SCIENTIFIC OPINION

Risks for animal health related to the presence of ochratoxin A (OTA) in feed

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Dieter Schrenk, Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo,
Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom,
Jean-Charles Leblanc, Elsa Nielsen, Evangelia Ntzani, Salomon Sand, Tanja Schwerdtle,
Christiane Vleminckx, Heather Wallace, Jürgen Gropp, Gunther Antonissen, Guido Rychen,
José Ángel Gómez Ruiz, Matteo Lorenzo Innocenti, Elena Rovesti and Annette Petersen

Abstract

In 2004, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks to animal health and transfer from feed to food of animal origin related to the presence of ochratoxin A (OTA) in feed. The European Commission requested EFSA to assess newly available scientific information and to update the 2004 Scientific Opinion. OTA is produced by several fungi of the genera *Aspergillus* and *Penicillium*. In most animal species it is rapidly and extensively absorbed in the gastro-intestinal tract, binds strongly to plasma albumins and is mainly detoxified to ochratoxin alpha (OTalpha) by ruminal microbiota. In pigs, OTA has been found mainly in liver and kidney. Transfer of OTA from feed to milk in ruminants and donkeys as well as to eggs from poultry is confirmed but low. Overall, OTA impairs function and structure of kidneys and liver, causes immunosuppression and affects the zootechnical performance (e.g. body weight gain, feed/gain ratio, etc.), with monogastric species being more susceptible than ruminants because of limited detoxification to OTalpha. The CONTAM Panel considered as reference point (RP) for adverse animal health effects: for pigs and rabbits 0.01 mg OTA/kg feed, for chickens for fattening and hens 0.03 mg OTA/kg feed. A total of 9,184 analytical results on OTA in feed, expressed in dry matter, were available. Dietary exposure was assessed using different scenarios based on either model diets or compound feed (complete feed or complementary feed plus forage). Risk characterisation was made for the animals for which an RP could be identified. The CONTAM Panel considers that the risk related to OTA in feed for adverse health effects for pigs, chickens for fattening, hens and rabbits is low.

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

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

Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated the risks to animal health and transfer from feed to food of animal origin related to the presence of ochratoxin A in feed. The previous assessment relating to the presence of ochratoxin A (OTA) as undesirable substance in animal feed was published by EFSA in 2004. The 2004 Opinion was used as starting point, nevertheless since 2004 new scientific information has become available on the risks for animal health and transfer to food of animal origin related to OTA in feed, which was incorporated in the present assessment. Information from the 2020 CONTAM Panel Opinion on OTA in food was also used for this Opinion.

OTA is produced by several fungi of the genera *Aspergillus* and *Penicillium*, including *P. verrucosum*, *A. ochraceus* and *A. carbonarius*. OTA is described as a heat stable toxin, nevertheless OTA reduction during heating at $175°C$ temperature and the formation of its degradation products have been shown. Feed materials and compound feed undergo, during production, different steps by which temperature impacts feed (e.g. conditioning, pelleting, expansion, extrusion, solvent extraction). No data on OTA isomers in feed have been identified.

Numerous analytical methods are available for OTA in food, both 'single toxin' and 'multi-toxin methods'. The most commonly used analytical methods for food are usually also used for feed. Reference materials for OTA in feed are commercially available and proficiency tests are offered by the EU reference laboratory for mycotoxins and plant toxins as well as by private providers.

OTA is rapidly and extensively absorbed in the gastro-intestinal tract in most animal species. In general OTA is characterised by a strong plasma protein binding. Inter-species differences have been observed with sheep having a lower plasma protein binding for OTA compared to other ruminants; turkeys had the highest plasma protein binding of different poultry species and donkeys had a lower plasma protein binding compared to pigs. In contrast to other animal species, a very low oral bioavailability and lower plasma protein binding has been observed in fish.

In cattle and sheep it has been suggested that ruminal microbiota play a major role in the extensive hydrolysis of OTA into ochratoxin alpha (OTalpha). Also in pigs OTalpha has been detected in urine at low amount. *In vitro* data showed that OTA in chickens, swine, goat and cows is metabolised via hydroxylation and dechlorination. OTA is excreted both via urine and faeces in all animal species.

Transfer to products of animal origin was described in the 2004 and 2020 CONTAM Panel Opinions, with very similar conclusions. Newly available studies confirm the conclusions from the previous Opinions that there is a low transfer of OTA from feed to milk in ruminants and donkeys as well as to eggs from poultry. In pigs, OTA has been found mainly in liver and kidney. For all other animal species no information is available about transfer from feed to food of animal origin.

Animal products used as feed materials in animal nutrition could contain OTA (from the transfer from feed to organs/tissues). Of certain relevance are kidney and blood which could contribute, in combination with naturally contaminated plant feed materials, to the exposure of animals, particularly carnivore species; nevertheless, this contribution is likely to be low, based on the limited use of these feed materials.

Studies in piglets indicate that OTA impairs function and structure of kidneys and liver, while in growing pigs, the long-term exposure affected the growing performance at the only tested level. A number of new studies were available on OTA toxicity in poultry. In growing chickens (chickens for fattening, chickens reared for laying and breeding), OTA caused an increase in weight of liver and kidney, a decrease in thymus weight and was associated with lesions in liver. Immunosuppression and a depression of zootechnical performance (e.g. body weight gain, feed/gain ratio) were found. A comparable picture results from studies with OTA in laying hens with significantly reduced egg mass production and deteriorated feed to egg ratio. A decrease in the growing performances of weaned rabbits was identified following exposure to OTA. No experimental data on OTA toxicity in horses was reported in the 2004 EFSA opinion. Although it has been suggested that, like other monogastric species, solipeds might be more susceptible to OTA than ruminants, no relevant reports documenting adverse effects of the mycotoxin in those species were retrieved from the recent literature. From the fish data available, the salmonids appeared to be relatively resistant species to OTA with no measurable effects observed up to the highest feed concentration dose tested (2 mg OTA/kg feed). Depression of zootechnical performance, increased intestinal permeability and alteration of hepatopancreatic tissue were found in various juvenile herbivorous fish species.

The CONTAM Panel considered as reference point (RP) for animal health adverse effects: for pigs 0.01 mg OTA/kg feed, for growing chickens and hens 0.03 mg OTA/kg feed, for rabbits 0.01 mg OTA/kg

feed. For ruminants, the CONTAM Panel concluded that it is not possible to derive an RP, due to lack of information. Nevertheless, several studies assessing the effects of OTA in castrated adult male sheep, calves, lactating ewes and goats demonstrate the protective function of the ruminal microbiota. This was shown for levels up to 3.5 mg OTA/kg complete feed in sheep. The CONTAM Panel identified 0.5 mg OTA/kg feed as reference point (RP) for herbivorous fish. Although an indication of toxicity in dogs and farmed mink is given, the CONTAM Panel could not derive RPs for these animal species and neither for cats, where no information could be retrieved.

A total of 10,757 analytical results on OTA in feed were initially extracted from the EFSA Database (sampling years 2012–2021). After assessment, data cleaning and conversion based on dry matter (DM), data on a total of 9,184 samples were made available. The highest OTA levels were reported for 'Horse beans' and 'Lucerne meal'. Among cereal grains, the highest levels were reported in 'Barley grain', and complete feed for pre-ruminant calves among the compound feed samples.

Dietary exposure was performed using different scenarios based on either model diets composed of feed materials or compound feed (complete and/or complementary). Forages were also included for ruminants and horses. Exposure was performed using either a mean or a high-exposure scenario (using the highest reliable percentile based on the number of samples available).

Risk characterisation was performed for those animal species for which an RP could be identified, namely chickens for fattening, laying hens, weaned pigs, pigs for fattening, sows and rabbits. Exposure was derived for salmonids but an RP could only be derived for herbivorous fish, therefore the risk for fish could not be characterised. The CONTAM Panel characterised the risk comparing the exposure against the relevant RP and expressing the exposure as a percentage of the RP. A percentage below 100 was considered a low risk. For weaned piglets the exposure amounted to 14–86% of the RP, for sows the exposure amounted to 11–49%, while for growing pigs the exposure amounted to 11–54% of the RP. For chickens for fattening and hens the exposure amounted to 2–17% of the RP and for rabbits to 16–55% of the RP. The intervals range between the lowest lower bound (LB) and highest upper bound (UB) for two exposure scenarios. The CONTAM Panel considers that this indicates a low risk for adverse health effects.

Uncertainty analysis was performed for the assessment. The uncertainties were identified and prioritised by the experts based on their potential input on the risk assessment output. For the animal species for which it was possible to characterise the risk (poultry (hens and chickens for fattening), pigs and rabbits), the CONTAM Panel considers that the risk related to OTA in feed for adverse effects is very likely (95–99% certain) to be low.

The CONTAM Panel concluded with a few recommendations. Further information is needed on OTA TK in animal species particularly in solipeds, dogs, cats and farmed mink. In addition, further data are required on the adverse effects of OTA in ruminants, solipeds, dogs, cats and farmed mink. Regarding the submission of OTA occurrence data to EFSA, it is urged to provide the adequate information on the feed samples analysed. This refers to reporting at least information on the expression of results and the moisture content (if the results are expressed in whole weight), and sufficient details on the samples analysed (e.g. target animals for the complete/complementary compound feed). The use of the most sensitive methods for the analysis of OTA in feed materials is recommended to reduce the uncertainties linked to the LB-UB estimations.

efsa JOURNAL

Table of contents

1. Introduction

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

Background

In 2004, the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of ochratoxin A (OTA) as undesirable substance in animal feed. The CONTAM Panel established for OTA a Lowest Observed Effect Level (LOEL) of 0.2 mg/kg feed for pigs based on effects on renal (diagnostic) enzyme levels and kidney function. For other animal species, no sufficient dose response data was available to establish No Observed Effect Level (NOEL) or LOEL, nevertheless adverse effects were observed in ruminants, chickens and various monogastric animals (e.g. dogs, cats, fish, etc.) (EFSA CONTAM Panel, 2004).

Since 2004, new scientific information has become available on the risks for animal health and transfer from feed to food of animal origin related to the presence of OTA in feed. The European Commission (EC) has therefore requested EFSA to assess newly available scientific information and to update the scientific Opinion of 2004 as regards the risk for animal health and the transfer from feed to food of animal origin.

Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the EC asked EFSA to provide an Opinion on the risks for animal health and transfer from feed to food of animal origin related to the presence of ochratoxin A in feed.

1.2. Additional information

The CONTAM Panel aims to derive RPs for adverse animal health effects expressed as the levels of OTA in complete feed therefore, the reference to OTA doses in 'feed' in this Opinion needs to be understood as 'complete feed', as laid down in Reg (EC) 767/2009[1] ('compound feed which, by reason of its composition, is sufficient for a daily ration').

1.2.1. Chemistry

The chemistry of OTA was discussed at length in the EFSA's OTA in food Opinion from 2020 (EFSA CONTAM Panel, 2020) this section provides a brief summary.

OTA is produced by several fungi of the genera *Aspergillus* and *Penicillium*, including *P. verrucosum, A.ochraceus* and *A. carbonarius*. In temperate climate zones OTA is produced by *P. verrucosum* at below 30°C and down to 0.8 a_w mainly in grains as maize, wheat, barley and rye. In warmer regions, *A. ochraceus* is found predominantly on peanuts and soybeans. The formation of OTA occurs mainly post-harvest. OTA has the chemical formula $C_{20}H_{18}ClNO_6$ with a molecular weight of 403.8 and the CAS No. 303-47-9. The chemical structure of the substance is given in Figure 1. Due to its isocoumarin moiety OTA exhibits a strong fluorescence after absorption of ultraviolet light.
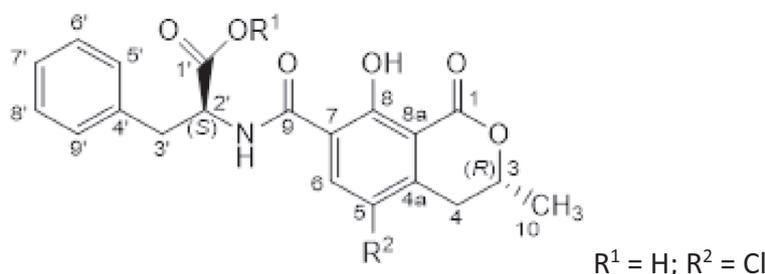

$R^1 = H; R^2 = Cl$

Figure 1: Chemical structure of ochratoxin A

---

[1] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, OJ L 229, 1.9.2009.

Besides OTA, other ochratoxins, such as ochratoxin B (OTB) and ochratoxin C, can be formed to a lesser degree, as described previously (EFSA CONTAM Panel, 2020). Also as described in the Opinion, parent mycotoxins may undergo alterations of their chemical structure due to chemical or biological reactions into modified forms. Modified forms of OTA have been shown in cell suspension cultures, for example wheat and maize, but also after processing at high temperatures (e.g. in coffee). The formation of glucuronides and sulfates of OTA has been reported in mammals (EFSA CONTAM Panel, 2020). Due to the limited information on ochratoxins other than OTA and on OTA modified forms, these are not included in this Opinion.

According to the literature, OTA is described as a heat stable toxin. However, OTA reduction during heating at elevated temperature and the formation of its degradation products have been studied by Cramer et al. (2008). They showed that when OTA was heated at 175°C and higher, its level decreased mainly due to diastereomer formation, that is, 2'R-ochratoxin A (2'ROTA). Sueck et al. (2019) presented similar observations, where OTA isomerised at 120°C. To date, 2'ROTA has been found in food products that required elevated manufacturing temperatures. Although diastereomer levels were relatively low, the presence of the isomer was confirmed in coffee, cocoa, bread and other products (Bittner et al., 2015; Zapaśnik et al., 2022; Bryła et al., 2023).

Feed materials and compound feed undergo, during production, different steps by which temperature impacts feed (e.g. conditioning, pelleting, expansion, extrusion, solvent extraction). No data on isomers in feed have been identified.

