cannot be excluded but is unlikely, based on low exposure.

Dogs

The indicative value (2013/165/EU) for dog food is 250 µg/kg feed. The estimated mean and 95th percentile occurrence in dog food based on mean and 95th percentile occurrence in grains possibly used for dog food is lower than the indicative value. There are no NOAELs/LOAELs for dogs.

Estimated exposure to T2 and HT2 and their modified forms is maximally 1.4 µg/kg b.w. per day and thus far below the LOAELs and NOAELs derived for other species, and not of concern.

10.2.4. Fumonisins and their modified forms

Pigs

For fumonisins a LOAEL of 200 µg/kg b.w. per day was derived for pigs by EFSA in 2005. Sows had the highest estimated exposure to fumonisins and their modified forms (60 % added to parent compound). The estimated mean and 95th percentile UB exposure was 19 and 51 µg/kg b.w. per day, respectively. This is up to 25 % of the LOAEL indicating no concern.

Ruminants

For ruminants, the lowest NOAEL identified by EFSA in 2005 was 600 µg/kg b.w. per day. In ruminants the highest mean exposure to fumonisins and 60 % addition of modified forms was 28 µg/kg b.w. per day. This is 5 % of the NOAEL and not of concern. Estimations of high exposure could not be done for the ruminant with highest exposure (lactating cows) due to lack of occurrence data, but is not expected to exceed the NOAEL.

Poultry

For poultry, EFSA identified a LOAEL of 2000 µg/kg b.w. per day in 2005. The highest estimated 95th percentile exposure to fumonisins and 60 % addition of modified fumonisins was 119 µg/kg b.w. per day in chickens for fattening. This is 6 % of the LOAEL, indicating no concern.

Horses

For horses the LOAEL identified by EFSA in 2005 was 200 µg/kg b.w. per day. The mean UB exposure to fumonisins and 60 % addition of modified fumonisins was 1.7 µg/kg b.w. per day. As this is less than 1 % of the LOAEL, it is of no concern.

Rabbits

For rabbits no NOAELs/LOAELs for fumonisins have been set. Mean UB exposure to fumonisins and their modified forms in rabbits is estimated to be 18 µg/kg b.w. per day. The mean occurrence level in feed is below the guidance value of 5000 µg/kg. Estimations of high exposures (P95) is 73 µg/kg b.w. per day, and not of concern since it is well below the LOAEL of 200 µg/kg b.w. per day set for the most sensitive species, pigs and horses.

Fish

In fish (carp) a LOAEL of 10 mg/kg feed was identified by EFSA in 2005. The lack of occurrence data for fumonisins and their modified forms in fish feed prevents an exposure assessment and a risk characterization in fish.

Cats and dogs

For cats and dogs, NOAELs/LOAELs are not available. The guidance value for pet food is 5 mg/kg feed. The estimated 95th percentile values for fumonisins and their modified forms are 1.5 and 1.7 mg/kg feed, respectively.

Estimated 95th percentile exposures to fumonisins and their modified forms are 22 and 25 µg/kg b.w. per day, for cats and dogs respectively and thus far below the LOAEL of 200 µg/kg b.w. per day derived for pigs and horses (sensitive to fumonisins) and thus not of concern.

10.3. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of the risks for animal and public health related to the presence of modified mycotoxins in food and feed has been performed following the guidance of the opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). In accordance with the guidance provided by the EFSA opinion (2006), the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model and model input (parameters).

10.3.1. Assessment objectives

The objectives of the assessment were specified in the terms of reference.

10.3.2. Exposure scenario/model/input parameters

Based on the fact that insufficient occurrence data for the modified mycotoxins covered by the terms of reference were available, exposure assessments have been carried out on the basis of exposure assessments for the parent compounds and adding literature derived factors to account for modified mycotoxins.

To establish total exposure levels by combining exposures to parent compound and its respective modified form, certain factors have been added to the overall exposures established in recently carried out risk assessments (EFSA CONTAM Panel, 2011a, b, 2013) for different consumer groups for ZEN, NIV and T2 and HT2, and for the different animal species for NIV, T2 and HT2. The uncertainties incurred in the derivation of the exposure levels (for both the different human consumer groups and animal species), and that apply also to the present evaluations, are already described in these assessments, and are they are therefore not repeated here.

The factors added to account for modified mycotoxins are based on limited data from the literature. Actual exposures to the different modified mycotoxins may well deviate.

Occurrence data on parent compounds include data generated with ELISA, which potentially detect both parent compounds and their modified forms. Adding certain factors to the parent compound to account for the modified forms may lead to an overestimation of overall occurrence.

The extraction methods for fumonisins are hampered by high variability and low accuracy which could lead to an underestimation of actual concentrations. Adding 60 % entrapped fumonisins to the reported fumonisin exposure levels could lead to an overestimation of actual exposures, since it is unclear to what extent these forms were extracted by methods used to generate the data present in the EFSA database.

Since no recent human exposure assessment is available for fumonisins, such an assessment has been carried out for the present opinion. The main uncertainty in this assessment is related to the big differences between the LB and UB estimates of occurrence and exposure levels owing to high LOQs associated with censored data.

F. verticillioides, which produces fumonisins, is known to grow predominantly on maize. Maize-based products are therefore considered to be the main dietary source. However, the fumonisin exposure assessment indicated that wheat-based cereals are a major source. This exposure from wheat based products is based on a few quantified samples and is associated with a high uncertainty. However, recent data in the literature confirm the presence in certain wheat products.

Overall, the human exposure assessment for fumonisins and consequently that for its modified forms are likely to be overestimations.

Since no recent animal exposure assessments are available for ZEN and fumonisins, such assessments have been carried out for the present opinion. In the preparation of the exposure assessments, the occurrence data collated by EFSA on these two substances were considered. The main uncertainty related to this dataset is that only a "light" quality procedure was followed (i.e. only checking of duplicates and consistency of information related to feed description, harmonisation of the unit of expression of the results and expressing fumonisins as total fumonisins equivalents). Normally, an outlier analysis is also performed in order to identify potential errors in reporting the results.

10.3.3. Other uncertainties

Since neither animal and human risks assessments nor any toxicological data are available for the modified mycotoxins covered by the terms of reference, toxicological/guidance values set for their parent compounds in previous evaluations have been used for the present risk assessments. Consequently, all the uncertainties regarding toxicity assessment identified in these previous assessments (EFSA CONTAM Panel, 2011a, b, 2013; FAO/WHO, 2012) and incurred with the guidance values set in Commission Recommendation 2006/576/EC are also applicable for the present assessment and are therefore not repeated here.

In the absence of any data, the present assessment is based on the assumption that modified mycotoxins have identical toxicological profiles and potencies as their parent compounds. This is based on data showing that the modified forms can be hydrolysed to the parent compounds in the GI tract. To what extent modified forms *per se* can exert toxic activity is not known.

Modified mycotoxins may easily be overlooked because of too high LOQs. This is a result of their relatively lower occurrence compared with the parent compound, which may however still be substantial, especially when dealing with a set of these compounds. A particular future issue in this regard is how to deal with left-censored data in order to arrive at an UB exposure. In the current opinion this does not apply, since levels of modified forms were estimated at a fixed contribution to the LB but also UB level of the parent compound.

10.3.4. Summary of uncertainties

In Table 26, 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 26: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the modified mycotoxins in food and feed

Sources of uncertainty	Direction[(a)]
Absence of occurrence data for animals and human consumer groups for modified mycotoxins	+/-
Lack of clarity about recovery of methods used for fumonisin analysis	+
Big difference between LB and UB estimates of occurrence and exposure owing to high LOQs associated with left censored data for fumonisins	+
Procedure applied for occurrence in feed data collection on zearalenone and fumonisins	+
Incomplete animal health NOAELs/LOAELs	+/-
Factors added to parent compounds to account for modified forms derived from limited data	+/-
Assumption that modified mycotoxins have similar toxicological profile and potency as their parent compounds	+/-

LOQ: limit of quantification; LB lower bound; UB: upper bound. NOAEL; No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level.
(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk, extent of potential over/underestimation might differ in direction.

The overall uncertainty incurred with the present assessment is considered as high. The exposure assessments to modified mycotoxins are likely to be overestimations.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Modified mycotoxins are metabolites of the parent (free) mycotoxin formed in the plant or fungus by conjugation with polar compounds. The most common conjugations are with glucose and modified glucose and others such as sulphate and glutathione.
- Modified mycotoxins have up to recently only occasionally been detected when analysing for mycotoxins.
- The present assessment covers modified forms of zearalenone, nivalenol, T-2 and HT-2 toxins and fumonisins. Concerning fumonisins, both covalently and non-covalently bound forms have been considered as modified forms.

Methods of analysis

- Protocols for modified mycotoxin analysis are mainly based on water/acetonitrile extraction followed by a "dilute & shoot" analysis by liquid chromatography – tandam mass spectrometry.
- Both targeted and untargeted methods are used and represent the methods of choice for modified mycotoxin detection. Immunochemical methods are simple alternatives for routine analysis but they are associated with higher uncertainties.
- Concerning fumonisins and their modified forms, variability due to different extraction strategies is high and can significantly affect the occurrence data.

- There is a lack of properly validated routine methods due to the lack of commercially available calibrants and reference materials.