1.2.2. Analytical methods

There are numerous published articles, including reviews, with descriptions of the analysis of OTA in feed. These include methods that only determine OTA but also the 'multi-toxin methods'. The analysis of OTA in food is well described in the CONTAM Panel Opinion from 2020 (EFSA CONTAM Panel, 2020). The most commonly used analytical methods for food are usually also used for feed. These methods are therefore only briefly summarised in this paragraph and the EFSA CONTAM Panel (2020) Opinion should be consulted for further details. The most commonly used methods for the determination of OTA in feed are liquid chromatography – mass spectroscopy/ mass spectroscopy (LC–MS/MS), liquid chromatography -fluorescence detector (LC-FLD) and enzyme-linked immunosorbent assay (ELISA) (see Section 3.2, Feed occurrence data). A wide range of ELISA kits, dipsticks and lateral flow devices are commercially available, with established procedures for screening purposes. The limits of quantification (LOQ) differ due to, for example, matrices and methods. For LC–MS/MS methods LOQs from about 0.4 μg/kg and up to 13 μg/kg are reported, while LOQs for LC-FLD and ELISA are in the range 0.04–2 μg/kg and 0.2–2 μg/kg, respectively. For extraction and clean-up, different methods are described including alkaline or acidic extraction and use of immunoaffinity columns. For multi-methods, where several mycotoxins are analysed at the same time, the use of the QuEChERS (Quick Easy Cheap Efficient Robust Safe) method, originally developed for extraction of pesticides, is reported. In the last few years much of the literature has focused on multi-mycotoxin methods especially using QuEChERS and LC–MS/MS in different feed materials (e.g. Nakhjavan et al., 2020; Nualkaw et al., 2020; Jo et al., 2021; Gonzales-Jartin et al., 2021; Konak et al., 2021; Seo et al., 2021; Bi et al., 2022; Mackay et al., 2022; Nochetto and Li, 2022). However, there has also been focus on other and often faster methods including those with biosensors that often involve specific antibodies, aptamers or molecular imprinting polymers in the analytical work (e.g. Bi et al., 2020; Gao et al., 2022; Zhang et al., 2022). Several reviews on the different analytical methods for the determination of OTA in food/feed have also been published within the last few years (e.g. Cai et al., 2020; Tittlemier et al., 2020; Adunphatcharaphon et al., 2022; Chen et al., 2022).

Reference materials for OTA in feed are commercially available and proficiency tests are offered by the EU reference laboratory for mycotoxins and plant toxins as well as by private providers.

1.2.3. Previous animal health risk assessments

EFSA has previously assessed OTA both in food (EFSA CONTAM Panel, 2020) and feed (EFSA CONTAM Panel, 2004).

In 2004 an Opinion of OTA in animal feed was published and no health-based guidance values were established for any animal species. The conclusions of the CONTAM Panel are briefly summarised in the following paragraph. Pigs were considered to be the most vulnerable species and a LOEL of 0.2 mg/kg feed was established. Immunotoxicity was observed at 0.5 mg/kg complete feed in chickens. In dogs, the CONTAM Panel concluded that renal tubular damage as well as necrotic changes

in lymphoid tissues occurred at 0.2 mg OTA/kg body weight (bw).[2] For all other animal species, data were either not available or insufficient for a quantitative assessment. The data used in the Opinion showed that no obvious linear relationship between feed levels and tissue concentrations could be established in pigs but residue concentrations in slaughtered pigs could be ranked as follows: serum > kidney > liver > muscle tissue and fat. In contrast, in chicken the highest levels of OTA were found in the liver, followed by the kidneys, while being substantially lower in other tissues. Also, for chickens no clear relationship between feed levels and tissue concentrations could be established. It was estimated that 0.11% of OTA in feed was transferred to eggs. It was shown that OTA could be transferred to milk from monogastric animals and humans, but low concentrations had also been found in milk from ruminants. In the Opinion it is mentioned that the transfer from feed to food of animal origin could be affected by other substances e.g. other mycotoxins.

In 2020 the EFSA Opinion on OTA in food applied a margin of exposure (MOE) approach. For the characterisation of non-neoplastic effects, a BMDL10 of 4.73 μg/kg bw per day was calculated from kidney lesions observed in pigs. For characterisation of neoplastic effects, a BMDL10 of 14.5 μg/kg bw per day was calculated from kidney tumours seen in rats.

The Opinion concluded that because of its long half-life OTA may accumulate in tissues of monogastric food producing animals such as pigs and thus be present in meat and meat products. Due to efficient degradation in the rumen, OTA levels in the milk and edible tissues of cows and other ruminants are relatively low. In fish, it was concluded that OTA has a short half-life and very low tissue levels.

1.2.4. Legislation

Directive 2002/32/EC[3] on undesirable substances in animal feed, includes, within Annex I, a list of substances which are tolerated in products intended for animal feed, subject to certain conditions. OTA is not included in Annex I. However, guidance values for OTA have been issued under Commission Recommendation 2006/576/EC[4] as amended by Commission Recommendation 2016/1319/EC[5]. The guidance values, given relative to a feed with a moisture content of 12%, are shown in Table 1. Guidance levels are established based on the same principles of the establishment of maximum levels. They are established taking into account the toxicity for animals (animal health) and considering the sensitivity of different animal species and the transfer from feed to food of animal origin. The guidance levels indicate the acceptability of feed placed on the market. The main difference with maximum levels is that for compliance with guidance levels a certain flexibility as regards the enforcement can be applied by the competent authorities.

In 2009, a new functional group of feed additives was established by Commission Regulation (EC) No 386/2009, i.e. *substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action*. No feed additives with a specific binding capacity for OTA have been approved.

In 2013, Commission Implementing Regulation (EU) No 1060[6] authorised the use of bentonite as a feed additive for all animal species, as a mycotoxin binder, with particular reference to Aflatoxin B1. Although not explicitly authorised for the binding of OTA bentonite has shown to bind OTA (Kihal et al., 2022).

---

[2] An incongruency was identified in the previous 2004 opinion with reference to the daily dose for dog in the Kitchen et al., 1977a, b, c as mg/kg feed but also mg/kg bw. A review of the papers revealed that that the dose used in the study is expressed as mg/kg bw.

[3] 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.

[4] Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. OJ L 118 M, 8.5.2007, p. 1111–1113.

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

[6] Commission Implementing Regulation (EU) No 1060/2013 of 29 October 2013 concerning the authorisation of bentonite as a feed additive for all animal species Text with EEA relevance OJ L 289, 31.10.2013, p. 33–37.

Table 1: Guidance values for OTA in feed in mg/kg based on a moisture content of 12% issued in Commission Recommendation 2006/576/EC as amended by Commission Recommendation 2016/1319/EC

Ochratoxin A	Feed materials*	
	– Cereals and cereals products**	0.25
	Compound feed for:	
	– Pigs	0.05
	– Poultry	0.1
	– Cats and dogs	0.01

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

**: The term 'Cereal and cereal products' includes not only the feed materials listed under heading 1 'Cereal grains and products derived thereof' of the list of feed materials referred to in part C of the Annex to Commission Regulation (EU) No 68/2013 of the 16 January 2013 on the Catalogue of feed materials (OJ L 29, 30.1.2013, p.1) but also other feed materials derived from cereals in particular cereal forages and roughages.

2. Data and Methodologies

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

2.1. Occurrence data submitted to EFSA

2.1.1. Data collection and validation

Occurrence data for the presence of OTA in feed were collected as part of the annual call for collection of chemical contaminants occurrence data in food and feed, in the framework of Articles 23 and 33 of Regulation (EC) No 178/2002[7]. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a).

Analytical data on OTA in feed were extracted from the EFSA Data Warehouse on 28 November 2022.

2.1.2. Data cleaning and analysis

To ensure the appropriate quality of the occurrence data used for the dietary exposure estimations, data cleaning and data validation steps were followed according to EFSA SOPs.[8] Together with duplicate samples, attention was paid to the information provided on analytical methods and their sensitivity, sampling strategy, feed classification, expression of the results, etc. Data providers were contacted when needed to confirm the information provided or to ask for missing information that was considered relevant for the exposure estimations (e.g. reported data initially identified as potential outliers).

The analytical results in the feed samples were initially expressed in different ways ('whole weight', 'dry matter', '88% dry matter'). The left-censored data[9] were treated by the substitution method using the lower bound (LB) and upper bound (UB) approach (WHO/IPCS, 2009; EFSA, 2010b). Applying the LB approach, results below the limit of detection (LOD)/limit of quantification (LOQ) were replaced by zero; for the UB approach, the results below the LOD were replaced by the value reported as the LOD; results below the LOQ and above the LOD were replaced by the value reported as the LOQ.

---

[7] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

[8] https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/SOP-040_S.pdf

[9] Left-censored data refer to the analytical results reported to be below the limit of detection (LOD) or limit of quantification (LOQ).

The EFSA guideline 'Use of LOQ cut-off values for dietary exposure to chemical contaminants' (EFSA, 2018) was used to identify possible ways to reduce the impact of the left-censored data on the LB-UB estimations. As described in this guideline, the distribution of the reported LOQs by analytical technique was assessed to establish a cut-off value to exclude samples reported with high LOQs.

2.2. Animal consumption data

The feeds consumed (and the feed intake) by the most relevant food producing and non-food producing animals can only be based on estimates, since no comprehensive feed consumption database exists covering the EU. The animal species and categories considered in this Opinion were: (i) ruminants (dairy cows (producing ~ 40 kg milk/day) for which non-forage feeds accounted for 70% of the diet (on a dry matter basis), beef cattle for which non-forage feeds accounted for 20% of the diet (on a dry matter basis), dairy sheep for which non-forage feeds accounted for 35% of the diet (on a dry matter basis), dairy goats for which non-forage feeds accounted for 75% of the diet (on a dry matter basis), lambs and kids for fattening for which non-forage feeds accounted for 50 and 40% of the diet (on a dry matter basis), respectively; (ii) pigs (weaned piglets, fattening pigs and lactating sows); (iii) poultry (chickens for fattening, turkeys and ducks for fattening, and laying hens); (iv) rabbits; (v) farmed fish (salmonids); (vi) companion animals (dogs, cats) and (vii) horses. The default values for average feed intakes and body weights used to calculate animal dietary exposure to OTA are described in Appendix A. These default values for feed intakes and body weight are based on published guidelines on nutrition and feeding (NRC, 2006; Leeson and Summers, 2008; EFSA FEEDAP Panel, 2017), and are extensively described by the CONTAM Panel in previous Scientific Opinions on the risks for animal and public health (EFSA CONTAM Panel, 2011, 2012). In May 2023 the CONTAM Panel modified/updated the default values[10] in line with current common practices and published guidelines.

2.3. Feed classification data

Feed samples were classified according to the Catalogue of feed materials as described in Commission Regulation (EU) 2022/1104[11].

2.4. Methodologies evidence collection and study appraisal

In preparation for the present Opinion, EFSA launched a call for an extensive literature search (ELS) and selection for relevant studies by screening of title and abstract. The ELS was performed between July and November 2022 and published on 24 March 2023 in the EFSA Journal (Urbani et al., 2023). The outsourced work focused on six search areas relating to (1) information on analytical techniques for quantification of OTA in feed, (2) information on occurrence/concentrations and formation of OTA in feed, (3) information on animal exposure to OTA via feed, (4) information on toxicokinetics (absorption, distribution, metabolism, excretion) in animals, (5) information on toxicity of OTA in animals, (6) information on the transfer of OTA from feed to animal derived food. The results of the search were screened for relevance against the inclusion/exclusion criteria defined ad hoc for the scope of the mandate (for full details see Urbani et al., 2023). The search was limited to the period between January 2003 and July 2022 to take into account the literature search performed for the previous 2004 EFSA Opinion (EFSA CONTAM Panel, 2004).

The total number of publications identified by the Contractors and identified as relevant for each area of interest were as follows: analytical techniques (423), occurrence (569), exposure (224), toxicokinetics (70), animal toxicity (428), transfer to food of animal origin (110).

The papers identified as relevant by the Contractors were screened by the WG and, by applying expert judgement, the relevant studies for the risk assessment were incorporated in the Opinion.

In addition to the systematic ELS, a 'snowballing approach'[12] was applied to identify further relevant studies to be considered for the assessment where relevant.

---

[10] The approach to estimate feed consumption and the default values for feed intake and body weight used to estimate the exposure to bromide was endorsed by the EFSA CONTAM Panel in its 133 Plenary meeting (https://www.efsa.europa.eu/sites/default/files/2023-07/Minutes_0.pdf)

[11] Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. OJ, 4.7.2022, L 177/4-L 177/74.

[12] Identifying papers that have been cited in papers found in a search.

2.5. Methodology applied for dietary exposure assessment

Model diets for each animal species and category were prepared to calculate the exposure to OTA. Similarly, to animal feed intakes, the model diets were derived from information described by the CONTAM Panel in previous Scientific Opinions (EFSA CONTAM Panel, 2011, 2012) and modified in May 2023 (see Section 2.2). The diets are described in the Appendix A.

The amendments introduced in May 2023 were also aimed at allowing a certain flexibility in the use of interchangeable feeding materials in relation to occurrence data availability and levels of contamination. With this scope, feed groups were identified, in line for Commission Regulation 2022/1104[13], and within each group, feed materials could be exchanged, provided the nutritional needs of the various animal species are met. Groups of feed materials are included in Appendix A.

Occurrence data in compound feeds and feed materials were used to derive a scenario of exposure for the consumption of compound feed only (when occurrence data allowed), and a scenario for the consumption of model diets using feed materials such as cereals and oil seeds, including forages for ruminants. The occurrence data on feed materials, forages and compound feeds reported in Table 7 were used to calculate animal exposure.

In the estimations of animal dietary exposure, two situations were considered: a mean occurrence scenario, in which the mean LB and UB values for each feeding stuff were used to estimate OTA dietary concentrations; and a high-occurrence scenario, in which the highest reliable percentile LB and UB values were used, up to the 95th percentile. The calculated mean and high concentrations of dietary OTA (reported in Appendix A) were combined with the estimated feed intake (also described in Appendix A) to obtain the estimated dietary exposure to OTA of the different animal species and categories in the two scenarios. The detailed results, summarised below, are tabulated in the Appendix C.

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics – absorption, distribution, metabolism and excretion

The TK of OTA in laboratory animals, humans and some target species has been recently detailed in the EFSA 2020 Opinion, based on the reviews by Ringot et al. (2006), Vettorazzi et al. (2014), Heussner and Bingle (2015) and Köszegi and Poor (2016). For more recent reviews see Tao et al. (2018) and Liu et al. (2022). The main steps of OTA kinetics are summarised in the following paragraphs.

3.1.1.1. Absorption

The dissociated (monoanionic and dianionic) toxin forms are best absorbed by passive mechanisms under acidic or slightly acidic conditions in the stomach(s) and jejunum, respectively. Indication has been provided of the role of drug transporters (MRP2, BCRP) in limiting OTA enteric absorption, which might possibly explain the wide species-related differences in oral bioavailability.

3.1.1.2. Distribution

Although wide species-related differences are reported, the toxin displays an unusually high affinity for serum albumin (up to 99%). This is strictly related to the long plasma half-life of OTA, as the albumin-bound toxin cannot be excreted through glomerular filtration. OTA is widely distributed with many studies reporting the highest concentrations in kidneys followed by liver and muscles. An active role in liver and kidney uptake has been reported for the OATPs[14] drug transporters; in addition, other drug transporters of the OAT family have been implicated in either the active uptake (basolateral expression) or the tubular reabsorption (apical expression) of the toxin in the kidney cells, explaining renal accumulation and toxicity. The ability to cross the placental barrier reported in certain species has been also related to the role played by the OAT drug transporter.

---

[13] Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials (Text with EEA relevance), C/2022/4474, OJ L 177, 4.7.2022, p. 4–74

[14] Organic anion transporting polypeptides (SLC superfamily), involved in the transport of organic anions through cellular membranes, active in uptake/excretion at hepatic and renal level.

3.1.1.3. Metabolism

OTA undergoes several phase I and phase II biotransformations, the majority of the metabolites displaying lower toxicity than the parent compound. The most important metabolic reaction, also from a quantitative viewpoint, is the hydrolytic cleavage into the non-toxic ochratoxin alpha (OTalpha) (Figure 2) and phenylalanine, which is mostly accomplished by ruminal and enteric microorganisms. Other pathways are of minor importance and metabolites identified following *in vitro* studies have not always been isolated *in vivo*. The cytochrome P450 enzymes (CYP)-dependent generation of OH-OTA derivatives occurs at both of the isocoumarin and phenylalanine moieties and leads to the formation of 4S-OH-OTA, 4R-OH-OTA, 7′-OH-OTA, 9′-OH-OTA, 5′-OH-OTA and some other minor metabolites in liver microsomes of several species including pigs, cows, goats and chickens. In the same experiments, the NADPH-dependent generation of the dechlorinated metabolite OTB and its subsequent hydroxylation have been also demonstrated (Figure 3).
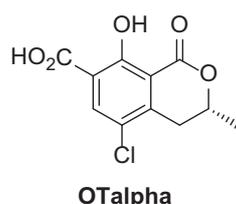
Figure 2: Ochratoxin Alpha (OTalpha)
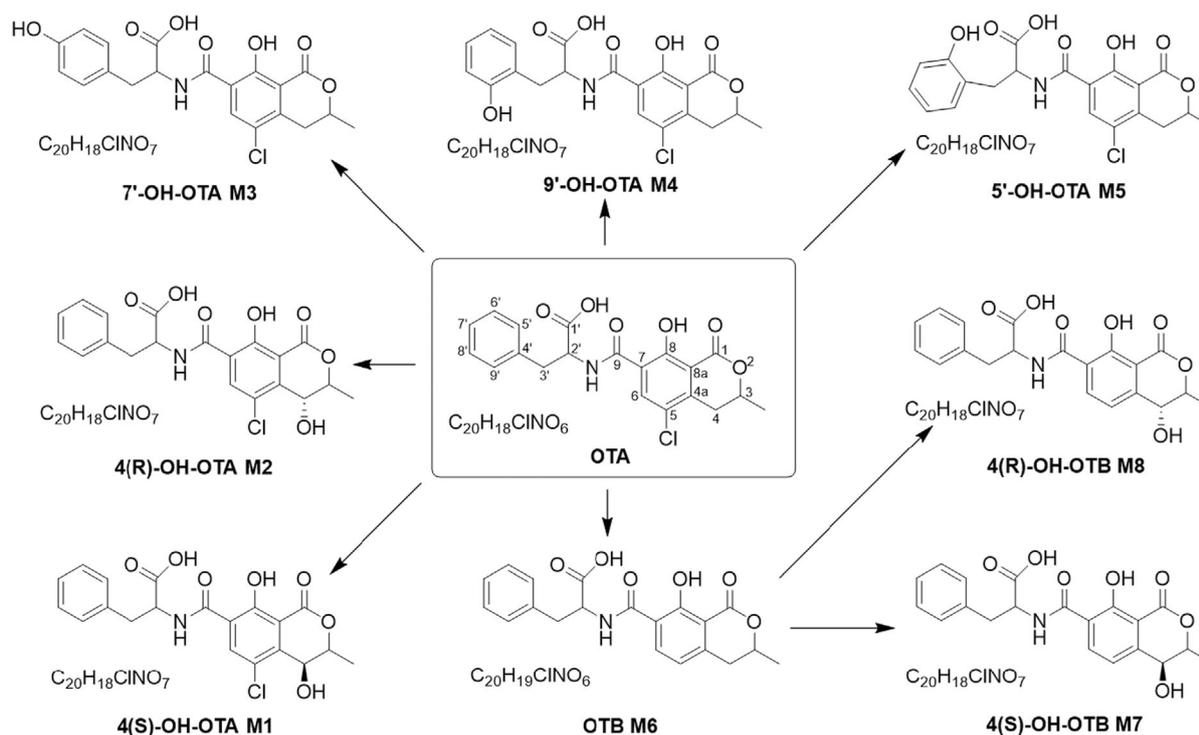
Figure 3: Metabolic breakdown of OTA in pigs, cows, goats and chickens (Yang et al., 2015 © 2015, Springer-;Verlag Berlin Heidelberg)

Glucuronide- and sulphate derivates of OTA itself and its metabolites have been described, although in some cases only indirect evidence of their formation *in vivo* has been provided.

3.1.1.4. Excretion

OTA and its metabolites are excreted via both the urinary and the faecal routes. As mentioned before (see Section 3.1.1.2), due to the strong binding to serum proteins occurring in most species, OTA renal excretion by glomerular filtration is limited and mostly takes place via tubular secretion by

means of drug transporters. The clearance of the toxin is also slow because of the remarkable tubular reabsorption which is mediated by OAT transporters[15] and favoured by acidic urines.

OTA and its metabolites are also detected in faeces. The contribution provided by biliary excretion, the enteric secretion mediated by MRP2 and BCRP[16] drug transporters as well as the entero-hepatic cycle remained to be established in many species.

Finally, excretion via milk has been documented in several species.

3.1.1.5. Species-related kinetics

Only limited information was provided in EFSA 2004 Opinion (EFSA CONTAM Panel, 2004) concerning OTA absorption, distribution, metabolism, excretion and toxicology (ADME) in pigs, ruminants, poultry and fish. To provide a more comprehensive picture, the CONTAM Panel decided to include also relevant papers published before 2004 which have been sometimes already mentioned but not described in detail in the 2004 Opinion.

Cattle

As reported in abovementioned the EFSA 2004 Opinion, both in young pre-ruminant calves and in adult individuals, the oral administration of OTA resulted in the generation of OTalpha as the main metabolite, which was not further biotransformed and was largely excreted in urine. *In vitro* studies performed with ruminal fluid from adult cattle indicate an extensive hydrolysis of OTA into OTalpha mainly by rumen protozoa. It was calculated that a cow should be able to efficiently degrade OTA up to a feed concentration of 12 mg/kg. No further data on ADME were mentioned. In the abovementioned Opinion it was summarised that, owing to an efficient rumen degradation, OTA levels in the milk and edible tissues of cows and other ruminants are low.

In vitro studies

In vitro experiments revealed a relatively strong binding between bovine serum albumin and OTA (Chu, 1971).

Since the publication of the 2004 Opinion (EFSA CONTAM Panel, 2004), a number of reviews have been published on *in vitro* OTA ruminal degradation (Mobashar et al., 2010; Upadhaya et al., 2010; Loh et al., 2020). It is confirmed that inocula from the three forestomach compartments (rumen, reticulum, omasum), but not from the abomasum, were able to extensively hydrolyse OTA into OTalpha. While the degrading capacity of protozoa was well acknowledged already in the 2004 Opinion, more recent investigations thereafter emphasise the role of the rumen bacterial community (Mobashar et al., 2012). According to several studies, ruminal degradation can be considered a first-order reaction following a mono-exponential decay, with half-life values ranging from 0.2–0.3 h to 4–5 h mainly according to the diet of donor animals of ruminal fluids. Diet affects the rate of OTA metabolism primarily via its influence on the composition of the rumen microbial populations, particularly the protozoal component. Accordingly, a higher OTA degradation rate was reported for rumen fluid from cows fed diets with a 60:40 concentrate:forage ratio compared to rumen fluid from individuals fed 100% hay (Müller et al., 2001).

The incubation of OTA with cow liver microsomes resulted in the formation of several hydroxy-derivatives, with 4(S)-OH-OTA largely prevailing over 4(R)-OH-OTA, 7'-OH-OTA and 9'-OH-OTA (Yang et al., 2015).

In vivo studies

Scant information is available on OTA ADME in cattle. In the only published paper on this topic (Sreemannarayana et al., 1988), OTA TK was investigated on milk-fed young male Holstein Friesian. This study was also mentioned in the 2004 Opinion but only briefly described. A first experiment was performed in pre-ruminating calves. Two individuals (60 kg bw each) were intragastrically (stomach tube) dosed with 0.5 mg OTA/kg bw, while two further calves (44 kg bw each) received 0.25 OTA/kg bw intravenous (i.v.) Blood samples were collected at fixed intervals up to 240 h. Urine and faeces were collected separately every 12 h until 120 h and then every 24 h until the end of the experiment (240 h). The collected specimens were analysed for both OTA and OTalpha with an high pressure

---

[15] OAT (SLC family), MRP and MRP2 (ABC family) are drug transporters involved in both OTA uptake and excretion in different organs.
[16] MRP2 and BCRP are drug transporters belonging to the ABC superfamily, involved in limiting enteric absorption and with a role in the enteric secretion, biliary excretion and entero-hepatic circulation.

liquid chromatography (HPLC) method (LOD = 0.05 ng/μL). Orally dosed calves showed a rapid and large absorption with a first serum peak of OTA at about 5 h and a second one at about 40 h; no measurable OTalpha levels were found in serum, pointing to a rapid clearance of the metabolite from the blood. While little amount of the toxin was detected in urine and faeces, most of the administered dose (average ~ 85%) was eliminated as OTalpha in urine, suggesting a significant role for enteric microbiota in performing OTA-cleavage, as demonstrated, for example, in pigs (Upadhaya et al., 2012). This conclusion is further supported by the lack of detectable amounts of OTalpha in serum and in the excreta from the preruminating calves treated i.v. in the described study. In those calves, little amount of OTA ($<$ 0.1 ng/μL) was recovered in serum 120 h after treatment.

The second experiment performed by Sreemannarayana et al. (1988) involved four ruminating calves (bw range 68–100 kg) which received a single oral dose of 2 mg OTA/kg bw. Blood, faeces and urine collection were as described above up to 120 h after dosing. In line with a rapid absorption, a first serum peak (about 2 ng/μL) was observed 2 to 4 h after treatment. After a decline until about 12 h, a further peak (about 0.5 ng/μL) was noticed at about 24 h, possibly indicating the occurrence of entero-hepatic circulation. Of note, serum OTalpha was already detectable (about 0.1 ng/μL) few hours after treatment and peaked (about 0.2 ng/μL) almost at 30 h. A slow decline was observed for both OTA and OTalpha, being still present at low levels (about 0.5 ng/μL) 120 h after dosing. As expected, OTalpha concentrations largely overweighed OTA levels in the excreta ($>$ 98%) thus confirming the extensive degradation of the toxin; the metabolite was predominantly excreted in urines (87%) rather than in faeces. The overall cumulative excretion (OTA + OTalpha) over 240 h amounted to about 90% of the administered dose.

Little is known about tissue distribution and milk excretion of OTA and OTalpha in cattle. Negligible concentrations of both OTA and OTalpha were found in tissues and milk of cows treated with either a single dose (0.03 mg OTA/kg bw) (Zhang et al., 2019) or after repeated dietary exposure to 5, 50 or 100 μg OTA/kg DM for 28 days (Hashimoto et al., 2015).

In summary, based on a very limited dataset, OTA appears to be rapidly and quite extensively absorbed in cattle. The serum concentration versus time curves may indicate the occurrence of entero-hepatic circulation and indicate a slow elimination mainly via urine in the form of OTalpha, also in line with the OTA strong binding to serum albumin. This metabolite arises from the extensive hydrolytic OTA degradation. In the rumen, this is accomplished not only by protozoa but also by bacterial populations and is affected by diet composition. Studies in pre-ruminant calves point to a role of the enteric microbiota in the generation of OTalpha. Negligible concentrations of both OTA and OTalpha were found in tissues and milk from cows under conditions of single or repeated exposure.

Sheep

Based on a limited database, in the EFSA 2004 Opinion it was reported that ovine microbial populations seem to have a lower capacity to degrade OTA into OTalpha, in agreement with the results of an *in vivo* study where sheep fed graded OTA concentrations exhibited significant OTA plasma levels whereas minor amounts of OTalpha were detected. A linear relationship was found between OTA concentrations in feed and OTA plasma levels.