Occurrence/Exposure

- Bran- and fiber-enriched products are more prone to contamination with mycotoxins including their modified forms.
- The EFSA occurrence database contains no data on modified mycotoxins covered by the present opinion. Therefore occurrence is based on limited information reported in the literature.
- Literature data show that modified forms of mycotoxins may add substantially to the overall mycotoxin levels, in particular for zearalenone and fumonisins
- For fumonisins, the major contribution comes from modified parent compound whereas for the other compounds, metabolites are the main contributors to modified forms
- In order to assess occurrence and exposure the Panel on Contaminants in the Food Chain (CONTAM Panel) added 100 %, 30 %, 10 % and 60 % to the levels of the parent compounds to account for the modified forms of zearalenone, nivalenol, sum of T-2 and HT-2 toxin and fumonisins, respectively.
- Occurrence data about modified mycotoxins in animal products (i.e. milk and dairy, meat, eggs) are not available, but occurrence in animal products is expected to be very low, since carry-over of *Fusarium* toxins from feed is considered insignificant for human exposure based on currently available data.

Hazard identification and characterisation

- Modified mycotoxins can be released, hydrolysed, biotransformed and absorbed in the gastrointestinal (GI) tract.
- Although there is no information on toxicity of conjugated mycotoxins addressed in the present opinion, literature data show that conjugates of xenobiotics can be of toxicological significance.
- Based on this the CONTAM Panel assumed equal toxicity of all modified forms as their parent compounds as a pragmatic approach.

Human health risk characterisation

- The risk characterization is performed by comparing the estimated combined exposure to parent and modified forms of mycotoxins with the established (provisional maximum) tolerable daily intake ((PM)TDI) levels for the respective parent compounds .
- Using the lower bound (LB) approach, no consumers with mean or 95th percentile exposure have combined exposure to zearalenone and modified zearalenone above the TDI set for zearalenone, indicating no concern. However, if the concentration of modified and parent zearalenone in cereals is closer to the upper bound (UB) level, high consumers (95th percentile) may have exposure up to 2.2 fold the TDI for zearalenone of 0.25 µg/kg body weight (b.w.) per day, which would be of concern.

- Exposure to the sum of nivalenol and modified nivalenol is not of concern, as the highest 95th percentile UB exposure across studies is less than 20 % of the TDI for nivalenol of 1.2 µg/kg b.w. per day.

- Exposure to the sum of T-2 and HT-2 toxin and their modified forms is considered not to be of concern since all the LB and UB exposures across studies were lower than the TDI of 0.1 µg/kg b.w. per day for the sum of T-2 and HT-2 toxin, with exception of the highest UB exposure which was similar as the TDI.

- The exposure to fumonisins and their modified forms could be of concern, especially in children age groups. In toddlers, 0.2-14 % (LB) to 43-66 % (UB) and in other children 0-38 % (LB) to 11-59 % (UB) across studies could exceed the PMTDI for fumonisins of 2 µg/kg b.w. per day. At high (95th percentile) exposure, the maximal exceedance was 2.5-fold (LB) to 3-fold (UB) the PMTDI.

Animal health risk characterisation

- In order to assess occurrence in feed and exposure of animals, the CONTAM Panel added equal factors for masked mycotoxins in feed as in food; 100 %, 30 %, 10 % and 60 % to the levels of the parent compounds to account for the modified forms of zearalenone, nivalenol, sum of T-2 and HT-2 toxin and fumonisins, respectively.

- Levels in feed have been compared with guidance values for feed, if established, and estimated exposures in animals are evaluated in relation to established NOAELs/LOAELs for different animals, when available. Furthermore, known differences in sensitivities between different species were taken into account when NOAELs/LOAELs were lacking.

- For fumonisins, contaminated feed has been used in the studies used for derivation of NOAELs/LOAELs, with the exception of poultry, for which the pure compound has been added to feed. For the other *Fusarium* toxins, experiments were carried out with application of pure compounds.

Zearalenone (ZEN) and its modified forms

Pigs

- The reported mean and P95 values for pig feed were below the guidance values in pig feed of 0.1 mg/kg feed in compound feed for piglets and gilts, and 0.25 mg/kg feed in feed for sows and fattening pigs.

- Risk characterization in pigs was based on the NOEL of 10 µg/kg b.w. per day for oestrogenic effects in pigs, which formed the basis for the human risk characterization in EFSA CONTAM Panel (2011a). Pigs are more senstitive to ZEN toxicity than other animal species.

- Based on available data in feed (mean and 95th percentile occurrence), there is no concern for exposure to ZEN and modified ZEN in piglets. Exposure in fattening pigs and sows receiving feed with average levels is also not of concern. Occurrence data were inadequate to conclude on risks regarding fattening pigs and sows receiving feed with higher than average contamination level.

Ruminants

- Addition of 100 % modified ZEN to the occurrence of ZEN as parent compound would lead to an exceedance of the guidance value of 0.5 mg/kg feed for dairy cattle for maize silage.

- EFSA has not derived a NOAEL/LOAEL for cattle, but cattle are considered to be less sensitive towards ZEN than pigs (EFSA, 2004). The estimated exposure after feeding with mixed feed containing 95th percentile concentration of ZEN and modified ZEN was in fattening cows and in dairy cows 4-5 fold the NOEL derived for pigs. The exposure in goats was lower. However, in the absence of a NOAEL/LOAEL the risk for cattle and goats cannot be characterised fully.

- According to EFSA (2004) next to pigs sheep are rather sensitive towards ZEN toxicity. Estimated exposure in sheep is lower than in pigs, and therefore not of concern.

Poultry

- There are neither NOAELs/LOAELs nor guidance values for poultry. According to EFSA (2004) poultry is less susceptible ZEN induced toxicity than pigs.

- The 95th percentile exposures in poultry to ZEN together with its modified forms are in the range of 2-fold the NOEL in pigs of 10 µg/kg b.w. per day, and the CONTAM Panel therefore considers exposures of poultry to ZEN and its modified forms not of concern.

Horses

- There are neither NOAELs/LOAELs nor guidance values for horses. Estimated exposure to ZEN and modified ZEN based on mean occurrence in horse feed is only 2 % of the NOEL in pigs of 10 µg/kg b.w. per day and thus not of concern.

Rabbits

- There are neither NOAELs/LOAELs nor guidance values for rabbits. Estimated exposure to ZEN and modified ZEN based on mean occurrence in rabbit feed is 10 µg/kg b.w. per day and thus similar to the no observed effect level (NOEL) derived for pigs. In the absence of information on susceptibility of rabbits towards ZEN a concern cannot be excluded.

Fish

- No occurrence data were available for fish feed, consequently no exposure assessment and risk characterisation could be carried out.

Cats and dogs

- There are neither NOAELs/LOAELs nor guidance values for cats and dogs. Exposure to ZEN and modified ZEN based on estimated 95th percentile occurrence in grains possibly used for pet food is maximally 30 % of the NOEL of 10 µg/kg b.w. per day derived for pigs and thus not of concern.

Nivalenol (NIV) and its modified forms

Pigs

- For NIV the CONTAM Panel established a LOAEL of 100 µg/kg b.w. per day for pigs (EFSA CONTAM Panel, 2013). The estimated exposures to NIV with the addition of 30 % modified NIV to the parent compound result in a mean exposure which is maximally 3% (95th percentile exposure) of the lowest observed adverse effect level (LOAEL), and is therefore not of concern.

Ruminants

- No NOAELs/LOAELs have been set for NIV in ruminants. Since EFSA noted in 2013 that the susceptibility of ruminants to nivalenol mediated toxicity is likely to be low, and that dairy cows had the highest 95th percentile exposure of 13 % of the LOAEL in pigs, the exposure of ruminants to NIV and its modified forms is not of concern.

Poultry

- For NIV the CONTAM Panel established a LOAEL of 53 µg/kg b.w. per day for poultry (EFSA CONTAM Panel, 2013). The highest 95th percentile exposure to NIV with additional 30 % modified NIV in poultry was only 7 % of the LOAEL and therefore not of concern.

Fish

- No occurrence data were available for fish, consequently no exposure assessment and risk characterisation could be carried out.

Other livestock and companion animals

- No NOAELs/LOAELs have been set for NIV in horses, rabbits, cats and dogs. The 95th percentile exposures in these species ranged between 0.6 and 2.3 µg/kg b.w. per day. This is much lower than the LOAEL of 53 µg/kg b.w. per day derived for poultry, the most sensitive species for which a NOAEL/LOAEL has been derived (EFSA CONTAM Panel, 2013). Therefore, exposure to NIV and its modified forms is most likely not of concern.

T-2 and HT-2 toxin (T2 and HT2) and their modified forms

Pigs

- In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 29 µg/kg b.w. per day for pigs. The highest 95th percentile exposure after adding 10 % modified T2 and HT2 to the exposure of the parent compound was 9 % of the LOAEL, and not of concern.

Ruminants

- In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 300 µg/kg b.w. per day for ruminants. In ruminants the highest 95th percentile exposure to T2 and HT2 with addition of 10 % modified forms was estimated to 3.4 % of the LOAEL, and not of concern.

Poultry

- In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 40 µg/kg b.w. per day for poultry. The highest estimated exposure to T2 and HT2 with addition of 10 % modified forms was maximally 20 % of the LOAEL and not of concern.

Rabbits

- In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 100 µg/kg b.w. per day for rabbits. The 95th percentile exposure to T2 and HT2 with addition of 10 % modified forms was 7 % of the LOAEL and not of concern.

Fish

- In 2011 EFSA derived a LOAEL for the sum of T2 and HT2 of 13 µg/kg b.w. per day for fish. No exposure data to the parent compound was available for fish. No exposure assessment to T2 and HT2 with addition of 10 % modified forms could be performed, and consequently the risk cannot be characterised.

Horses

- No NOAEL/LOAEL has been set for horses. The estimated 95th percentile exposure in horses to T2 and HT2 with 10 % addition of modified forms was 1.8 µg/kg b.w. per day. This is lower than in pigs (sensitive to T2 and HT2), for which no concern was identified, indicating that exposure to the sum of T2 and HT2 with 10 % addition of modified forms is not of concern in horses.

Cats

- The guidance value for cat food is 50 µg/kg feed. The mean estimated occurrence of T2 and HT2 and its modified forms in cat food is below the guidance value, however, the 95th percentile is in the range of 75 – 82 µg/kg feed exceeding the guidance value. The 95th percentile estimated exposure in cats was 0.71 µg/kg b.w. per day. No NOAEL/LOAEL is available for cats but it is known that this species is sensitive toward T2 and HT2 induced toxicity. A concern cannot be excluded but is unlikely based on low exposure.

Dogs

- The indicative value (2013/165/EU) for dog food is 250 µg/kg feed. The estimated mean and 95th percentile occurrence in grains possibly used for dog food is lower than the indicative value. There is no NOAEL/LOAEL for dogs. Estimated exposure to T2 and HT2 and their modified forms is far below the LOAELs and NOAELs derived in other species and thus not of concern.

Fumonisins and their modified forms

Pigs

- For fumonisins a LOAEL of 200 µg/kg b.w. per day was derived for pigs by EFSA in 2005. The highest estimated 95th percentile exposure to fumonisins with the addition of 60 % modified forms to the parent compound was up to 16 % of the LOAEL, indicating no concern in pigs.

Ruminants

- For ruminants, the lowest NOAEL identified by EFSA in 2005 was 600 µg/kg b.w. per day. In ruminants the highest mean exposure to fumonisins with addition of 60 % modified forms was 5 % of the NOAEL and not of concern. Estimations of high exposure could not be done for ruminants due to lack of occurrence data, but is not expected to exceed the NOAEL.

Poultry

- For poultry, EFSA identified a LOAEL of 2 000 µg/kg b.w. per day in 2005. The highest estimated 95th percentile exposure to fumonisins and 60 % addition of modified fumonisins was 6 % of the LOAEL, indicating no concern.

Horses

- For horses the LOAEL identified by EFSA in 2005 was 200 µg/kg b.w. per day. The mean exposure to fumonisins with 60 % addition of modified fumonisins was less than 1 % of the LOAEL, and of no concern.

Rabbits

- The mean occurrence level in feed of fumonisins with addition of 60 % masked forms is below the guidance value of 5 000 µg/kg set for fumonisins. For rabbits no NOAELs/LOAELs for fumonisins have been set. Estimations of high exposures (P95) is 73 µg/kg b.w. per day, and not of concern since it is well below the LOAEL of 200 µg/kg b.w. per day set for the most sensitive species, pigs. and horses.

Fish

- In fish (carp) a LOAEL of 10 mg/kg feed was identified by EFSA in 2005. The lack of occurrence data for fumonisins and their modified forms in fish feed prevents an exposure assessment and a risk characterization in fish.

Cats and dogs

- The guidance value for fumonisins in pet food is 5 mg/kg feed. The estimated 95th percentile values for fumonisins and their modified forms are below the guidance value. NOAELs/LOAELs are not available for cats and dogs. Estimated 95th percentile exposures to fumonisins and their modified forms for cats and dogs are maximally 12 % of the LOAEL of 200 µg/kg b.w. per day derived for pigs and horses and most likely not of concern.

RECOMMENDATIONS

- There is a need for more information on the chemical structures of modified mycotoxins.
- There is a need for further work to identify modified mycotoxins not yet characterised.
- Nomenclature including abbreviations should be standardised for mycotoxins and their modified forms.
- There is a need for properly validated and sensitive routine analytical methods for modified mycotoxins.

- The fate of modified forms of mycotoxins upon food and feed processing should be further investigated.
- There is a need for more occurrence data on mycotoxins and their modified forms in food and feed, in particular for fish and pet food.
- There is a need for toxicological data on modified mycotoxins.
- Re-assessments of animal health effects of zearalenone and fumonisins are needed in order to set NOAELs/LOAELs for these compounds

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APPENDICES

Appendix A. Occurrence data for fumonisins

The occurrence data correspond to the European data that were considered by the JECFA in its risk assessment (FAO/WHO, 2012). The dataset contained 3 654 analytical results obtained on food samples, generated with analytical methods based on gas or liquid chromatography and ELISA, collected between 2000 and 2010 and reported by 11 European countries. Around 44 % of the analytical results corresponded to the sum of fumonisins B_1, B_2 and B_3 whereas the other results corresponded to the sum of fumonisins B_1 and B_2 only. As done in the JECFA evaluation, the results corresponding to the sum of fumonisins B_1 and B_2 were used together with the ones corresponding to the sum of fumonisins B_1, B_2 and B_3, without any adjustment.

Tables A1 and A2 present the mean levels used for the exposure assessment. Two estimates were produced depending on the assessment made on the results below the LOD/LOQ: the lower bound estimate (LB), replacing all results reported as below the LOD/LOQ by 0, and the upper bound estimate (UB), replacing all results reported as below the LOD/LOQ to their respective LOD/LOQ.

Table A1: Distribution of fumonisins levels in grains and grain-based products, expressed in µg/kg

Foodex level	Food groups	N[(a)]	LC[(b)]	Mean LB-UB[(c)]	P95 LB-UB[(c)]
1	**Grains and grain-based products**	**2 981**	**53**	170 - 215	811 – 812
2	**Grains for human consumption**	**186**	**58**	102 - 161	577 – 589
3	Corn grain	127	46	145 - 186	669
Ahg	Other grains (wheat, barley, maize, rice)	58	84	8 - 106.8	-
3	*Grains unspecified*	*1*	*100*	*0 - 40*	-
2	**Grain milling products**	**1 366**	**47**	279 - 315	1 321
3	Maize milling products	1 204	41	316 - 349	1 498
4	Maize flour	535	30	468 - 497	2 302
4	Maize semolina	589	46	213 - 246	1 018
4	Maize starch	15	100	0 - 61.7	-
4	Other and unspecified maize milling products	65	80	64.5 - 119.3	231 – 331
Ahg	Milling products from other grains	106	97	5.3 - 72.7	0 – 260
Ahg	*Milling products from unspecified grains*	*56*	*89*	*7.4 - 64.5*	-
2	**Bread and rolls**	**128**	**70**	112 - 199	389 – 404
4	Maize bread	8	13	1 220 – 1 229	-
4	Tortilla bread	25	60	93 – 198	-
Ahg	Bread from other grains	60	80	17.2 - 124.3	111 – 260
Ahg	*Bread from unspecified grains*	*35*	*71*	*33.8 - 91*	-

Table A1: Distribution of fumonisins levels in grains and grain-based products, expressed in µg/kg (continued)

Foodex level	Food groups	N [(a)]	LC [(b)]	Mean LB-UB[(c)]	P95 LB-UB[(c)]
2	**Pasta**	**112**	**74**	**137 - 201**	**883**
Ahg	Pasta from maize grain	4	25	188 - 208	-
Ahg	Pasta from other grain (wheat)	60	95	24.2 - 91.8	26.9 – 200
Ahg	*Pasta from unspecified grains*	*48*	*52*	*273 - 336*	-
2	**Breakfast cereals**	**770**	**58**	**41.1 - 84.9**	**176 - 260**
Ahg	Breakfast cereals made from corn	556	54	41.9 - 82.7	176 - 247
Ahg	Breakfast cereals made from other grains	43	91	4.8 - 89.8	-
Ahg	*Breakfast cereals made from grain mixtures or unspecified grains*	*171*	*65*	*47.6 - 91.1*	*248 - 260*
2	**Fine bakery wares**	**415**	**52**	**109 - 164**	**601 - 638**
3	Pastries and cakes	2	100	0 – 35	-
3	Biscuits (cookies)	409	51	110 - 165	601 - 638
4	Biscuits salty	394	49	114 - 162	609 - 639
4	Biscuits sweet	15	93	20 - 231	-
3	*Fine bakery wares unspecified*	*4*	*100*	*0 - 100*	-
2	***Grains and grain-based products unspecified***	*4*	*100*	*0 – 39*	-

(a): N: number of samples.
(b): LC: percentage of censored results.
(c): Mean LB-UB, P95 LB-UB, mean and 95th percentile contamination level presented as lower bound estimate - upper bound estimate. When the lower and upper bound estimates are equal, only one estimate is given. In case of too few observations (less than 60 for the 95th percentile), the estimation may be biased and is not consequently not provided.
Note: The numbers for the occurrence values are all given with 3 figures, but this does not reflect precision.
Ahg: ad-hoc food groups created for the purpose of the assessment. In italics: food groups not taken into account in the exposure assessment.