In vitro studies

No major differences between ovine and bovine rumen fluid were found in the rate of OTA hydrolytic cleavage to OTalpha, the major role being played by protozoa (Kiessling et al., 1984). In a further experiment, Xiao et al. (1991a) found that the rate of OTA hydrolysis (12.5 mg/10 mL) was greatly affected by the diet and the ruminal pH. Rumen fluid samples from grain-fed sheep had lower pH (5.5 vs. 6.5–7.2) and did hydrolyse OTA 2- to 5-fold less than samples from sheep fed hay, likely due to a negative effect of the above conditions on microbial populations involved in OTalpha generation. In addition, since OTA is a weak acid, a low pH would favour the passive absorption of undissociated OTA from rumen wall into the bloodstream.

In vivo studies

To investigate OTA kinetics, four Suffolk ewes were first treated i.v. with 0.2 mg OTA/kg bw (Xiao et al., 1991b). Blood was sampled at fixed times up to 144 h post dosing and the same protocol was adopted for urine and faeces. OTA serum concentrations declined rapidly up to about 10 h after treatment and then more slowly up to 120 h when the toxin was no longer detectable; the kinetic profile corresponded to a bi-exponential decline, with distribution and elimination half-lives of 1.5 h and 17.5 h, respectively. The relatively short elimination half-life is in line with a lower extent of OTA

binding to serum albumin in sheep compared to other species (e.g. pigs, cattle). The cumulative excretion data indicated that 90 to 98% of the injected dose was recovered as OTA in the urine (no toxin in faeces); only ~ 2.4% and 4% of the administered dose were recovered as OTalpha in the urines and faeces, respectively. No other OTA metabolites were identified. Taken together these results point to a negligible role of tissue OTA biotransformations and a limited occurrence of biliary excretion and entero-hepatic circulation following OTA i.v. treatment.

Female sheep fed on different diets (grain or hay) received a single OTA intraruminal dose (0.5 mg/kg bw) and ruminal fluid samples were taken at 1 h intervals up to 10 h after dosing. In keeping with the results of the *in vitro* experiments, OTA concentrations declined at a much faster rate in hay-fed sheep becoming undetectable 6 h after treatment, while still measurable after 10 h in grain-fed sheep. The corresponding half-lives for rumen OTA disappearance and the pH of ruminal fluid were 0.65 versus 3.38 h and 6.9 versus 5.6 for hay- or grain-fed sheep, respectively (Xiao et al., 1991a).

According to Blank and Wolfram (2009), six crossbred castrated male sheep (Weißkopf-Texel) with a mean body weight of ~89 kg were offered a diet consisting of 70% concentrates and 30% grass silage. After 3 weeks adaptation, they were treated once with 150 g contaminated meal containing 2.46 mg OTA (~ 0.03 mg/kg bw). Blood samples were taken by venipuncture at T0 and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h after dosing. Faeces and urine were collected daily over a period of 6 days. One week later, the treatment was repeated and rumen fluid samples were collected before and 1, 4, 7, 10 and 24 h post-feeding using an oro-ruminal probe. OTA and OTalpha were detected using an HPLC method (LOD = 0.2 ng/mL). OTA was found to be rapidly absorbed with a serum peak (~ 14 ng/mL) at 6.5 h, suggesting that absorption may already occur in the forestomach. A slow decline ensued, with a $t_{1/2}$ of about 17 h; OTalpha serum levels were below 1 ng/mL over the whole collection period. OTA disappearance from ruminal fluid fitted a mono-exponential decay; almost 90% of OTA was cleaved to OTalpha after 9 h. OTA was mainly eliminated as OTalpha, accounting for about 90% of the elimination form in either urine (the predominant route) or faeces. Overall, 84% of the administered dose was eliminated after 6 days in faeces (27%) and urine (54%) as the sum of the parent compound and its metabolite.

OTA kinetics was also investigated after the prolonged exposure to a contaminated diet. Höhler et al. (1999) treated three groups of four sheep with diets consisting of 70% concentrates and 30% hay (DM basis) for 4 weeks with OTA dietary concentrations of 0, 2 or 5 mg/kg of concentrate feed, corresponding to 0, 0.22 or 0.55 mg OTA/kg bw, respectively. Blood samples were taken once a week while faeces and urine were collected over a 7-d period (from the 3rd to 4th week). OTA and its metabolites were measured with an LC-FLD method. Relatively constant serum OTA levels in the range 10.2–10.8 ng/mL were detected in the 2 mg/kg diet group; higher (7- to 11-fold) and increasing concentrations (74.4 ng/mL at week 1 and 111.7 at week 4) - not proportional to OTA intake- were measured in the 5 mg/kg diet group. Relatively constant but low levels of OTalpha were instead measured in serum samples from both 2- (3.4–2.0 ng/mL) and 5 mg/kg diet group (15.9–18.5 ng/mL) pointing to an incomplete cleavage of the mycotoxin. OTA was largely eliminated as OTalpha through the urinary route; at the end of week 3 of treatment, 0.5% and 11.7% of the administered dose were excreted in the faeces as OTA and OTalpha, respectively; in urine, the percentages amounted to 3.6 and 68.6, respectively. No OH-OTA was detected in any of the analysed samples.

A further study (Blank et al., 2003) was designed to achieve greater insight into the systemic availability and excretion pattern of OTA in sheep repeatedly exposed to dietary concentrations also found under farming conditions. Twelve 1-year-old male lambs (39.3 ± 1.6 kg) were allotted to four groups of three animals each and offered an OTA contaminated diet (70% concentrates and 30% grass silage) corresponding to a daily intake of 0, 387, 774 and 1,161 μg OTA A, respectively (0, 9.5, 19.0 and 28.5 μg OTA/kg bw per day) for 29 days. Blood sampling was performed on days 1, 5, 9, 13, 23 and 29, while faeces and urine were quantitatively collected over a period of 7 days (day 15–21). Ruminal fluid was sampled on days 24 and 25 at fixed intervals up to 13 h after the morning feeding. There was a linear increase in serum OTA concentrations (range 1.5–18.2 ng/mL) with respect to both the increasing intake of the mycotoxin and the duration of the exposure; OTalpha displayed a similar trend, but serum concentrations were much lower (range 0.5–2.3 ng/mL). OTA concentrations in ruminal fluid showed a linear decrease over time, reaching concentrations approximating the LOQ (0.2 ng/mL) at the last sampling timepoint (13 h after the morning feeding); apart from animals exposed to 9.5 μg OTA kg bw/day, OTalpha rumen fluid concentrations increased with time after feeding and OTA dose. The half-lives for OTA disappearance from rumen, however, were not significantly affected by the dose. As regards faecal and urinary excretion, there was a dose-related increase in both OTA and OTalpha concentrations, the metabolite being predominantly excreted in

faeces and urine as well. Overall, treated sheep excreted 74–80% of the ingested OTA and the cumulative excretion of OTA and OTalpha was 2–3 fold higher in urine than in faeces.

The kinetics of OTA after a single- and a repeated administration was studied in six 2-year-old Lacaune sheep (late lactation) (Boudra et al., 2013). Sheep were fed *ad libitum* with hay and provided with a concentrate mixture of 0.8 kg pelleted wheat and soybean meal/day; they were allotted to two groups (n = 3/group) and used for two different studies. In the first one, sheep were administered with a single dose of 0.005 or 0.03 mg OTA/kg bw; after a 4-day washout, the same toxin amounts were administered daily for 24 days. Blood samples were taken at fixed intervals up to 96 h after treatment. Faeces and urine were quantitatively collected for 48 h (collection time not clearly reported). After a single exposure, a rapid absorption of OTA and a rapid conversion to OTalpha occurred, since measurable amounts of both compounds were detected in serum as early as 1 h after dosing. C_{max} values were dose-dependent and were observed, on average, 6 and 8 h after treatment for OTA and OTalpha, respectively. Also, area under the curve $(AUC)_{(0\rightarrow last)}$ was significantly affected by the dose. Compared to the single dosing, the repeated exposure led to an increase in OTA- but not in OTalpha serum concentrations, particularly for the high dosage (0.03 mg OTA/kg bw); by contrast, t_{max} values were reduced to almost the half (3 h) for both compounds. Large individual differences were noticed in urinary and faecal excretion of the mycotoxin and its metabolite. In line with previous studies, OTalpha was the prevalent excretion form in urine and faeces. Upon single exposure, there was a direct relation between the administered dose and amount of either compound in urine or faces. In repeatedly exposed ewes this was the case only for faeces and similar OTA urinary concentrations were detected for both 0.005 or 0.030 mg OTA/kg bw-dosed animals. This suggests a more efficient detoxification by the microbiota under conditions of repeated exposure. Milk excretion was documented for both OTA and OTalpha, with low transfer rate (see also transfer Section 3.2.1).

In summary, also in sheep OTA is extensively hydrolysed to OTalpha mostly in the rumen. The exposure to high dosages and dietary factors such as the prevalence of concentrates versus hay or other events known to lower rumen pH will depress the rate and the extent of OTA conversion; the above conditions will result in an increase in OTA bioavailability also due to the weak acid nature of the toxin, with the potential to distribute into tissues and undergo milk excretion. No other OTA metabolites have been reported. Both OTA and OTalpha are rapidly and extensively absorbed at ruminal and enteric level and more slowly eliminated mainly as OTalpha in urine and to a much lesser extent in faeces. The relatively short elimination half-life, however, points to a lower extent of binding to serum albumin when compared to other target species. While milk excretion has been documented, no data on tissue distribution could be retrieved.

Goats

Goats were not mentioned in the EFSA 2004 Opinion. There is little information on OTA TK in goats. *In vitro* studies (Upadhaya et al., 2011) were performed to investigate factors affecting rumen OTA hydrolysis to OTalpha. In keeping with the results of similar experiments carried out in cattle and sheep, the rate of hydrolysis was significantly higher when the toxin was incubated with rumen fluid from goats fed a 100% roughage diet as compared to a 50:50 concentrate:roughage diet. OTA degradation to OTalpha was also depressed at acidic pH. Finally, the populations of *Bacillus licheniformis*, a ruminal bacterium expressing carboxypeptidase A, were found to play an active role in OTA degradation. The *in vitro* OTA biotransformation was investigated in goat liver microsomes (Yang et al., 2015). Metabolite profile was quali-quantitatively similar to that described in cow preparations (see above), except for 4(*S*)-OH-OTA which was generated to a much lower extent (less than a half).

Only one *in vivo* study could be retrieved (Nip and Chu, 1979). The fate of labelled toxin (3H-OTA) was investigated in two goats after a single oral administration of 0.5 mg/kg bw. Blood, urine, milk and faeces were collected at fixed intervals up to 168 h (7 days). Seven days after treatment, more than 90% of the total radioactivity was reported to be excreted; namely 53% and 38% was found in faeces and urine, respectively while total radioactivity found in milk and serum accounted for only 6% and 2.4% of the fed radioactivity, respectively. The measured radioactivity was found to be due to OTA and at least two further metabolites (I and II) which remained unidentified. Faeces represented the major OTA excretion route, accounting for 6% and 22% of the administered mycotoxin after 24 h and 7 d, respectively. In keeping with total radioactivity results, only 3% of the administered dose was excreted via urine within 7 d (2% in the first 24 h).

In summary, and unlike the other ruminant species, OTA and its metabolites seem to be mostly excreted via the faecal route in goats, although it is not known whether this may be related to an incomplete absorption/degradation of the toxin and/or to biliary excretion/entero-hepatic circulation.

Solipeds

No information on ADME in solipeds was provided in EFSA 2004 Opinion (EFSA CONTAM Panel, 2004).

Horses

Only one study was retrieved since the 2004 Opinion (Minervini et al., 2013), reporting the results of a survey performed in 12 stallions, 2 ponies, 7 cycling mares and 17 pregnant mares (age range 2–18 years) fed with hay and commercial feed or oats. About 84% of the plasma samples were found to contain OTA (median 121.4 pg/mL, range 53–705 pg/mL). To study placental transfer, serum samples were collected from the 17 mares and from the umbilical cords of their foals after delivery. Fourteen serum samples from the pregnant mares contained OTA (median 106.5 pg/mL, range 70–348 pg/mL), but measurable concentrations of the toxin were detected only in 50% of their foals (median 96.6 pg/mL, range 70–253 pg/mL). No correlation was found between OTA levels in umbilical and mare serum samples. No other information on OTA ADME was made available.

In summary, OTA placental transfer was reported in mares exposed to OTA via a naturally contaminated diet.

Donkeys

In a recent study, Lippolis et al. (2020) investigated the OTA contamination in pregnant hinnies and their foals. Seven Martina Franca hinnies fed with hay and concentrates (a cereal mixture) were included in the study. The incidence of feed samples which tested positive for OTA was 32% and ranged from 0.3 to 2.7 μg/kg. Blood specimens collected 15 days before delivery and at delivery revealed the presence of OTA at median levels of 78 and 97 ng/L, respectively. In 34 blood samples collected 2–3 months after delivery median OTA concentrations amounted to 109 ng/L. Interestingly, blood samples from foals at delivery (umbilical cord) tested negative for OTA, while the median value for those collected 2–3 months after delivery (a total of 33) was 52 ng/L. Milk samples collected at the same timepoints showed median OTA concentrations of 7.5 ng/L. Results indicate that, unlike equine placenta, donkey placenta does not seem to be crossed by OTA, the maternal transfer of the toxin occurring only via milk.

The TK of a single oral OTA dose was investigated in donkeys (Kang et al., 2023). Four Dezhou male donkeys (124 $\pm$ 2 kg bw) were orally administered (gavage) with *A. ochraceous* culture material providing 2.5 mg OTA/kg bw. Blood, faeces and urine were collected at fixed intervals up to 120 h after the treatment and OTA was determined by an LC-FD method (LOD 0.2 μg/L or μg/kg, LOQ 1 μg/L or μg/kg). OTalpha and other metabolites were not determined. The toxin was quickly absorbed as it was detected in plasma as early as 5 min after treatment; plasma OTA concentrations progressively increased peaking at 12 h (10.3 $\pm$ 2 μg/mL) and showing a slow decline thereafter (e.g. less than 2 μg/mL at 96 h). The elimination half-life amounted to 24.5 $\pm$ 2.5 h, which is for example about one third that reported in the literature for pigs, likely pointing to a lower protein binding extent than in swine. In the study, OTA elimination in urine was already noticeable at 6 h, increased up to 12 h and then declined to reach 'low levels' from 36 h onwards; faecal elimination was instead apparent only at 12 h and became negligible at 56 h. Overall, while the absorption rate was about 89%, urinary and faecal excretion over 120 h accounted for only about 11 and 12% of the administered OTA dosage, respectively. It is not known whether the amount of toxin not accounted for may be explained by extensive biotransformation and/or tissue accumulation.