Table A2: Distribution of fumonisins levels in other food products, expressed in µg/kg

Foodex level	Food groups	N[(a)]	LC[(b)]	Mean LB-UB[(c)]	P95 LB-UB[(c)]
1	**Vegetables and vegetable products**				
3	Sweet corn (Zea mays var. saccharata)	33	76	81 - 104.7	-
Not taken into account in the exposure assessment: vegetables and vegetable products other than sweet corn (N =2)					
1	**Foods for infants and young children**				
Ahg	**Cereal-based food for infants and young children**	221	95	2.8 - 99.3	0 - 260
Ahg	Cereals which are or have to be reconstituted	72	94	5.8 - 136.3	28 - 260
3	Biscuits, rusks and cookies for children	45	89	3.4 - 92.5	-
3	Cereal-based meals	5	100	0 – 147	-
3	*Cereal-based food unspecified*	*99*	*99*	*0.5 – 73*	*0 - 230*
Not taken into account in the exposure assessment: foods for infants and young children other than cereal-based (N = 16)					
1	**Foods for special nutritional use**				
3	*Formulas for metabolic disorder**	*181*	*56*	*181 - 230*	*1 027*

Table A2: Distribution of fumonisins levels in other food products, expressed in µg/kg (continued)

Foodex level	Food groups	N[(a)]	LC[(b)]	Mean LB-UB[(c)]	P95 LB-UB[(c)]
1	**Composite dishes**				
2	**Cereal-based dishes**	16	19	307 - 362	-
Not taken into account in the exposure assessment: composite dishes other than cereal-based (N = 3)					
1	**Snacks, desserts and other foods**				
2	**Snack food**	181	64	53 - 163	250 - 340
3	Corn chips	27	67	56 - 212	-
3	Corn curls	17	88	4.5 - 58.6	-
3	Tortilla chips	39	49	66 - 173	-
3	Popcorn	83	61	56 - 165	290 - 340
3	*Snacks unspecified*	*15*	*80*	*49 - 153*	-
Not taken into account in the exposure assessment: desserts (N = 2), other foods (N = 1) and potato and fish-based snacks.					
1	**Other food groups**				
Not taken into account in the exposure assessment: legumes, nuts and oilseeds (N = 1), animal and vegetable fats and oils (N = 4), alcoholic beverages (N = 1), products for special nutritional use, juice, water and non alcoholic beverages, eggs and egg products, fish and other seafood, fruit and fruit products (N = 2), herbs, spices and condiments (N = 1), meat and meat products (N = 1), milk and dairy products (N = 1), starchy roots and tubers (N = 6), sugars and confectionary.					

(a): N: number of samples.
(b): LC: percentage of censored results.
(c): Mean LB-UB, P95 LB-UB, mean and 95th percentile contamination level presented as lower bound estimate - upper bound estimate. When the lower and upper bound estimates are equal, only one estimate is given. In case of too few observations (less than 60 for the 95th percentile), the estimation may be biased and is not consequently not provided.
Note: The numbers for the occurrence values are all given with 3 figures, but this does not reflect precision.
Ahg: ad-hoc food groups created for the purpose of the assessment.
* Some occurrence data are available for 'Formulas for metabolic disorder', however no consumption evens of such products is reported in the Comprehensive European Food Consumption Database.
In italics: food groups not taken into account in the exposure assessment.

Appendix B. Food consumption data for fumonisins

Food consumption data were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive database) which was built in 2010 from existing national information on food consumption at the individual level (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). The Comprehensive database comprises consumption data of 66,642 individuals from 32 surveys carried out in 22 different European countries covering the following age-groups: infants (< 1 year old), toddlers (≥ 1 year to < 3 years old), children (≥ 3 years to < 10 years old), adolescents (≥ 10 years to < 18 years old), adults (≥ 18 years to < 65 years old), elderly (≥ 65 years to < 75 years old) and very elderly (≥ 75 years old). Consumption data were collected with 24h dietary recalls covering one or two days, 48 h dietary recalls, or through dietary records covering 3 to 7 days.

In view of performing a chronic exposure assessment, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), only individuals with at least two days of reporting were considered, which represented a total of 53 728 individuals from 28 surveys and 17 European countries. The average consumption level was estimated at the individual level for the different food groups taken into account in the exposure assessment (see Section 1).

Appendix C. Exposure assessment for fumonisins

Chronic exposure was assessed at the individual level by multiplying the mean consumption for each food with the corresponding mean concentration, summing up the respective intakes throughout the diet, and finally dividing the results by the individual's body weight. The mean as well as the 95th percentile of exposure were then derived for each population group (i.e. [survey and age class] combinations). The percentage of individuals with an exposure higher than the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg b.w. per day was also estimated.

Table C1 presents lower and upper bound estimates of chronic dietary exposure to fumonisins across the different population groups. Table C2 summarizes the percentage of individuals with an exposure above the PMTDI estimated across the surveys available for each age class. Whereas the mean exposure estimates are provided for all surveys and age groups, the 95th percentile and percentage of subject above the PMTDI are provided only for those with more than 60 subjects were used to derive the ranges of the 95th percentiles and percentages of subjects above the PMTDI.

Overall, the mean exposure levels estimated are below the PMTDI, but the 95th percentile estimates are in the region of the PMTDI for some surveys. Indeed, in the children age groups, the mean exposure levels range between 0.04 and 1.17 µg/kg b.w. per day at the LB and 0.34 and 1.77 µg/kg b.w. per day at the UB. The 95th percentile of exposure was between 0.24 and 3.14 µg/kg b.w. per day at the LB and between 1.43 and 4.05 µg/kg b.w. per day at the UB. In the surveys covering adult age groups (including adolescents), the mean exposure levels remain below the HBGV, being between 0.04 and 0.69 µg/kg b.w. per day at the LB and 0.31 and 1.04 µg/kg b.w. per day at the UB. However, the 95th percentile of exposure is in the region of the HBGV for some surveys, being between 0.08 and 1.58 µg/kg b.w. per day at the LB and 0.53 and 2.19 µg/kg b.w. per day at the UB. The percentage of individuals with an exposure above the PMTDI was ranging from 0 % (LB) up to 35 % (UB) in the children age groups (infants, toddlers, other children and adolescents), whereas it was ranging from 0 (LB) up to 0.5 % (UB) in the adults age groups (adults, elderly, very elderly).

Tables C3 and C4 present for each age group the minimum and maximum percentage contribution of main food groups to the total average exposure at the lower and upper bound respectively.

The two main contributors (i.e. representing more than 10 % of the total exposure in at least one population group) identified in all age groups are the 'Bread other than maize based' and 'Pasta', representing respectively up to 98 % and 74 % of the total LB exposure. 'Cereal based dishes' is also identified as main contributor in all age groups excepted the infants, representing up to 80 % of the total LB exposure. They are followed by "Fine bakery wares", which are representing up to 18 % of the total LB exposure. The 'Breakfast cereals' (maize based or other than maize based) are also identified as a main contributor in some groups of toddlers, other children and adolescents, representing up to 12 % of the total LB exposure.

Table C1: Chronic exposure to fumonisins, expressed in µg/kg b.w. per day across population groups

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]	P95 LB-UB[(c)]	Percentage above PMTDI LB-UB[(d)]
		Infants			
Bulgaria	NUTRICHILD	860	0.12 - 0.63	0.53 - 1.99	0.1 - 5.0
Italy	INRAN_SCAI_2005_06	16	0.04 - 0.34	-*	-*
		Min	**0.04 - 0.34**	**0.53 - 1.99**	**0.1 - 5.0**
		Median	-	-	-
		Max	**0.12 - 0.63**	**0.53 - 1.99**	**0.1 - 5.0**
		Toddlers			
Belgium	Regional_Flanders	36	0.43 - 1.34	-*	-*
Bulgaria	NUTRICHILD	428	0.26 - 1.34	0.58 - 2.32	0 - 9.3
Finland	DIPP_2003_2006	497	0.82 - 1.65	1.61 - 3.26	1.4 - 27
Germany	DONALD_2006_2008	261	0.50 - 1.30	1.36 - 2.39	1.9 - 11
Italy	INRAN_SCAI_2005_06	36	0.36 - 1.32	-*	-*
Netherlands	VCP_kids	322	0.53 - 1.32	1.30 - 2.43	1.6 - 13
Spain	enKid	17	0.18 - 0.93	-*	-*
		Min	**0.18 - 0.93**	**0.58 - 2.32**	**0 - 9.3**
		Median	**0.43 - 1.32**	**1.33 - 2.41**	**1.5 - 12**
		Max	**0.82 - 1.65**	**1.61 - 3.26**	**1.9 - 27**
		Other children			
Belgium	Regional_Flanders	625	0.39 - 1.17	1.05 - 2.16	0.3 - 7.2
Bulgaria	NUTRICHILD	433	0.20 - 1.13	0.45 - 2.01	0 - 5.3
Czech Republic	SISP04	389	0.22 - 0.96	0.50 - 1.83	0.3 - 3.6
Denmark	Danish_Dietary_Survey	490	0.14 - 0.89	0.24 - 1.43	0 - 1.2
Finland	DIPP_2003_2006	933	0.60 - 1.22	1.05 - 2.09	0.1 - 6.3
Finland	STRIP	250	0.69 - 1.41	1.80 - 2.87	4.0 - 18
France	INCA2	482	0.20 - 0.86	0.39 - 1.51	0 - 0.8
Germany	DONALD_2006_2008	660	0.55 - 1.17	1.32 - 2.15	1.1 - 6.2
Greece	Regional_Crete	839	1.17 - 1.77	3.14 - 4.05	18 - 35
Italy	INRAN_SCAI_2005_06	193	0.29 - 1.13	0.87 - 2.31	0 - 9.3
Latvia	EFSA_TEST	189	0.27 - 0.71	0.98 - 1.70	0 - 2.6
Netherlands	VCP_kids	957	0.43 - 1.11	0.94 - 1.94	0.1 - 4.2
Spain	enKid	156	0.29 - 0.88	1.01 - 1.79	1.9 - 3.2
Spain	NUT_INK05	399	0.38 - 1.03	0.95 - 1.85	0 - 2.8
Sweden	NFA	1 473	0.50 - 1.14	1.41 - 2.36	1.8 - 9.2
		Min	**0.14 - 0.71**	**0.24 - 1.43**	**0 - 0.8**
		Median	**0.38 - 1.13**	**0.98 - 2.01**	**0.1 - 5.3**
		Max	**1.17 - 1.77**	**3.14 - 4.05**	**18 - 35**
		Adolescents			
Belgium	Diet_National_2004	584	0.12 - 0.50	0.36 - 1.02	0
Cyprus	Childhealth	303	0.69 - 1.04	1.58 - 2.19	1.3 - 6.9
Czech Republic	SISP04	298	0.20 - 0.78	0.64 - 1.52	0.3 - 1
Denmark	Danish_Dietary_Survey	479	0.08 - 0.48	0.14 - 0.87	0
France	INCA2	973	0.13 - 0.52	0.27 - 0.98	0 - 0.1
Germany	National_Nutrition_Survey_II	1 011	0.21 - 0.51	0.60 - 1.11	0.2 - 0.7
Italy	INRAN_SCAI_2005_06	247	0.20 - 0.71	0.58 - 1.37	0 - 0.8
Latvia	EFSA_TEST	470	0.22 - 0.58	0.81 - 1.30	0.2 - 1.3
Spain	AESAN_FIAB	86	0.11 - 0.43	0.24 - 0.91	0