In summary, based on a limited dataset, in donkeys OTA appears to be rapidly and extensively absorbed, and partly (about 23% of the administered dose) and more slowly eliminated via faeces and urine in similar proportion. No information on the biotransformation or distribution of OTA has been retrieved. Under conditions of 'natural' exposure, the toxin has been detected in the milk at ng/L levels and is likely not transferred to foals via the placental route.

Dogs, cats

No relevant data on OTA TK in dogs and cats were identified.

Poultry

In 2004, EFSA CONTAM Panel reported an overall absorption of OTA of ~ 40% in chickens, and a shorter serum half-life (4 h) compared to mammals. Residual levels of OTA were reported in kidney, live and muscle tissue, with the highest level in kidney and liver tissue. In addition, OTA residues were

demonstrated in eggs (range 1.3–7.9 ng/g) in different studies where laying hens are given feed contaminated with high concentrations of OTA (1.3–10 mg/kg).

Broiler chickens

In vitro *studies*

Based on *in vitro* experiments with liver microsomes prepared from livers of Beijing Huandu chickens for fattening, the amount of 7′-OH-OTA was the largest of different hydroxylated metabolites. Besides, the other hydroxylated metabolites found were 4S-OH-OTA, 4R-OH-OTA, 9′-OH-OTA, 5′-OH-OTA, with the amount of 4R-OH-OTA remarkably lower compared to mammalian species. Also, the dechlorination product OTB was observed in chicken liver microsomes (Yang et al., 2015).

In vivo *studies*

The metabolism of OTA in chickens was characterised *in vivo* by administering a single dose of OTA analytical standard (5 mg/kg bw) by oral gavage to six Beijing Huandu chickens for fattening (three males/three females, mean bw 1.0–1.2 kg) (Yang et al., 2015). In this study, animals were fasted for 12 h prior to OTA administration. OTA metabolites were identified by ultra-high performance liquid chromatography/ quadrupole time-of-flight– mass spectroscopy UPLC/Q-TOF-MS. In accordance with the *in vitro* investigation, six different metabolites were also confirmed *in vivo* in excreta of chickens, including five hydroxylated metabolites of OTA: 4S-OH-OTA, 4R-OH-OTA, 7′-OH-OTA, 9′-OH-OTA, 5′-OH-OTA and one dechlorination product of OTA (OTB). Besides, in this study two hydroxylated metabolites of OTB (4S-OH-OTB and 4R-OH-OTB) were found in the excreta. The hydroxylation of the phenylalanine moiety of OTA is an important metabolic pathway in chickens, with 7′-OH-OTA identified as the main metabolite (20 ± 2.5%) of different OTA components present in excreta of chickens. Furthermore, the 4S-OH-OTA isomer was the major and 4R-OH-OTA was the minor metabolite in chickens.

TK and plasma protein binding of OTA was investigated in eight 4-week-old Ross 308 chickens for fattening (four males/four females, mean bw 1.27 kg) by Devreese et al. (2018). Four animals received a bolus of 0.25 mg OTA/kg bw by gavage and the other four birds were given 0.25 mg OTA/ kg bw by injection in the wing vein. In this study, animals were fasted for 8 h before the administration of OTA. The experiment was performed in a two-way crossover design, after a 6-day wash-out period the protocol was repeated, but the birds that received an i.v. injection of OTA then received OTA orally and vice versa. Venous blood samples were collected at fixed intervals up to 36 h. Plasma levels of OTA were measured by LC–MS/MS with an LOQ of 5.0 ng/mL. Based on the relative comparison of AUC following oral vs. i.v. administration, orally dosed birds showed a rapid and almost complete absorption, with a mean oral bioavailability of 93% and 110% in male and female chickens for fattening, respectively. The C_{max} of 47 and 75 ng/mL was reached on average after 4.6 and 1.4 h in male and female chickens for fattening, respectively. Additionally, a secondary peak after oral as well as intravenous administration (4–6 h) was observed in the plasma concentration-time profiles. This might suggest the occurrence of entero-hepatic circulation of OTA. Plasma protein binding was high (ranging between 90% and 97%) and the mean V_d was 20 L/kg after i.v. administration. Elimination of OTA in chickens for fattening was characterised by a low clearance (0.6–0.7 L/h per kg) and a long elimination half-life ($t_{1/2el}$) (22–24 h), although the latter was characterised by a high standard deviation, especially in male chicken for fattening (6 h) suggesting a large inter-individual variation. No difference between male and female birds was observed for any of the TK parameters.

Laying hens, breeder hens and roosters

To get more insights in the biliary excretion of OTA, Armorini et al. (2015) conducted an experiment in laying hens (Isa Brown, 30 weeks old, mean bw 1.80 kg). Forty-five laying hens were divided into three experimental groups, control group received diet without OTA, second group was fed a diet contaminated with 0.010 mg OTA/kg, and the last group was fed with OTA 0.2 mg/kg feed. Diets were experimentally contaminated by mixing naturally highly contaminated ground maize (280 mg OTA/kg) with the basal diet. Animals were housed individually and fed with 100 g/day of experimental diet for 6 weeks. Average levels of OTA, detected by LC-FD analysis, in bile were 6 ng/mL and 140 ng/mL and in kidney tissue 0.5 ng/g and 1 ng/g for the groups with 0.01 or 0.2 mg OTA/kg feed, respectively. OTA was only detected in one liver sample (0.5 ng/g) (LOD 0.5 ng/g, LOQ 1.0 ng/g) of an animal fed the highest OTA level.

Devreese et al. (2018) investigated also TK and plasma protein binding in 20-week-old hens and roosters of Lohmann Brown-Lite layer strain (mean bw 2.05 kg). OTA was characterised by a high mean oral bioavailability of 85 and 99% in roosters and laying hens, respectively. The C_{max} of 56 and 49 ng/mL was reached on average after 0.75 and 1.88 h in roosters and laying hens, respectively. Similarly, as in chickens for fattening, also a secondary peak in the plasma concentration-time profile was observed 4 h after administration, which might point to the occurrence of entero-hepatic circulation of OTA. Plasma protein binding was high (ranging between 88% and 94%) and the mean Vd was 16–19 L/kg after oral administration. Elimination of OTA in layer chickens was characterised by a low clearance (0.9–1 L/h/kg) and a long elimination half-life ($t_{1/2el}$)(12–14 h). However, distribution and elimination parameters were characterised by a large inter-individual variation. No difference between laying hens and roosters was observed for any of the TK parameters. The oral bioavailability and half-life values of layer chickens mentioned by Devreese et al. (2018) are larger than those reported in the 2004 Opinion (40% and 4 h, respectively) based on Galtier et al. (1981). This difference can in part be explained by the difference in the quantification limits of the analytical methodology used in these studies, thin layer chromatography (TLC) with an estimated LOQ of 100 ng/mL versus LC–MS/MS with an LOQ of 5 ng/mL.

Turkeys

TK and plasma protein binding was evaluated in 5 week-old-Hybrid Converter turkey poults (mean bw 1.71 kg) and compared to other avian species (Devreese et al., 2018). The experiment was performed as previously described. OTA was also in turkey poults almost completely absorbed (88–110%). After 0.75–0.81 h since administration, a higher mean C_{max} (179–201 ng/mL) was observed in turkey poults compared to other poultry species. A minor secondary peak in the plasma concentration-time profile was observed 6 h after oral administration, which might indicate the occurrence of entero-hepatic circulation of OTA. Plasma protein binding was very high (97–99%) and the mean Vd (5–10 L/kg) and the clearance of OTA (0.3–0.4 L/h/kg) were lower compared to other poultry species. Similarly to the other poultry species evaluated in this study, TK parameters in turkey poults were also characterised by large inter-individual variation and no impact of sex could be demonstrated.

Ducks

Finally, Devreese et al. (2018) also evaluated the TK properties and plasma protein binding in eight 6-month-old Muscovy ducks (mean bw 2.68 kg). OTA in ducks was also characterised by a high oral absorption rate (94–104%). The mean C_{max} of 35–48 ng/mL was reached faster in ducks (0.31 h after administration) compared to other poultry species. In ducks, plasma protein binding was also high (82–89%), however lower compared to other poultry species. Consequently, the mean Vd was higher in ducks (28–29 L/kg) compared to other poultry species. Elimination of OTA in turkey poults was characterised by a low clearance (0.7 L/h/kg) and a long elimination half-life ($t_{1/2el}$) (17 h). Similarly, as in the other poultry species evaluated in this study, TK parameters in ducks were also characterised by large inter-individual variation and no impact of sex could be demonstrated.

In summary, the overall absorption of OTA in poultry is high, and OTA is extensively bound to serum proteins and widely distributed in different tissues, especially to the kidneys and liver. There is indication of entero-hepatic circulation in chickens and turkeys. Especially in turkeys a very high plasma protein binding and a lower Vd are observed, while in ducks a lower plasma protein binding and a higher Vd were demonstrated. OTA is metabolised via hydroxylation and dechlorination in chickens. Furthermore, clearance of OTA was lower in turkeys compared to the other poultry species. Elimination occurs via the bile and the excreta.

Pigs

In pigs, in the 2004 opinion (EFSA CONTAM Panel, 2004) an overall absorption of OTA of about 60% and a long serum half-life of 72–120 h were mentioned. It was indicated that also in pigs OTA was mainly eliminated via urinary and biliary excretion. Furthermore, placental transfer to piglets was suggested by one study, but could not be confirmed by other available studies. The metabolism and rate of excretion of OTA might be influenced by the presence of other xenobiotics (including other mycotoxins and veterinary drugs) by interfering with the OTA serum protein binding. In addition, an extensive plasma protein binding of more than 99% was shown and an elimination half-life of 6 days was reported in pigs.

Since the previous EFSA assessments, different new studies mainly focussed on the tissue distribution and excretion of OTA and the correlations between OTA levels in different tissues,

biological fluids and contaminated feed with respect to food safety assessment and the validation of possible biomarkers of exposure.

In vitro studies

In vitro analysis was performed with liver microsomes isolated from six Changbai swines, mean bw 20–25 kg, three males and three females. Different phase I metabolisation products were identified including five hydroxylated metabolites, i.e. 4S-OH-OTA, 4R-OH-OTA, 7'-OH-OTA, 9'-OH-OTA, 5'-OH-OTA and one dechlorination product of OTA (OTB), with similar levels of 4S-OH-OTA as 4R-OH-OTA (Yang et al., 2015).

In vivo studies

In the bile of pigs fed a naturally OTA contaminated diet (100 μg/kg) Kühn et al. (1995) demonstrated the occurrence of OTA glucuronide conjugates following enzymatic deconjugation with β-glucuronidase before extraction of the sample. In the three analysed samples in this study, 17–57% of total OTA content was contributed by OTA glucuronide conjugates. No further metabolites were investigated.

A positive correlation was demonstrated between the OTA concentration in plasma and levels in kidney ($R^2 = 0.81$) and liver ($R^2 = 0.61$) after four consecutive weeks of exposure of 6-week-old castrated male (n = 12) and female (n = 12) crossbred piglets (P76 × Naima dam) (Aoudia et al., 2009). In this study, OTA contaminated pelleted feed was prepared by mixing in contaminated wheat, at a final confirmed contamination level of 120 μg OTA/kg feed. The average plasma OTA concentration was 68 ng/mL and 83 ng/mL, after 2 and 4 weeks of exposure, respectively. In this study the average concentration of OTA in the kidney (13 ng/g) was higher than in liver (1 ng/g) after 4 weeks of exposure, with 0.15% and 0.07% of the total ingested OTA being recovered in the kidneys and liver, respectively.

A similar positive linear correlation (0.81) was observed between the OTA serum and kidney levels in wild boars (n = 101) in Poland (Grajewski et al., 2012). Following a single ingested dose of 66 μg OTA/kg bw Blank and Wolffram (2005) reported a daily urinary excretion of 5.5% of the ingested OTA.

Pleadin et al. (2016) and Altafini et al. (2017), both, analysed the levels of OTA in different tissues and biological fluids after feeding piglets with OTA contaminated feed. Pleadin et al. (2016) fed five female piglets (hybrid Zeger type) for 4 weeks of the fattening period with a diet contaminated with 250 μg OTA/kg feed. Altafini et al. (2017) administered four hybrid piglets (two males and two females) either 50 μg OTA/kg feed or 500 μg OTA/kg feed for 15 days. In both studies, pure OTA crystalline standard was used to artificially contaminate the feed. In both studies the highest OTA concentrations were found in kidneys and lungs, followed by liver and bile, heart, spleen, muscle tissue, fat and brain. Altafini et al. (2017) observed a direct relation between the dietary OTA and OTA concentrations in the different tissues, particularly evident in plasma, lung, heart and muscle and the least in kidney. Levels of OTA in bile were quite close to those observed in kidneys and liver. Pleadin et al. (2016) observed significantly higher mean OTA levels in urine (16 μg/L) compared to serum (5 μg/L).

Tkaczyk et al. (2021) administered a multi-mycotoxin contaminated diet for 14 days to 24 pigs (Polish Landrace × Polish Large White crossbreeds, mean bw 28.5 kg). A control diet was compared with diets containing 0.114 and 0.226 mg OTA/kg feed respectively. A strong correlation between OTA dietary levels and levels of OTA and OTalpha in urine was found. OTalpha levels in pig urine were on average two-times lower than OTA levels.

In summary in pigs, OTA is characterised by a high serum protein binding and is distributed mainly to kidneys, lung and liver, with levels detected in these different tissues correlating directly with the dietary contamination level. OTA underwent metabolism via three different major pathways, namely hydroxylation, dechlorination and glucuronidation (demonstrated only *in vivo*). OTalpha was found in urine, indicating hydrolysis of the peptidic bond of OTA. Urine and bile are the major routes of excretion of OTA.

Rabbits

The only available TK study in rabbits is the one of Galtier et al. (1981), where a bolus of 2 mg crystalline OTA/kg bw was administered orally or intravenously to male rabbits (Fauve de Bourgogne, n = 6, bw = 2–3 kg). Plasma OTA level was measured by spectrofluorometric analysis. Based on the relative comparison of AUC following oral versus i.v. administration, the bioavailability of orally administered OTA was 56%. The maximum plasma concentration after oral administration was already

observed after 1 h, while only after 10 h in pigs. The absorption rate constant was 11 times higher in the rabbit (k_a = 10.890 h^{-1}) than in pigs (k_a = 0.995 h^{-1}). Vd was approximately 10 times larger compared to pigs, but five times smaller compared to layer chickens. Ochratoxin A was eliminated approximately 109 times faster in rabbits compared to pigs, but nine times slower compared to layer chickens. Transfer to milk of rabbit after a single intravenous administration based on the milk/plasma concentration ratio was 0.1–0.2. However, Ferrufino-Guardia et al. (2000) observed a much lower milk/plasma ratio of only 0.015 upon feeding lactating rabbits with naturally contaminated diet (10–20 µg OTA/kg bw per day). Besides the conventional metabolites as described in the introduction part of this section, *in vitro* studies with rabbit liver and kidney microsomes have demonstrated the formation of the phase I metabolite 10-hydroxyochtratoxin A (10-OH OTA) (Stormer et al., 1983; Xiao et al., 1996; Zepnik et al., 2001).

In summary, based on a limited dataset, OTA in rabbits is characterised by a fast oral absorption. In rabbits OTA is also widely distributed in organs and tissues. Only limited *in vitro* data is available about the biotransformation, indicating hydroxylation and dechlorination.

Fish

The 2004 opinion (EFSA CONTAM Panel, 2004) reported limited data on the toxicokinetics of OTA in fish. The TK profile of OTA following oral or i.v. administration of 0.05 µg/g bw in carp was investigated in carp (*Cyprynus carpio*).

A serum half-life of 0.68 h after oral dosing and 8.3 h after i.v. administration was observed (Hagelberg et al., 1989). In contrast to other animal species, a low bioavailability could be demonstrated (1.6%) and a high fraction (22%) of OTA in blood remained unbound in fish.

In rainbow trout i.v. injected with ^{14}C-OTA (Fuchs et al., 1986), radioactivity was mainly due to the toxin and mostly found in kidney, urine and bile, with negligible values in muscles.

Bernhoft et al. (2017) studied tissue distribution and elimination of OTA in the Atlantic salmon (*Salmo salar*). Groups of 2 (tanks) × 25 juvenile post-smolt salmons (12 months old, both genders, 58 g bw) were given diets (48% crude protein (CP), 25% crude fat (CF)) with no added OTA, with 0.8 mg and 2.4 mg pure OTA/kg, respectively, for 8 weeks. The analysed levels in feed were below LOQ (0.015 µg/kg), 0.72 and 2.00 mg OTA/kg, respectively. OTA concentrations in plasma, liver and muscle were measured on 10 fish each per group after 3, 6 and 8 weeks. After 8 weeks, also concentrations in kidneys and skin were measured. For studying OTA elimination, plasma, liver, kidney, muscle and brain samples were collected from the high-dose group at 2, 4, 8, 18 and 48 h after last feeding on three fish each per sample.

The main results are given in Table 2. Final body weight of the fish did not differ. OTA was mainly found in liver and kidney. OTA concentrations in liver of both dose groups decreased during the study and significantly for the high OTA group; the levels after 8 weeks in both OTA groups were about 50% of the level at week 3. Liver and kidney concentrations of OTA were significantly correlated (r = 0.82). The concentrations in muscle and brain were all < LOQ (0.09 µg/kg).

Table 2: Plasma and tissue TK parameters in Atlantic salmons after 8 week-exposure to OTA

OTA mg/kg feed	µg OTA/kg tissue			C_{max} (µg/kg)		t_{max} (h)		$t_{1/2\lambda1}$ (h)	
	Liver	**Kidney**	**Skin**	**Plasma**	**Liver**	**Plasma**	**Liver**	**Plasma**	**Liver**
0.8	1.01	0.16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2.4	2.61	1.03	< LOQ	0.3	6.1	2	4	1.2	1.4

LOQ: 0.09 µg/kg tissue, 0.004 µg/kg plasma; n.s.: not sampled.

The maximum OTA concentration in liver and kidney was reached after 4 h, it subsequently decreased in liver bi-exponentially with $t_{1/2\lambda1}$ = 1.4 h and $t_{1/2\lambda2}$ = 10.2 h. Elimination half time for OTA in kidneys was 4.7 h. In both organs, OTA increased slightly between 18 and 48 h after last feeding, indicating redistribution between two or more body compartments. As in liver, OTA concentrations in plasma decreased bi-exponentially ($t_{1/2\lambda1}$ = 1.2 h and $t_{1/2\lambda2}$ = 19.8 h). OTA in muscle peaked twice at comparably low concentrations, at 4 h and at 18 h (t_{max}), C_{max} was of 0.4 µg/kg.

In summary OTA in salmon has a rather low bioavailability and is rapidly eliminated with negligible concentrations in tissues.

Table D.1 within Appendix D summarises the TK parameters for the various animal species.

Summary

OTA is rapidly and extensively absorbed in the gastro-intestinal tract in all animal species studied. In general OTA is also characterised by a strong plasma protein binding. However, inter-species differences have been observed; with sheep having a lower plasma protein binding compared to other ruminants, turkeys having the highest plasma protein binding of different poultry species. Also donkeys are characterised by a lower plasma protein binding compared to pigs. In contrast to other animal species, a very low oral bioavailability and lower plasma protein binding has been observed in fish.

As a consequence of the inter-species differences in plasma protein binding as well as in the volume of distribution and elimination half-life have been demonstrated with a relative short elimination half-life in sheep and donkeys. Based on the plasma/serum concentration versus time curves of cattle, chicken for fattening, layer chickens and turkeys entero-hepatic circulation of OTA is suggested.

In cattle and sheep it has been suggested that ruminal protozoa and gastro-intestinal bacterial community play a major role in the extensive hydrolysis of OTA into OTalpha. This hydrolytic capacity of forestomach compartments to degrade OTA is influenced by diet (ratio concentrate:forage) and the ruminal pH. In different *in vivo* studies in ruminants, OTalpha has been identified as the major metabolite. Also in pigs OTalpha has been detected in urine of pigs at low amount. *In vitro* data showed that OTA in chickens, swine, goat and cows are metabolised via hydroxylation and dechlorination. Hydroxylation and dechlorination was only demonstrated *in vivo* in laying hens. Additionally, *in vivo* glucuronidation was demonstrated in pigs.

Finally, OTA is mainly excreted via urine and faeces in all animal species. In cattle, sheep, donkeys and rabbits transfer of low levels via milk has been described. In poultry, OTA is also transferred to eggs. In horses placental transfer was reported in mares exposed to OTA, while this was not observed in donkeys.

3.1.2. Transfer from feed to food of animal origin

Transfer to product of animal origin has been described in the Opinions from 2004 to 2020 and is summarised below.

As described in Section 1.2.3, the data used in the 2004 Opinion showed that residues could be found in slaughtered pigs in kidney, liver, muscle tissue and fat after exposure to OTA. In chicken OTA was found in liver and kidney and at low levels in other tissues. Neither for pigs nor for chickens there was a clear relationship between dose and concentration in tissues. It was estimated that 0.11% of OTA in feed was transferred to eggs. It was shown that OTA could be transferred to milk from monogastric animals, but low concentrations had also been found in milk from ruminants.

Transfer to food of animal origin was also described in the 2020 Opinion and in more details than in the 2004 Opinion but the conclusions in the two Opinions are very similar. Besides, there was also a comprehensive chapter on occurrence in the literature in the 2020 Opinion. Below is a summary of the transfer from feed to food of animal origin as included in the 2020 Opinion.

In monogastric species such as pigs, pre-ruminant calves and rabbits, OTA may accumulate in the meat and organs due to high bioavailability, limited conversion rate into OTalpha and long half-life in such animals.

Poultry species appear to eliminate OTA faster than monogastric mammalian species, resulting in a low OTA accumulation in tissues and blood. It also seems that transfer of OTA into eggs appears to occur only when OTA intake is very high. For example, no OTA could be detected in eggs when hens were fed diets containing 0.3, 1 or 2 mg OTA/kg of feed, but OTA was found in eggs of laying hens fed OTA at 10 mg/kg bw.

Ruminants such as cows, sheep and goats are, in contrast to monogastric animals, capable of degrading OTA to the virtually non-toxic OTalpha in their rumen; consequently, only a small amount of intact OTA is available for tissue distribution and milk excretion. The ability to detoxify OTA is strictly related to a functional rumen and may change when the feed composition is altered; for example, a high proportion of protein-rich concentrates in feed which may depress the hydrolytic capacity of rumen microorganisms allowing a higher amount of OTA to enter the systemic circulation. Under normal conditions, transfer into cow milk has been shown to be low. Analysis of commercially available milk samples in Italy showed that only 3 out of 83 samples contained OTA at levels between 70 and 110 ng/L, with an LOQ of 5 ng/L. Out of 132 French milk samples, only three were found to contain OTA levels above the LOQ of 5 ng/L and it has been estimated that 0.01% of the OTA in cattle feed would be transferred into cow milk. The low transfer to milk was confirmed in a study where lactating

Holstein cows were fed a diet containing 100 μg OTA per kg of dry matter for 28 days and no OTA was detected in the milk or in liver, kidney, muscle or fat with an LOQ of 0.1 μg/kg. For dairy ewes, also a very low transfer rate (< 0.02%) for OTA from feed into milk was determined.

The occurrence of OTA was shown in commercial samples of blue-mould ripened cheeses at levels ranging from 0.25 to 3.0 μg/kg and provided circumstantial evidence that the OTA does not originate from a contamination of the milk but is formed during ripening and storage of the cheese. OTA was also detected in the rind (1–262 μg/kg) and interior (18–146 μg/kg) of traditional handmade semi-hard cheeses.

For fish it was concluded that the oral bioavailability of OTA appears to be low, and the toxin distributes mainly to liver and kidney. Only trace amounts were transferred to edible tissues, i.e. muscle, making fish a negligible source of exposure.

In the 2020 Opinion occurrence data from Member States were included and used for exposure assessment. These data show that most of the samples in the group of Meat and Meat products (FoodEx level 1) were left censored and for subgroups (FoodEx level 3) as rabbit, meat, beef, pork and goat meat as well as sheep liver all samples were left censored. At FoodEx level 3 the group 'Ham, pork' has the lowest % of left-censored data and the highest concentrations of OTA between 3.0 and 3.1 μg/kg (mean LB-mean UB). It should be noted that contamination of processed animal products not only can originate from OTA in feed but also can be formed during the process, e.g. due to environmental contamination and subsequent mycelia growth on ham surfaces It has been shown that OTA can be produced by *Penicillium nordicum*, which can grow on pork meat during ripening (EFSA CONTAM Panel, 2020).

For unprocessed meat and meat products the food group at FoodEx level 3 with the highest concentrations was the group 'Chicken meat' with mean LB and mean UB of respectively 0.12 μg/kg and 1.8 μg/kg (98% left-censored data).

In the literature OTA was also found in ripened cheese (73% left-censored) at concentrations of 2.2–2.9 μg/kg (mean LB-mean UB). No OTA was found in soft cheese. Altogether, it was concluded that the occurrence in cheese is very likely due to environmental contamination during the processing (EFSA CONTAM Panel, 2020).

It was concluded that the residues found in the open literature were similar to the data submitted to EFSA from Member States.

Concentrations of OTA in processed foods such as sausages may also originate from OTA containing animal organs and blood that can be used as ingredients in the production of processed foods. Some relevant studies have been retrieved from the literature that were not included in the previous Opinions. In the study by Nip and Chu (1979; see also Section 3.1.1) the transfer from feed to milk from goats was found to be very low (< 0.03%) as for cows.

No residues were found in eggs (LOQ = 0.15 μg OTA/kg) from hens fed with feed containing 2 mg OTA/kg for 3 weeks (Denli et al., 2008). Hassan et al. (2012a), (see also Section 3.1.4) exposed hens to diets contaminated with OTA of 0, 0.1, 0.5, 1.0, 3.0, 5.0 or 10.0 mg/kg feed for a period of 21 days. Laying hens were inseminated using artificial insemination. OTA levels were assessed in six eggs on alternate days as well as after the withdrawal of OTA. No OTA was found in the dosing group of 0.1 or 0.5 mg/kg. For the other dosing groups, the mean concentrations increased with increasing dose rate and the highest means varied between 3.65 mg OTA/kg (1.0 mg/kg; Day 17) and 17.86 mg OTA/kg (10.0 mg; Day 7). After withdrawal of OTA the levels decreased to non-detectable after 24–28 days.

Pozzo et al. (2013b, see also Section 3.1.4) determined the residues in tissues from chickens that were feed for 35 days with a diet with 0.1 mg OTA/kg added. At the end of the study the mean levels in kidney and liver were respectively 3.6 μg OTA/kg and 1.9 μg OTA/kg while no OTA was found in breast or thigh (LOD = 2 μg/kg). In the study by Armorini et al. (2015) (see also Section 3.1.1) laying hens were fed for 6 weeks with a diet contaminated with 0.01 mg OTA/kg or 0.2 mg OTA/kg. In kidney, respectively, 2 and 12 samples out of 15 samples were positive for the presence of OTA with mean concentrations of the positives of 0.45 μg OTA/kg and 1.04 μg OTA/kg for the two dosing groups respectively. In liver, respectively, none and one sample (0.47 μg OTA/kg) were positive for each group.

Pleadin et al. (2016) determined the residues in pig tissues after feeding for 28 days with OTA-fortified feed (250 μg OTA/kg of feed). The mean residues in the tissues were (in μg/kg): 13.87 in kidney, 7.28 in liver, 4.72 in muscle and 4.11 in fatty tissues.