Table C1: Chronic exposure to fumonisins, expressed in µg/kg b.w. per day across population groups (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB(b)	P95 LB-UB(c)	Percentage above PMTDI LB-UB[(d)]
Spain	enKid	209	0.18 - 0.62	0.65 - 1.48	0 - 0.5
Spain	NUT_INK05	651	0.25 - 0.70	0.59 - 1.28	0 - 0.2
Sweden	NFA	1 018	0.43 - 0.86	1.18 - 1.75	0.3 - 2.5
		Min	**0.08 - 0.43**	**0.14 - 0.87**	**0**
		Median	**0.20 - 0.60**	**0.59 - 1.29**	**0 - 0.6**
		Max	**0.69 - 1.04**	**1.58 - 2.19**	**1.3 - 6.9**
		Adults			
Belgium	Diet_National_2004	1 304	0.10 - 0.40	0.28 - 0.81	0 - 0.2
Czech Republic	SISP04	1 666	0.11 - 0.45	0.35 - 0.92	0.1 - 0.2
Denmark	Danish_Dietary_Survey	2 822	0.05 - 0.34	0.09 - 0.59	0
Finland	FINDIET_2007	1 575	0.28 - 0.53	0.54 - 0.99	0
France	INCA2	2 276	0.09 - 0.37	0.18 - 0.72	0
Germany	National_Nutrition_Survey_II	10 419	0.16 - 0.41	0.44 - 0.86	0 - 0.2
Hungary	National_Repr_Surv	1 074	0.07 - 0.38	0.14 - 0.67	0
Ireland	NSIFCS	958	0.06 - 0.35	0.12 - 0.62	0
Italy	INRAN_SCAI_2005_06	2 313	0.12 - 0.46	0.38 - 0.86	0
Latvia	EFSA_TEST	1 306	0.14 - 0.40	0.58 - 1.03	0.2 - 0.5
Netherlands	DNFCS_2003	750	0.11 - 0.40	0.29 - 0.77	0.1
Spain	AESAN	410	0.11 - 0.36	0.42 - 0.84	0
Spain	AESAN_FIAB	981	0.08 - 0.33	0.19 - 0.64	0
Sweden	Riksmaten_1997_98	1 210	0.33 - 0.63	0.83 - 1.25	0 - 0.3
United Kingdom	NDNS	1 724	0.06 - 0.34	0.14 - 0.63	0 - 0.1
		Min	**0.05 - 0.33**	**0.09 - 0.59**	**0**
		Median	**0.11 - 0.40**	**0.29 - 0.81**	**0**
		Max	**0.33 - 0.63**	**0.83 - 1.25**	**0.2 - 0.5**
		Elderly			
Belgium	Diet_National_2004	518	0.07 - 0.31	0.21 - 0.67	0
Denmark	Danish_Dietary_Survey	309	0.05 - 0.31	0.08 - 0.53	0
Finland	FINDIET_2007	463	0.28 - 0.53	0.53 - 0.99	0
France	INCA2	264	0.07 - 0.34	0.15 - 0.67	0
Germany	National_Nutrition_Survey_II	2 006	0.13 - 0.38	0.34 - 0.74	0
Hungary	National_Repr_Surv	206	0.06 - 0.35	0.12 - 0.58	0
Italy	INRAN_SCAI_2005_06	290	0.11 - 0.43	0.26 - 0.86	0
		Min	**0.05 - 0.31**	**0.08 - 0.53**	**0**
		Median	**0.07 - 0.35**	**0.21 - 0.67**	**0**
		Max	**0.28 - 0.53**	**0.53 - 0.99**	**0**
		Very elderly			
Belgium	Diet_National_2004	712	0.06 - 0.31	0.18 - 0.64	0
Denmark	Danish_Dietary_Survey	20	0.04 - 0.31	-*	-*
France	INCA2	84	0.07 - 0.32	0.14 - 0.55	0
Germany	National_Nutrition_Survey_II	490	0.13 - 0.37	0.34 - 0.73	0.2
Hungary	National_Repr_Surv	80	0.07 - 0.39	0.13 - 0.64	0
Italy	INRAN_SCAI_2005_06	228	0.11 - 0.46	0.22 - 0.84	0
		Min	**0.04 - 0.31**	**0.13 - 0.55**	**0**
		Median	**0.07 - 0.35**	**0.18 - 0.64**	**0**
		Max	**0.13 - 0.46**	**0.34 - 0.84**	**0.2**

(a): N: number of subjects. (b) mean LB-UB: mean lower bound - upper bound. (c) P95 LB - UB: 95th percentile lower bound - upper bound. (d): Percentage above PMTDI: percentage of individuals with an exposure above the PMTDI lower bound - upper bound. * P95 and percentage of individuals with an exposure above 1 µg/kg b.w. per day for dietary surveys/age classes with less than 60 subjects were not reliable and therefore not presented. Note: The numbers for the exposure values (mean, P95) are all given with 3 figures, and the percentage above HBGV with 2 figures, but this does not reflect precision. When LB and UB estimates are identical, only one estimate is provided.

Table C2: Percentage above the PMTDI across surveys in total population from fumonisins

Age class	Percentage above the PMTDI in total population					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Infants	0.1	5.0	-*	-*	0.1	5.0
Toddlers	0.0	9.3	1.5	12	1.9	27
Other children	0.0	0.8	0.1	5.3	18	35
Adolescents	0.0	0.0	0.0	0.6	1.3	6.9
Adults	0.0	0.0	0.0	0.0	0.2	0.5
Elderly	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	0.0	0.0	0.0	0.0	0.2	0.2

*Estimates based on only two dietary surveys.

Table C3: Minimum and maximum relative contribution (%) of main food groups to the fumonisins total lower bound exposure

Food category	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Maize grain for human consumption	0	0 - 0.1	0 - 0.6	0 - 0.9	0 - 4.9	0 - 0.3	0 - 1.0
Other and unspecified grains for human consumption	2.0 - 8.7	0 - 3.5	<0.05 - 3.1	0.3 - 3.0	0.4 - 4.5	0.3 - 2.5	0.6 - 2.0
Maize milling product	0 - 0.7	0 - 0.8	0 - 3.7	0 - 3.7	0 - 4.4	0 - 7.1	0 - 4.4
Other and unspecified grain milling products	1.9 - 3.5	0 - 12	0 - 4.1	<0.05 - 6.5	0 - 4.3	0 - 4.6	0.1 - 4.8
Maize bread and tortilla	0	0 - 0.3	0 - 0.9	0 - 1.3	0 - 21	0 - 4.0	0
Bread other than maize based	3.3 - 65	18 - 94	13 - 94	11 - 66	8.0 - 97	40 - 98	47 - 86
Maize based breakfast cereals	0 - 0.1	0 - 0.4	0 - 12	0 - 11	0 - 5.8	0 - 2.9	0 - 2.3
Breakfast cereals other than maize based	0 - 0.1	<0.05 - 11	0.1 - 7	0 - 5.5	0.6 - 11	0.2 - 3.1	0.1 - 4.7
Pasta	14 - 74	1.0 - 26	1.0 - 52	0.3 - 52	0 - 42	0.4 - 34	1.9 - 33
Fine bakery wares	0 – 11	0.3 - 15	0.7 - 24	0.6 - 16	<0.05 - 12	0 - 15	0.6 - 18
Sweet corn	0 - 0.7	0 - 3.5	0 - 5.8	0 - 7.2	0 - 6.3	<0.05 - 2.0	0.2 - 1.7
Food for infants and young children	2.0 - 11	0.2 - 4.4	0 - 0.4	0	0	0	0
Cereal based dishes	0	0 - 41	0 - 76	0 - 76	0 - 80	0 - 19	0 - 18
Maize based snacks	0 - 3.2	0 - 8.4	<0.05 - 8.2	<0.05 - 1.9	0.1 - 1.3	0 - 0.1	0
Snacks other than maize based	0 - 0.1	0 - 2.0	0 - 1.7	0 - 2.7	0 - 1.2	0 - 0.4	0 - 0.4

Note: The numbers for the percentage contributions are all given with 2 figures but this does not reflect precision. A “0” indicates the absence of contribution to the total exposure.

Table C4: Minimum and maximum relative contribution (%) of main food groups to the fumonisins total upper bound exposure

Food category	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Maize grain for human consumption	0	0	0 - 0.2	0 - 0.3	0 - 2.0	0 - 0.1	0 - 0.3
Other and unspecified grains for human consumption	5.2 - 12	0 - 11.2	0.2 - 9.7	2.2 - 9.7	2.4 - 11	1.9 - 7.3	1.7 - 6.1
Maize milling product	0 - 0.1	0 - 0.2	0 - 1.1	0 - 1.1	0 - 1.2	0 - 1.9	0 - 1.1
Other and unspecified grain milling products	5.1 - 5.2	0 - 5.9	0 - 9.0	0.1 - 11	0 - 11	0 - 11	0.2 - 11
Maize bread and tortilla	0	0 - 0.2	0 - 0.8	0 - 1.2	0 - 5.8	0 - 1.0	0
Bread other than maize based	2.5 - 40.1	23 - 83	23 - 83	27 - 75	28 - 93	53 - 93	57 - 84
Maize based breakfast cereals	0	0 - 0.2	0 - 3.8	0 - 3.4	0 - 1.9	0 - 0.8	0 - 0.6
Breakfast cereals other than maize based	0	<0.05- 4.2	0.1 - 12	0 - 5.0	0.4 - 9.6	0.1 - 3.9	0.1 - 5.3
Pasta	3.8 - 30	1.8 - 23	2.4 - 20	0.7 - 19	0.1 - 16	0.8 - 17	1.0 - 16
Fine bakery wares	0 - 24	1.8 - 20	2.1 - 30	2.3 - 24	<0.05 - 18	0 - 13	2.9 - 15
Sweet corn	0 - 0.2	0 - 2.2	0 - 1.2	0 - 1.5	0 - 1.5	<0.05 - 0.4	0.1 - 0.4
Food for infants and young children	19 - 50	2.4 - 30	0 - 3.9	0 - 0.1	0 - 0.1	0	0
Cereal based dishes	0	0 - 13	0 - 57	0 - 58	0 - 50	0 - 5.6	0 - 5.0
Maize based snacks	0 - 2.3	0 - 5.6	<0.05 - 4.8	<0.05 - 2.1	0.1 - 1.0	0	0
Snacks other than maize based	0	0 - 1.3	0 - 2.0	0 - 2.4	0 - 0.9	0 - 0.2	0 - 0.2

Note: The numbers for the percentage contributions are all given with 2 figures but this does not reflect precision. A “0” indicates the absence of contribution to the total exposure.

Appendix D. Data management and validation of occurrence data on zearalenone and fumonisins in feed

All the data related to the presence of fumonisins (total fumonisins, fumonisin B1, fumonisin B2, fumonisin B3) and zearalenone in feed, collected between 2008 and 2014, submitted to EFSA and validated at the date of the 23rd of July 2014 were considered.

The data control quality procedure consisted in checking for potential duplicates, checking the consistency of information related to the feed description, harmonising the unit of expression of the results throughout the dataset and expressing the fumonisins as a total fumonisins equivalent.

The feed description considered in this assessment corresponds to the Commission Regulation (EU) No 575/2011 on the Catalogue of food materials[22]. For sake of consistency, the results were all expressed on a 88 % dry matter basis. When the information required to convert the result into this unit was missing, the random hot-deck imputation technique, as described in the 2012 EFSA report on update of the monitoring of levels of dioxins and PCBs in food and feed (EFSA, 2012) was applied in order to approximate the missing value. The total fumonisins was estimated by summing fumonisins B_1, B_2 and B_3 when available, by summing fumonisins B_1 and B_2 when fumonisin B_3 was missing, or by considering the result provided for total fumonisins when no detailed data was provided for fumonisins B_1, B_2 and B_3. Results which were available only for fumonisins B_1 or B_2 were not further considered. As done in the JECFA evaluation, the results corresponding to the sum of fumonisins B_1 and B_2 were used together with the ones corresponding to the sum of fumonisins B_1, B_2 and B_3, without any adjustment.

Some high limits of quantification (LOQ) were observed in the zearalenone dataset. In order to prevent such LOQs adding a bias to the description of zearalenone levels in feed, the maximum left-censoring limit accepted was set at 100 µg/kg. It corresponded to the guidance value defined for zearalenone in compound feed (Commission Recommendation 2006/576/EC).

A total of 8 602 analytical results were initially extracted from the EFSA chemical occurrence database. The cleaning process led to the exclusion of:

- 111 analytical results corresponding to duplicate submission,
- 50 analytical results which were referring to sub-samples, for which an average value at the sample level was retained for further analysis,
- 35 analytical results which were available only for fumonisin B_1 or B_2,
- 302 analytical results on zearalenone associated with an LOQ above 100 µg/kg,

After the adjustment in total fumonisins equivalent, the final dataset contained 5 000 analytical results: 3 233 for zearalenone and 1 767 for fumonisins.

[22] Commission Regulation (EU) No 575/2011 of 16 June 2011 on the Catalogue of feed materials. OJ L159, 17.6.2011, p. 25-65.

Appendix E. Descriptive statistics of of occurrence data on zearalenone and fumonisins in feed

Table F1 shows the distribution of zearalenone across feed groups. Around 41 % of the results available are for 'Complete feed', which can allow to assess the exposure levels of a number of animal species from the direct consumption of complete feeds, without considering the raw materials. The overall quantification rate of zearalenone in complete feed is 52 % with an average MB level at 28.7 µg/kg 88 % dry matter. Regarding the raw materials, 'Maize' is the cereal with the highest quantification rate (53 %). The average zearalenone MB level in 'Maize' is estimated at 66.7 µg/kg 88 % dry matter. High levels of zearalenone are also observed in 'Forages and roughage, and derived products' with average MB level at 252 µg/kg 88 % dry matter. Overall, these results are in line with data compiled in Opinion of the CONTAM Panel related to Zearalenone as undesirable substance in animal feed (EFSA, 2004).

Table F2 shows the distribution of fumonisins across feed groups. Around 35 % of the results available are for 'Complete feed', which can be used to assess the exposure levels from the direct consumption of complete feeds. Fumonisins are less frequently detected than zearalenone, the overall quantification rate in 'Complete feed' being of 22 %. The average MB level is at 206 µg/kg 88 % dry matter. A high level of censorship (above 80 %) is also observed in all raw materials, maize excepted. Fumonisins are found in almost half the samples, with an average MB level at 571 µg/kg 88 % dry matter.

Table E1: Zearalenone concentrations (µg / kg 88 % dry matter) across feed groups

Feed groups	N[(a)]	LC[(b)]	Mean	Median	P95	P97.5	P99
			presented as MB [LB-UB][(c)]				
Compound feed	**1 980**	**45.3**	**38.6 [36.1 - 41.9]**	**9.85 [9.70 - 10.7]**	**93.4**	**200**	**500**
Complete feed	1 316	51.8	28.7 [25.4 - 32.0]	11.4 [0 - 14.1]	82.9 [82.9 - 84.5]	162	310
Piglets (weaning diets)	*262*	*60.3*	*16.6 [14.3 - 19.0]*	*4.93 [0 – 7.00]*	*47.8 [47.8 - 47.8]*	*59.1*	-
Growing/Fattening pigs	*18*	*61.1*	*24.6 [18.4 - 30.8]*	*12.6 [0 - 22.8]*	-	-	-
Breeding pigs	*254*	*47.2*	*27.2 [23.3 - 31.1]*	*14.1 [11.1 - 21.3]*	*101*	*150*	-
Lactating sows	*29*	*48.3*	*77.2 [72.4 - 82.0]*	*15.0 [2.05 - 25.1]*	-	-	-
Fattening calves (weaning diets)	*106*	*43.4*	*26.6 [24.0 - 29.1]*	*14.3 [14.3 - 18.3]*	*68.2*	-	-
Fattening cattle	*14*	*57.1*	*53.5 [47.3 - 59.7]*	*15.3 [0 - 20]*	-	-	-
Lactating/dairy goats	*28*	*64.3*	*10.8 [8.93 - 12.7]*	*2.74 [0 - 5.49]*	-	-	-
Lactating/dairy sheep	*75*	*58.7*	*15.7 [14.2 - 17.2]*	*2.56 [0 - 5.12]*	*73.1*	-	-
Dairy cows	*247*	*51.4*	*19.2 [17.0 - 21.5]*	*7.20 [0 – 10.0]*	*67.5*	*80.3*	-
Poultry (starter diets)	*54*	*24.1*	*59.7 [58.5 - 60.9]*	*20.6*	-	-	-
Fattening chickens (broilers)	*72*	*44.4*	*41.2 [36.5 - 46.0]*	*15.0 [10.0 – 25.0]*	*128*	-	-
Fattening turkeys	*11*	*45.5*	*50.8 [44.3 - 57.2]*	*18.7 [17.7 - 29.6]*	-	-	-
Laying hens	*79*	*55.7*	*59.5 [54.9 - 64.1]*	*13.6 [0 - 24.3]*	*183*	-	-
Fattening rabbits	*13*	*84.6*	*58.8 [50.1 - 67.6]*	*12.3 [0 - 24.6]*	-	-	-
Pet foods, birds, cats, dogs	*4*	*100.0*	*9.73 [0 - 19.5]*	*8.82 [0 - 17.6]*	-	-	-
Other and unspecified complete feed [(d)]	*50*	*54.0*	*35.3 [27.9 - 42.8]*	*19.2 [0 - 29.9]*	-	-	-
Complementary feed (incomplete diet)	224	48.2	139 [136 - 142]	14.4 [7.78 - 17.0]	500	905	-
Piglets (weaning diets)	*17*	*29.4*	*10.9 [10.2 - 11.7]*	*8.20 [8.20 - 9.12]*	-	-	-
Growing/Fattening pigs	*66*	*42.4*	*129 [127 - 131]*	*20.1 [20.1 - 20.6]*	*905*	-	-
Fattening cattle	*48*	*35.4*	*113 [111 - 114]*	*17.5 [17.5 – 20.0]*	-	-	-
Dairy cows	*19*	*26.3*	*35.2 [32.8 - 37.5]*	*19.9 [19.9 - 21.2]*	-	-	-
Other and unspecified complementary feed [(e)]	*74*	*71.6*	*221 [215 - 227]*	*5.04 [0 - 10.1]*	*221*	-	-
Compound feed, unspecified	440	24.1	16.9 [17.2 - 20.5]	5.98 [9.92 - 10.0]	52	83.3	178
Cereal grains, their products and by-products	**981**	**61.7**	**46.6 [42.2 - 50.8]**	**6.20 [0 - 10.3]**	**164**	**325**	**561**
Barley	120	84.2	105 [98.2 - 110]	5.13 [0 - 10.2]	133	-	-
Maize	354	46.6	66.7 [63.7 - 70.8]	10.0 [10.0 – 20.0]	333	510	690