These studies confirm the conclusions from the previous Opinion that there is a low transfer of OTA from feed to milk in ruminants as well as to poultry and eggs while the transfer in pigs is larger.

Since the previous Opinion, only one new study in donkeys has been retrieved besides two reviews on transfer (Ganesan et al. (2021), and Tolosa et al. (2021)). The study in donkeys showed that transfer of OTA to milk can take place (Lippolis et al., 2020; see also Section 3.1.1).

For all other animal species no information is available about transfer from feed to food of animal origin.

Animal products used as feed materials in animal nutrition could contain OTA (from the transfer from feed to organs/tissues). Of certain relevance are kidney and blood which could contribute, in combination with naturally contaminated plant feed materials, to the exposure of animals, particularly carnivore species; nevertheless, this contribution is likely to be low, based on the limited use of these feed materials.

3.1.3. Adverse effects and mode of action

The adverse effects and modes of action of OTA have been described in detail in the recent CONTAM opinion of OTA in food (EFSA CONTAM Panel, 2020). A few reviews have been published since then (Awuchi et al., 2021; Ganesan et al., 2022; Liu et al., 2022) overall confirming the existing wealth of knowledge on the toxin. In summary, the toxin accumulates in the kidney, which is confirmed as OTA target in most species, with alterations in structure and functions and the development of tumours in rats and mice. Although OTA genotoxicity has been reported both *in vitro* and *in vivo*, the mechanisms of mutagenicity and chromosomal damage are unclear. Contributing factors may include oxidative DNA damage, potential DNA adducts, unresolved 'replication stress' and damage to the mitotic spindle (EFSA CONTAM Panel, 2020). Both direct and indirect genotoxic and non-genotoxic modes of action might each contribute to tumour formation. The reproductive functions (offspring alterations, sperm cell abnormalities) and the immune system are also negatively affected by OTA exposure. Recent research highlights a negative effect of OTA on gut integrity and microbiota especially in poultry (e.g. for ducks see Wang et al., 2019), with dysbiosis, depression of the immune function, increase in permeability and pathogen translocation (for a review, see Zhai et al., 2021).

At cellular level, inhibition of protein synthesis and ATP production, induction of apoptosis and autophagy, activation of multiple signalling pathways (e.g. ELK, ERK, MAPK, p38, JNK, caspases, etc.), alteration of gene expression, particularly the Nrf2 pathway triggering oxidative stress are among the molecular mechanisms reported to be involved in OTA-mediated cell damage.

3.1.4. Effects in food producing and non-food producing animals

In the 2004 EFSA Opinion, it has been concluded the following:

- Pigs are generally considered to be the most sensitive farm animal species to the nephrotoxicity of ochratoxin A.
- Chickens are also sensitive species, and it is assumed that ochratoxin A is the most important cause of poultry nephropathy.
- Ruminants are less sensitive than monogastric species. Herbivores such as horses, rabbits and related species are likely to be more sensitive than ruminants,
- Other monogastric animal species including dogs, cats and fish are expected to be sensitive to renal toxicity and immunosuppressive effects, as these have been observed in all species tested so far.

In the current chapter, the results of the most recent publications on OTA related to adverse effects identified in the ELS, together with some of the studies already included in the 2004 Opinion, are reviewed and summarised. The studies are presented according to the animal species, categories and year of publication.

3.1.4.1. Pigs

In the 2004 EFSA Opinion, a no observed adverse effect level (NOAEL) could not be identified for pigs, while a LOEL (0.2 mg/kg feed) was established based on effects on renal (diagnostic) enzyme levels and kidney function. The results of 10 recent papers (eight in weaned piglets and two in growing pigs) are summarised in Table 3. The studies are presented according to increasing OTA doses.

Four studies aimed at assessing the effects of OTA at a low dose of 0.05 mg/kg feed in weaned piglets. Marin et al. (2016) investigated the effects of OTA on blood chemistry and liver metabolism in weaned piglets. Twelve weaned piglets were fed for 33 days a maize-soybean-meal based feed

contaminated or not with 0.05 mg OTA/kg complete feed (confirmed by analysis). Exposure to OTA resulted in a significant decrease in the concentrations of total protein, albumin and nitric oxide in plasma, and interleukin-6 in the liver. OTA exposure also resulted in a significant increase of alanine aminotransferase and triglycerides in plasma and of superoxide dismutase in the liver. Marin et al. (2017, 2018) investigated the OTA effects on inflammation and oxidative stress parameters (glutathione peroxidase, catalase and superoxide dismutase, total antioxidant capacity (TAC), cytokine synthesis) in gut and kidney in respectively 12 and 10 weaned piglets fed for 30 days a maize-soybean-meal based feed contaminated or not with 0.05 mg/kg OTA (confirmed by analysis). Exposure to OTA resulted in a significant decrease of IL-6 synthesis (mRNA and protein expression) in colon, IL-4 in duodenum, IL-10 in colon, tendency of IL-1beta and IL-6 decrease in kidney (Marin et al., 2017). Marin et al. (2018) observed impacts of OTA on kidney functions by a significant increase of serum creatinine.

Marin et al. (2019) assessed the effects of OTA on blood chemistry and microRNA profiling in kidneys of weaned piglets. Fifteen weaned piglets were fed for 28 days a maize-soybean-based meal contaminated or not with 0.05 or 0.2 mg OTA/kg feed (confirmed by analysis). At the highest dose 0.2 mg OTA/kg feed, significant effects were observed in serum creatinine and in renal parenchyma architecture, no significant changes were observed at 0.05 mg OTA/kg feed. Bernardini et al. (2014) evaluated the effects of an OTA (0.181 ± 0.034 mg/kg feed, confirmed by analysis) contaminated diet on growth performances, blood parameters, systemic cytokine levels, cell oxidative stress markers and reactivity of immune system of 30 weaned piglets for 42 days compared to 30 control piglets non-exposed to OTA. No effects were seen on the performance parameters of the animals, although OTA diminished the protein content in the serum and increased levels of TNF-alpha and IL-10 in plasma. *HO-1* mRNA, indicative for cell oxidative stress, was decreased in the kidney but increased in the liver. Gan et al. (2017) also studied the effects of OTA in feed of nine piglets at either 0.4 mg/kg or 0.8 mg/kg (not confirmed by analysis) for 42 days. When compared to control animals, histopathological lesions of kidney (e.g. hyperchromatic nuclei, nuclear atrophy, etc.) and spleen (e.g. hyperchromatic nuclei, lymphocyte depletion, etc.) were observed at both OTA doses. Impairment of zootechnical performances (e.g. body weight gain, feed/gain ratio) was also observed at both doses. Raja et al. (2008) have determined the effects of a high dose of OTA (2.5 mg/kg feed, confirmed by analysis) given for 84 days to weaned piglets (5). In contaminated animals, serum total protein and albumin were increased, creatinine, urea, ALT, AST and ALP were increased, and metastatic calcification of kidneys, liver, intestine and myocardium was noted. Aukema et al. (2004) also assessed the effects of two high doses of OTA (2 and 4 mg/kg feed, confirmed by analysis) for 28 days in weaned piglets. Both doses resulted in significant renal fibrosis and increased concentration in serum creatinine.

In the growing pig, Malagutti et al. (2005) reported a long-term study to assess the effect of a low OTA level on zootechnical parameters and OTA concentrations in meat. Sixty-four growing pigs were fed for 119 days a maize-soybean based meal contaminated or not with 0.025 mg OTA/kg feed. At the end of the experiment, OTA exposed animals had significantly lower body weight (163.4 vs. 170.9 kg), daily weight gain (1,030 vs. 1,094 g/d) and increased feed to gain ratio (FGR) (3.77 vs. 3.53). A second study was performed in growing pigs to assess the OTA effects on serum biochemistry (Pleadin et al., 2012). Groups of four growing pigs were fed for 30 days a maize-soybean-meal diet either contaminated with 0.3 mg OTA/kg feed (not confirmed by analysis) or untreated. The exposure to OTA resulted in a significantly higher serum creatinine, urea, potassium and alkaline phosphatase levels and lower glucose and total protein levels.

Summary on pigs

Overall, the studies in piglets indicate that OTA in concentrations from 0.2 mg/kg feed when given for a 4-week period impairs function and structure of kidneys and liver. The lowest level tested (0.05 mg/kg feed) may already impact physiological function of kidneys and liver of piglets. In growing pigs, a long-term exposure (119 days) to 0.025 mg OTA/kg feed affected the growing performance.

The CONTAM Panel considers the concentration of 0.025 mg OTA/kg feed for pigs as a lowest observed adverse effect level (LOAEL).

The CONTAM Panel considers 0.01 mg OTA/kg feed as reference point (RP) for adverse animal health effect for pigs, derived by applying an UF of 3 to the LOAEL[17] and rounding from 0.008 to 0.01.

---

[17] The use of an UF of 3 is in line with EFSA's 'Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data' (EFSA Scientific Committee, 2012) and ECHA's 'Guidance on information requirements and chemical safety assessment Chapter R.8' (ECHA, 2012).

Table 3: New studies on adverse effects on pigs which have become available since the 2004 Opinion (EFSA CONTAM Panel, 2004)

N/group, breed gender	Dosage (mg/kg feed) and duration	Endpoint(s)	Result(s)	Lowest effect concentration (mg/kg feed)	Reference
12 TOPIGS-40 crossbred weaned 4-week-old, females, piglets, 2 groups	0.05 for 33 days	Blood chemistry and liver metabolism	Decrease in total protein concentration, albumin and nitric oxide in plasma, interleukin-6 in the liver. Increase of alanine aminotransferase and triglycerides in plasma and of superoxide dismutase in the liver.	Effects at 0.05	Marin et al. (2016)
12 TOPIGS-40, crossbred piglets, 4-week-old, females, 2 groups	0.05 for 30 days	Inflammation and oxidative stress parameters (glutathione peroxidase, catalase and superoxide dismutase, (TAC), NO) cytokines in gut and kidney	Decrease of IL-6 and IL-10 synthesis in colon, IL-4 synthesis in duodenum, IL-1beta and IL-6 in kidney. Increase of serum creatinine.	Effects at 0.05	Marin et al. (2017, 2018)
15 crossbred TOPIG hybrid [(Landrace × Large White) × (Duroc × Pietrain)] pigs, 3 groups, 5 pigs per group	0.05 or 0.2 for 28 days	Blood chemistry and microRNA profiling in kidneys	Increase in serum creatinine and modification of the renal parenchyma architecture	Effects at 0.2	Marin et al. (2019)
60 castrated male weaned pigs (Large-White), 2 groups	0.181 ± 0.034 or 0.00045 ± 0.00005 for 42 days (Control group)	Growth performances, blood parameters, systemic cytokine levels, cell stress markers and reactivity of immune system	No effects were seen on the performance parameters of the animals. Decrease in serum protein content. Increase in of TNF-alpha and IL-10 levels in plasma	Effects at 0.181 ± 0.034	Bernardini et al. (2014)
27 crossbred [(Landrace × Yorkshire) × Duroc] 6-weeks old pigs, 3 groups	0.4 or 0.8 for 42 days. (no indication on OTA analysis in feed)	Endoplasmic reticulum (ER) stress, MAPK signalling pathway and autophagy in kidney and spleen	Histopathological lesions of kidney and spleen. Effects on zootechnical performances	Effects at 0.4	Gan et al. (2017)
10 crossbred (Landrace × local non-descript) 10 weeks old piglets, 2 groups	2.5 for 84 days	Clinico-pathological and pathomorphological changes were examined in piglets exposed to OTA	Increased haemoglobin concentration, decrease in PCV, leukocytosis, lymphocytosis and monocytosis Decrease serum total protein, albumin, globulin Increased Creatinine, urea, ALT, AST and ALP. Metastatic calcification of kidneys, liver, intestine and myocardium	2.5	Raja et al. (2008)

N/group, breed gender	Dosage (mg/kg feed) and duration	Endpoint(s)	Result(s)	Lowest effect concentration (mg/kg feed)	Reference
24 pigs (12 male, 12 female), weaned at 18 days of age, 3 groups	2 and 4 for 42 days	Alteration of phosphoinositide signalling pathway in connection with induced renal injury	Renal fibrosis and increased concentration in serum creatinine	Effects at 2	Aukema et al. (2004)
64 (Large White × Landrace) growing pigs, around 41 kg), 2 groups of 32, housed in pens holding 4 animals	0.025 for 119 days	Examine the effect of a low OTA level on zootechnical parameters and OTA concentrations in meat	OTA exposed animals had significantly lower body weight (163.4 vs. 170.9 kg; −4%), daily gain (1,030 vs. 1094 g/day; −6%) and feed efficiency (−7%)	0.025	Malagutti et al. (2005)
8 pigs, male, Zegers hybrid type, 90 days old, 2 groups	0.3 (not confirmed by analysis) for 30 days	OTA effects on serum biochemistry in growing pigs	Exposure to OTA resulted in a significantly higher serum creatinine, urea, potassium and alkaline phosphatase levels and lower glucose and total protein levels	0.3	Pleadin et al. (2012)

3.1.4.2. Poultry

The CONTAM Panel concluded in the opinion on Ochratoxin A in 2004, 'Chickens are also sensitive species, and it is assumed that ochratoxin A is the most important cause of poultry nephropathy. Quantitative dose response data, however, allowing establishment of a NOEL or LOEL are not available. Immunotoxicity was observed at 0.5 mg/kg complete diet'.

A total of 37 studies was reviewed. Three studies identified in the literature search were not further considered due to weaknesses in reporting (Rao et al., 2018; Solcan et al., 2013b) and in design (Hameed et al., 2012). In particular, in Rao et al. (2018), controversial description of experimental groups was identified, no statistics, no data on mortality; in Solcan et al. (2013b) no statistics are reported, no data on zootechnical performance, mortality or symptoms of toxicity were provided; in Hameed et al. (2012), no replicates were used and unclear data on the number of euthanised animals was reported. Adverse effects were described in growing chickens in 17 publications, in hens in four, in turkeys in four and in ducks in two studies. Another four studies investigated in-ovo effects and three described OTA effects on gene expression, but these are not included in the Opinion as they could not be used to establish a RP.