Table E1: Zearalenone concentrations (µg / kg 88 % dry matter) across feed groups (continued)

Feed groups	N[(a)]	LC[(b)]	Mean	Median	P95	P97.5	P99
			presented as MB [LB-UB][(c)]				
Malt	12	66.7	38.4 [23.6 - 53.2]	25.5 [0 - 51.1]	-	-	-
Mixed grains	27	48.1	28.7 [22.4 - 35.0]	19.6 [17.5 - 20.2]	-	-	-
Oats	30	86.7	7.96 [4.42 - 11.8]	2.57 [0 - 5.15]	-	-	-
Triticale	36	80.6	5.09 [2.76 - 7.86]	3.03 [0 - 6.06]	-	-	-
Wheat	381	65.9	16.3 [11.5 - 20.1]	6.06 [0 - 10.1]	63.4	91.8	127
Wheat bran	*69*	*76.8*	*19.7 [12.9 - 27.0]*	*5.10 [0 - 10.2]*	*77.8*	-	-
Wheat feed	*66*	*80.3*	*14.6 [8.46 - 18.2]*	*5.04 [0 - 10.0]*	*40.3*	-	-
Wheat middlings	*88*	*51.1*	*15.2 [13.5 - 17.4]*	*6.04 [0 - 10.07]*	*50*	-	-
Wheat, other and unspecified	*158*	*63.3*	*16.2 [11.1 - 19.4]*	*6.11 [0 - 10.2]*	*70.8*	-	-
Cereal grains, other and unspecified [(f)]	21	57.1	76.7 [74.7 - 79.8]	5.15 [0 - 10.1]	-	-	-
Forages and roughage, and products derived	**160**	**64.4**	**252 [240 - 254]**	**30.7 [0 - 21.7]**	**1134**	**-**	**-**
Cereals straw	39	94.9	12.9 [8.18 - 17.7]	5.00 [0 - 10.0]	-	-	-
Grass, field dried, [Hay]	17	64.7	610 [603 - 611]	19.8 [0 - 20.0]	-	-	-
Lucerne; [Alfalfa]	10	30.0	552 [550 - 553]	238	-	-	-
Maize silage	63	63.5	233 [216 - 235]	45.6 [0 - 32.7]	790	-	-
Forages, other and unspecified	31	38.7	296 [280 - 299]	128 [128 - 134]	-	-	-
Oil seeds, oil fruits, and products derived	**72**	**76.4**	**27.3 [22.8 - 31.4]**	**5.00 [0 - 10.0]**	**140**	**-**	**-**
Sunflower seed	45	84.4	16.8 [12.9 - 21.6]	5.00 [0 - 10.0]	-	-	-
Toasted soya (beans)	20	60.0	55.2 [51.7 - 59.0]	10.0 [0 - 20.0]	-	-	-
Oil seeds, other and unspecified[(g)]	7	71.4	15.5 [4.28 - 15.3]	12.5 [0 - 16.0]	-	-	-
Miscellaneous [(h)]	**15**	**80.0**	**9.51 [2.46 - 16.6]**	**8.79 [0 - 10.0]**	**-**	**-**	**-**
Feed, other and unspecified[(i)]	**25**	**36.0**	**35.6 [34.0 - 38.2]**	**13.0 [12.0 - 13.0]**	**-**	**-**	**-**

(a) N: number of samples. (b) LC: percentage of left censored results. (c) MB (LB-UB): mean, 95th, 97.5th and 99th percentiles presented as the middle bound estimate (lower bound estimate; upper bound estimate). When the middle, lower and upper bound estimates are equal, only one estimate is given. (d) Other and unspecified complete feed: Calves (pre-ruminant) (3), Fattening ducks (5), Fattening geese (1), Fattening sheep (1), Fish (1), Lambs (weaning diets) (2), unspecified (37). (e) Other and unspecified complementary feed: Breeding pigs (8), Calves (pre-ruminant) (8), Fattening calves (weaning diets) (6), Fattening geese (1), Fattening rabbits (3), Horses (2), Lactating sows (1), Lambs (weaning diets) (7), Pet food, birds (1), Pet food, dogs (1), Poultry (starter diets) (8), unspecified (28). (f) Cereal grains, other and unspecified: Millet (1), Rice (3), Rye (4), Sorghum; [Milo] (3), Spelt (9), unspecified (1) (g) Other oilseeds: Niger seed (1), Palm kernel expeller (1), Rape seed (4), Safflower seed (1). (h) Miscellaneous: Caramelized sugar (2), Feed beer (1), Products from the bakery and pasta industry (6), Products from the pastry industry (1), Starch (2), unspecified (4). (i) Feed, other and unspecified: Fermentation (by-)products (3), Land animal products and products derived thereof (2), Legume seeds and products derived thereof (2), Milk products and products derived thereof (1), Minerals and products derived thereof (4), Other seeds and fruits, and products derived thereof (3), Tubers, roots, and products derived thereof (5), unspecified (5). -: P95, P97.5 and P99 are not reliable when the number of samples is respectively below 60, 180 and 298 and therefore not presented. Note: The numbers for the concentration values are all given with 3 figures, but this does not reflect precision

Table E2: Fumonisins concentrations (µg / kg 88 % dry matter) across feed groups

Feed groups	N[(a)]	LC[(b)]	Mean	Median	P95	P97.5	P99
			presented as MB [LB-UB][(c)]				
Compound feed	**762**	**76**	**202 [133 - 272]**	**73.9 [0 - 148]**	**787 [730 - 810]**	**1549 [1498 - 1549]**	**2418 [2403 - 2433]**
Complete feed	636	78	206 [135 - 276]	73.9 [0 - 148]	760 [721 - 803]	1549 [1547 - 1549]	2418 [2403 - 2430]
Piglets (weaning diets)	*307*	*85*	*139 [73.2 - 205]*	*73.9 [0 - 148]*	*400 [351 - 449]*	*753 [704 - 792]*	*1557 [1547 - 1568]*
Growing/Fattening pigs	*43*	*60*	*308 [246 - 369]*	*150 [0 - 244]*	-	-	-
Breeding pigs	*13*	*92*	*212 [79.2 - 344]*	*150 [0 - 300]*	-	-	-
Lactating sows	*68*	*72*	*274 [152 - 395]*	*150 [0 - 300]*	*1020 [970 - 1070]*	-	-
Fattening cattle	*10*	*60*	*52.3 [24.0 - 80.6]*	*32.5 [0 – 55.0]*	-	-	-
Dairy cows	*21*	*81*	*69.9 [25.6 - 114]*	*50.0 [0 - 100]*	-	-	-
Poultry (starter diets)	*33*	*18*	*330 [314 - 345]*	*117 [92.0 - 120]*	-	-	-
Horses	*112*	*97*	*91.8 [11.1 - 173]*	*74.6 [0 - 149]*	*150 [0 - 300]*	-	-
Complete, other and unspecified[(d)]	*29*	*34*	*1043 [993 - 1093]*	*210 [170 - 310]*	-	-	-
Complementary feed (incomplete diet)	75	59	237 [181 - 292]	80.0 [0 - 151]	1080	-	-
Piglets (weaning diets)	*15*	*93*	*117 [52.5 - 181]*	*74.3 [0 - 149]*	-	-	-
Fattening cattle	*22*	*32*	*198 [187 - 208]*	*57.5 [50.0 - 89.2]*	-	-	-
Complementary, other and unspecified[(e)]	*38*	*61*	*307 [229 - 385]*	*150 [0 - 300]*	-	-	-
Compound feed, unspecified	51	76	110 [34.4 - 185]	50.0 [0 - 100]	-	-	-
Cereal grains, their products and by-products	**537**	**69**	**349 [284 - 413]**	**110 [0 - 151]**	**1681 [1509 - 1681]**	**3388 [3388 - 3389]**	**4860**
Barley	63	81	136 [48.7 - 225]	30.0 [0 - 40.6]	550 [35.0 - 550]	-	-
Maize	277	51	571 [516 - 626]	150 [0 - 300]	3388	4750	-
Mixed grains	18	94	161 [21.1 - 301]	150 [0 - 300]	-	-	-
Oats	35	97	82.7 [10.8 - 155]	39.1 [0 - 78.1]	-	-	-
Wheat	124	91	83.5 [28.9 - 138]	50.0 [0 - 100]	150 [30.0 - 300]	-	-
Wheat bran	*35*	*100*	*50.2 [0 - 100]*	*50.0 [0 - 100]*	-	-	-
Wheat feed	*29*	*100*	*50.2 [0 - 100]*	*50.0 [0 - 100]*	-	-	-
Wheat, other and unspecified	*60*	*82*	*119 [59.7 - 179]*	*49.3 [0 - 77.8]*	*582 [532 - 632]*	-	-
Cereal grains, other and unspecified[(f)]	20	75	220 [118 - 321]	150 [0 - 300]	-	-	-
Forages and roughage, and products derived	**160**	**99**	**128 [5.88 - 250]**	**150 [0 - 300]**	**150 [0 - 300]**	**-**	**-**
Cereals straw	37	100	50 [0 - 100]	50.0 [0 - 100]	-	-	-
Forage meal; [Grass meal]; [Green meal]	22	100	150 [0 - 300]	150 [0 - 300]	-	-	-
Maize silage	38	95	170 [24.7 - 315]	150 [0 - 300]	-	-	-
Forages, other and unspecified	63	100	141 [0 - 282]	150 [0 - 300]	150 [0 - 300]	-	-
Legume seeds and products derived[(g)]	**11**	**100**	**139 [0 - 278]**	**150 [0 - 300]**	**-**	**-**	**-**
Oil seeds, oil fruits, and products derived	**264**	**98**	**137 [7.29 - 268]**	**150 [0 - 300]**	**150 [0 - 300]**	**150 [0 - 300]**	**-**

Table E2: Fumonisins concentrations (µg / kg 88 % dry matter) across feed groups (continued)

Feed groups	N [(a)]	LC [(b)]	Mean	Median	P95	P97.5	P99
			presented as MB [LB-UB][(c)]				
Palm kernel expeller	20	100	146 [0 - 292]	150 [0 - 300]	-	-	-
Rape seed	28	100	147 [0 - 293]	150 [0 - 300]	-	-	-
Sunflower seed	56	98	85.3 [0.90 - 170]	50.0 [0 - 100]	-	-	-
Toasted soya (beans)	148	97	154 [12.0 - 296]	150 [0 - 300]	150 [0 - 300]	-	-
Soya (bean) meal	*32*	*97*	*140 [4.47 - 276]*	*150 [0 - 300]*	-	-	-
Soya beans, extruded	*106*	*97*	*163 [15.4 - 311]*	*150 [0 - 300]*	*150 [0 - 300]*	-	-
Soya, other and unspecified [(h)]	*10*	*100*	*106 [0 - 212]*	*150 [0 - 300]*	-	-	-
Oil seeds, other and unspecified [(i)]	12	92	147 [8.33 - 287]	150 [0 - 300]	-	-	-
Other seeds and fruits, and products derived [(j)]	**16**	**94**	**388 [256 - 520]**	**150 [0 - 300]**	**-**	**-**	**-**
Feed, other and unspecified [(k)]	**17**	**100**	**126 [0 - 252]**	**150 [0 - 300]**	**-**	**-**	**-**

(a) N: number of samples. (b) LC: percentage of left censored results. (c) MB (LB-UB): mean, 95th, 97.5th and 99th percentiles presented as the middle bound estimate (lower bound estimate; upper bound estimate). When the middle, lower and upper bound estimates are equal, only one estimate is given. (d) Other and unspecified complete feed: Calves (pre-ruminant) (1), Fattening chickens (broilers) (1), Fattening rabbits (2), Fattening sheep (1), Fattening turkeys (2), Fish (2), Lactating/dairy sheep (1), Laying hens (7), Pet food, birds (2), Pet food, dogs (1), unspecified (9). (e) Other and unspecified complementary feed: Breeding pigs (1), Calves (pre-ruminant) (4), Dairy cows (9), Fattening calves (weaning diets) (1), Fattening rabbits (3), Growing/Fattening pigs (2), Lactating sows (9), Lactating/dairy sheep (1), Poultry (starter diets) (3), unspecified (5). (f) Cereal grains, other and unspecified: Millet (3), Rice (4), Rye (3), Sorghum; [Milo] (5), Spelt 2), Triticale (3). (g) Legume seeds and products derived: Carob, dried (1), Mung beans (1), Peas (2), Sweet lupins (1), Vetches (1), unspecified (5). (h) Soya, other and unspecified: Soya (bean) expeller (1Soya (bean) hulls (6), unspecified (3). (i): Oil seeds, other and unspecified: Groundnut expeller, partially decorticated (7), Linseed (2), Niger seed (2), Vegetable oil and fat (1). (j): Other seeds and fruits, and products derived: Broken chestnuts (1), Buckwheat (2), Citrus pulp (11), Perilla seed (1), Pine nut (1). (k) Feed, other and unspecified: Fermentation (by-)products (3), Minerals and products derived thereof (2), Miscellaneous (5), Other plants, algae and products derived thereof (1), Tubers, roots, and products derived thereof (6). -: P95, P97.5 and P99 are not reliable when the number of samples is respectively below 60, 180 and 298 and therefore not presented. Note: The numbers for the concentration values are all given with 3 figures, but this does not reflect precision

GLOSSARY AND ABBREVIATIONS

GLOSSARY

- **Masked mycotoxins –** mycotoxins structurally modified by plants, as living organisms, as part of their defence against xenobiotics. Due to this modification, "masked mycotoxins cannot be detected by routine analysis", but can be cleaved in the GI tract of mammals to release their parent compound (original definition).

- **Modified mycotoxins** – mycotoxins structurally modified as an effect of a metabolic process exerted by a living organism (i.e. plants, fungi, mammals), or as an effect of food processing. Extractable and not extractable forms are included, as well as matrix entrapped toxins. Masked mycotoxins senso strictu are included in this definition.

- **Hidden fumonisins** – matrix entrapped fumonisins (see definition).

- **Matrix entrapped mycotoxins** – these compounds are strongly associated to the matrix macroconstituents, as an effect of specific/aspecific complexation. Although the chemical structure of the parent compound is not modified, this physical entrapment may cause an underestimation of the mycotoxin content in the food/feed samples.

- **Matrix bound mycotoxins** – these forms are covalently bound to the matrix unsoluble constituents (i.e. cell walls, fibers, etc.) and, thus, are not directly accessible to the extraction solvent. They have to be liberated from the matrix by chemical or enzymatic treatment prior to chemical analysis.

- **Extractable conjugated mycotoxins** – these forms are directly accessible to the solvent, can be extracted and detected by proper analytical methods. Masked mycotoxins senso strictu are included in this definition.

- **Targeted analytical methods** – any analytical method that is aimed at the determination of a specific analyte or of a group of analytes. The analytical procedure should be properly validated for the target "known" analytes.

- **Untargeted analytical methods** – an analytical method, mainly based on mass spectrometry followed by data mining and elaboration, aimed at the acquisition of undefined information from a sample ("profiling"). Information about "known" and "unknown" analytes can be obtained in the post-acquisition data elaboration.

- **Direct analytical determination** – modified mycotoxins are directly extracted from the matrix by solvent extraction. The detection method is based on the analyte chemical structure and implies a proper validation using calibrators and/or reference materials. The final analysis returns the cocnentration of the parent compound together with the concentration of their modified forms.

- **Indirect analytical determination** – the matrix is chemically or enzymatically treated before extraction to release parent mycotoxins from their modified forms. The extraction and detection steps are thus aimed at the determination of parent compounds. The final result return the mycotoxin concentration as the sum of parent and modified forms.

ABBREVIATIONS

3Ac-DON	3-acetyldeoxynivalenol
15Ac-DON	15-acetyldeoxynivalenol
Ac-DON	Acetyldeoxynivalenol
ACN	Acetonitrile
AcOH	Acetic acid
APCI	Atmospheric Pressure Chemical Ionization
b.w.	Body weight
CaCO-2	Heterogeneous human epithelial colorectal adenocarcinoma cells
CAS	Chemical Abstracts Service
CID	Collision-induced dissociation
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DAS	Diacetoxyscirpenol
DAI	Daidzein
DOM-1	Deepoxy-DON
DON	Deoxynivalenol
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionisation
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FB	Fumonisin B
FT-ICR	Fourier transform ion-cyclotron resonance
FUS-X	Fusarenon-X
GC	Gas chromatography
GEMS	Global Environment Monitoring System
GEN	Genistein
Glc	Glucoside
Glc*p*	Glucopyranoside
GlcA	Glucuronide
GSH	Glutathione
GST	Glutathione-S-transferase
HBGV	Health based guidance value
HFB	Hydrolyzed fumonisin B
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
HT2	HT-2 toxin
IAC	Immunoaffinity column
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KOH	Potassium hydroxide
LB	Lower bound
LC	Liquid chromatography/left-censored
LC-FLD	Liquid chromatography – fluorescence detection
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
MB	(LB-UB): mean
MCF-7 cells	Michigan Cancer Foundation-7 cells
MeOH	Methanol
MRM	Mutliple reaction monitoring
MS	Mass spectrometry

MS/MS	Tandem mass spectrometry
MW	Molecular weight
NCM-FB	N-carboxymethyl fumonisin B
NDF	N-deoxyfructosyl
NDF-FB	N-deoxyfructos-1-yl fumonisin B
NEO	Neosolaniol
NIV	Nivalenol
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OPA	Ortho-phthalaldehyde
OTα	Ochratoxin α
OTA	Ochratoxin A
OTB	Ochratoxin B
P95	95th percentile
PAT	Patulin
PCB	Polychlorinated biphenyl
PHFB	Partially hydrolysed fumonisin B
PMTDI	Provisional maximum tolerable daily intake
QTL	Quantitative trait loci
Q-TOF	Quadrupole and time-of-flight
QuEChERS	Quick, easy, cheap, effective, rugged and safe
RAL	ß-resorcylic acid lactone
RES	Resveratrol
RP-HPLC/ESI-ITMS	Reverse phase high performance liquid chromatography – electrospray – ion trap mass spectrometry
RP-HPLC/ESI-TOFMS	Reverse phase high performance liquid chromatography – electrospray – time of flight - mass spectrometry
Sa/So ratio	Sphinganine/sphingosine ratio
SCF	Scientific Committee on Food
SGLT	Sodium-dependent glucose transporter
SPE	Solid-phase extraction
Sulf	Sulphate
T2	T-2 toxin
TDI	Tolerable daily intake
TLC	Thin layer chromatography
TOF	Time of flight
UB	Upper bound
UDP	Uridine 5'-diphosphate
UF	Uncertainty factor
UGT	UDP glucosyltransferase
UV	Ultraviolet
WHO	World Health Organization
ZAL	Zearalanol
ZAN	Zearalanone
ZEL	Zearalenol
ZEN	Zearalenone