Growing Chicken

Kumar et al. (2004) evaluated the effects of ochratoxin A (OTA) on *Escherichia coli*-challenged chickens for fattening. One hundred and eighty-four 1-day-old broiler chicks were divided into two groups of 92 chicks each, with one group fed a control mash diet and the other fed a mash diet containing 2 mg OTA/kg (by adding a seed culture containing 80 mg OTA/kg). On day 14, each group was further subdivided into two groups with one group inoculated with *E. coli* O78 ($1/10^7$ colony-forming units/0.5 mL) by the intraperitoneal route, whereas the other group was not inoculated with *E. coli*. Four birds from each group were sacrificed at 1, 2, 3, 5, 7, 10, 14 and 21 days post-inoculation to record pathological changes in liver, kidneys, heart, lungs, bursa, spleen and thymus. Ochratoxin contaminated diets induced changes in kidneys (swollen proximal convoluted tubules, degeneration of tubular epithelium and interstitial nephritis) and liver (degeneration and mononuclear cell infiltration) from 10 DPI onwards in chicks fed OTA alone and those infected with *E. coli*. There was atrophy of the lymphoid organs along with depletion of lymphocytes. Gross and histopathological changes were more severe in chickens exposed to OTA and inoculated with *E. coli* than the chickens exposed to OTA alone or those infected with *E. coli*, indicating combined action of these two. The authors concluded that the immunosuppressive action of OTA favours secondary infections.

Elaroussi et al. (2006) fed groups of 80 one-day-old chickens (Ross) diets contaminated with 0, 0.4 and 0.8 mg OTA/kg (by addition of an ethanolic extract from a seed culture) for 5 weeks. Blood and

organ samples were taken from eight birds per group. Immune response parameters were measured, the sheep red blood cells (SRBC) test as an indicator for humoral immune response (five 19-day-old chickens/group) and the cutaneous delayed hypersensitivity test with phytohemagglutinin for cell-mediated immune response (ten 37-day-old chickens). Mortality amounted to 1, 3 and 7 birds (out of 80), respectively, for the three groups. OTA contaminated diets resulted in decreases of body weight, feed consumption, FGR (all dose dependent) and of relative thymus weight. However, relative gizzard weight increased. The serum concentrations of thyroid hormones T3 and T4 were dose dependently affected, T3 increased and T4 decreased. Haematology showed a significant and dose-dependent decrease of RBC, WBC, packed cell volume (PCV) and haemoglobin. Humoral immune response and cell-mediated immunity was depressed in both groups fed ochratoxin compared with the control group.

Elaroussi et al. (2008) fed groups of 80 one-day-old chickens (Ross) diets contaminated with 0, 0.4 and 0.8 mg OTA/kg (by addition of an ethanolic extract from a seed culture) for 5 weeks. OTA at both levels resulted in significant increase of relative weights of kidney, liver, of the concentrations of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), uric acid, creatinine in blood. Significant decrease was found for bursa Fabricius weight, serum Ab titres against Newcastle disease virus, total serum proteins, albumin, globulin. Histology showed marked degenerative changes in kidney and bursa and mononuclear cell infiltration in liver. The authors suggested that OTA reduces immunity, decreases the response to vaccination and thereby increases susceptibility to infections.

Stoev (2010) evaluated the possible long-term toxic or carcinogenic effects of OTA exposure. Twenty 14-day-old specific pathogen free chicks (Plymouth Rock, a dual-purpose breed) were allocated to three groups with five male and five female chickens each. A control group received feed without added OTA, feed for the OTA group was supplemented with 5 mg OTA/kg (from a seed culture containing about 2 mg OTA/g). All chicks were examined for the occurrence of various neoplastic tissues at the end of the 2 years experimental period. After 10 experimental months, one male chick of the OTA group was dead. Several large grey-white neoplastic spots were seen in the diaphragmatic and abdominal surfaces of the liver in the same chick, which were subsequently diagnosed as adenocarcinoma. After 18 and 20 months, two other males of the same group died with diagnosed lymphosarcoma in the kidney and carcinoma in the ureters, respectively. At study end, benign cystic adenomas were found in the kidneys of one male and one female. Gross pathology examination at the end of the 2-year experimental period revealed that all chicks from OTA-treated group were emaciated and their bones were sometimes thin or soft. Pathomorphological investigation of kidneys showed degenerative changes (granular or hydropic degeneration, karyomegaly, karyopyknosis or karyorrhexis) in epithelial cells of the proximal convoluted tubules, cystiform dilatation of the lumen of some tubules, tubular atrophy in other tubules, mononuclear proliferation in the mucosa of ureters and moderate proliferation of connective tissue and mononuclear cells in the renal interstitium, were seen in chicks of OTA-treated group. The author considered the neoplasms (tumours) and pathological changes observed in experimental chicks to be due to OTA exposure as no such pathological changes or neoplasms were observed in the control group. OTA was found to provoke strong degenerative changes in liver and kidneys, degenerative changes and depletion of cells in lymphoid organs, oedematous and degenerative changes in the brain, muscular haemorrhages and fatty changes in the bone marrow.

Sakthivelan and Sudhakar Rao (2010) investigated the influence of OTA contaminated diets on the zootechnical performances of chickens. Sixty chickens for fattening (one-day-old Vencobb chicks, India) were randomly divided into six replicates of 10 chicks each and exposed to diets containing 0, 1 and 2 mg added OTA/kg, respectively, for 28 days. A seed culture was used as OTA source. Both OTA levels significantly depressed final body weight with a moderate effect on feed intake. Feeding OTA significantly reduced also total serum protein and albumin levels in chickens for fattening.

Some pathological responses of male White Leghorn chicks (a layer breed) to OTA contaminated diets were investigated by Hassan et al. (2012c). For this purpose, 350 one-day-old chicks were divided into five groups. One group was kept as control, while the other four groups were fed diets contaminated with 0.1, 0.5, 1.0 and 1.5 mg OTA/kg from a seed culture, respectively, for 21 days. The intended values were analytically confirmed. A significant decrease in the feed intake and body weight gain of the chicks was observed in OTA-treated groups (from 0.5 mg OTA/kg onwards). Clinical signs exhibited by the chicks already at the lowest dose tested included severe diarrhoea, dullness, depression, increased water intake and ruffled feathers. Gross pathological lesions on liver and kidneys included lighter in coloration, friable and haemorrhagic. A significant increase in the weight of liver and kidney was observed in OTA fed chicks, significant already at 0.1 mg OTA/kg. Histologically, liver and

kidneys of chicks showed degenerative and necrotic changes. Serum biochemical profile indicated a severe damage to liver and kidneys in OTA fed chicks.

The immunological responses of male White Leghorn chicks (a layer breed) to dietary OTA were investigated by Hassan et al. (2012b). For this purpose, 350 one-day-old chicks were divided into five groups. One group was kept as control, while the other four groups were fed diets contaminated with 0.1, 0.5, 1.0 and 1.5 mg added OTA/kg, respectively, for 21 days. OTA was obtained by acetonitrile-water extraction of a seed culture; the intended levels of the experimental diets were analytically confirmed. The basal ration was given for the consecutive 14 days. At 14- and 16-days of age, random chicks (n = 10) from each group were used for analyses of phagocytic function of the reticuloendothelial system or for measuring the lymphoproliferative responses to intradermally administered phytohemagglutinin-P (PHA-P). At 30-days of age, abdominal macrophages were collected from 15 chicks/group and utilised for determination of their phagocytic potential. Antibody titres (i.e. total antibodies, IgM and IgG) against SRBC were determined at 7 and 14 days of age after a primary and a booster (given 14 days after primary) dose of the antigen administered intravenously. Data from the present study showed that the relative weight of the bursa of Fabricius of chicks fed OTA for 14 and 21 days and the spleen of chicks fed OTA for 21 days were significantly lower than their control counterpart. Phagocytic function of reticuloendothelial system, evaluated by carbon clearance and lymphoproliferative response to PHA-P of chicks, were significantly lowered by OTA. Total antibody SRBC titres were depressed by OTA, as IgG, more markedly after booster application. The number of abdominal macrophages displaying phagocytosis of SRBC, the number of SRBC/macrophage and nitrite production were each significantly lower in cells from chicks of the OTA groups. The authors confirm the immunosuppressive effect of oral OTA regarding functional impairment in some of the components of the immune system.

Solcan et al. (2013a) evaluated the nephrotoxic effect of OTA in chickens for fattening. Forty Ross 308 chickens for fattening (6 days old) were divided into two groups: one group received daily, by gavage a daily dose of 50 µg OTA/kg body weight for up to 21 days (OTA purity was 99.5%), while the control group received only the diluent (sunflower oil). After 21 days, the chickens were euthanised and the kidneys removed for analysis by histopathology and immunohistochemistry to detect an anti-apoptotic marker (Bcl-2). Macroscopically the kidneys were enlarged, showed degeneration and gout deposits. Histologically, glomerulonephrosis and tubulonephrosis were common lesions in all chicks. In two of the five chicks exposed to OTA for 21 days, focal tubular cell proliferation, multiple adenoma-like structures and Bcl-2-positive epithelial cells were identified in layers of the renal papilla and in convoluted tubules. Transmission electron microscopy of the proximal convoluted tubules identified abnormal forms of mitochondria.

Nedeljković-Trailović et al. (2013) studied the influence of OTA on blood serum proteins in chickens for fattening. The 42-day study was performed on a total number of 48 Hybro-chickens for fattening divided into four groups. After the pre-experimental period of 14 days, three experimental groups of chickens for fattening (n = 12) were formed and fed diets 0.5, 1.0 and 1.5 mg added OTA/kg during the next seven consecutive days. A 99% pure OTA was used as OTA source. In the same period, a control group was fed a diet with no OTA added. After the period of toxin addition, blood samples were taken from six animals in each group. The remaining animals from all four groups were fed diets without OTA until the 42nd day of the study, when blood samples were taken again. The total level of blood serum proteins was affected by treatment with different doses of OTA. A slight however significant and dose-dependent increase of albumins was observed together with a decrease of globulin fraction. But the Albumin/Globulin (A/G) ratio increased suggesting that the globulins were the dominant protein fraction in blood serum. Significance was only reached at the intermediate and the high OTA level. The decline of the concentration of globulins as well as the increase in albumin in relation to the control was persistent, it was observed still after a 3-week withdrawal. The authors concluded that increasing dietary OTA levels (0.5, 1.0 and 1.5 mg/kg) have possibly dose-dependent cumulative effects on the blood serum protein status in chickens for fattening, and that the effect lasts even longer after OTA withdrawal.

Pozzo et al. (2013a,b) published a study, in which day-old male chickens for fattening were fed a control (basal) diet) and the same basal diet contaminated by 0.1 mg OTA/kg for 35 days. Dietary OTA concentration was analytically confirmed. Group size was 18. Growth and slaughter performance traits were recorded, haematology and routine blood chemistry assayed. In the 35-day study with a low number of chickens per group, 0.1 mg OTA/kg feed did not affect the animal performance, slaughter traits, organ weights, haematological parameters, liver enzymes or renal function. However, a decrease was observed for thymus weight, and serum concentrations of total protein, albumin and the globulins

in the OTA group. OTA did not affect lipid peroxidation or parameters indicating oxidative stress. Although no clinical sings of an intoxication were observed, feeding OTA contaminated diet resulted in moderate degenerative lesions in the liver, spleen and bursa of Fabricius.

Singh et al. (2015) studied the effect of increasing dietary OTA levels on performance of 200 chickens. Groups of four replicates with 10 one-day-old chickens for fattening each were fed for 6 weeks diets containing 0, 0.1, 0.2, 0.3 and 0.4 mg added OTA/kg feed, respectively. A seed culture was used as OTA source. Final body weight was significantly depressed by 0.2 mg OTA/kg and more. Cumulative feed consumption was also lower in these groups, but reaching significance only at the highest OTA level. FGR of the group with 0.4 mg OTA/kg was significantly lower than that of all other groups, FGR of the groups with the intermediate OTA levels was inferior to the groups with no added and 0.1 mg added OTA/kg feed. Relative weight of liver, kidneys, spleen and Bursa of Fabricius was not affected by the lowest OTA concentration, but reduced by higher OTA levels.

Sailaja et al. (2017) conducted an ultrastructural study in kidney of broiler chicken fed low levels of OTA. Forty-eight Vencobb broiler chicks were allocated into four groups and fed diets contaminated with 0, 0.03, 0.06, 0.09 mg OTA/kg, respectively, for 5 weeks with *at libitum* access to feed and water. OTA concentrations in feed were analytically confirmed. Six birds from each group were then sacrificed after 35 days and kidney tissues were analysed by transmission electron microscopy. Swelling of epithelial cells, loose intercellular junctions and brush borders, varied sizes of nuclei and karyolysis swelling of mitochondria were observed already at the lowest dose (0.03 mg OTA/kg feed). Other effects as disrupted nucleoli and chromatin material were detected at 0.06 and 0.09 mg OTA/kg feed, respectively. Since complete histopathology was not performed, and clinical consequences cannot be derived, the CONTAM Panel did not consider this study to set a reference point.

The study of Hameed et al. (2017) was designed to investigate the effects of OTA on oxidative stress in broiler chicks. A total of 60 one-day-old broiler chicks was divided into four equal groups. Group A served as control, three other groups received feed contaminated with different levels of OTA (1.6, 3.2 and 6.4 mg/kg, respectively, analytically confirmed) for the first 10 days of life. All birds were fed the control diet from day 11 until day 31. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status of blood plasma (TAS), RBCs hemolysate and supernatant of tissue homogenates (liver, kidney and muscles) were estimated in all groups on days 11 and 31 in order to determine the oxidative stress in chicks. The results revealed a significant dose-dependent decrease in the SOD, GPx and TAS levels in all OTA fed groups. This effect was found persistent even after 21 days of OTA withdrawal in all tissues except RBCs hemolysate. The CONTAM Panel concluded that OTA at dietary levels between 1.8 and 6.4 mg/kg induces oxidative stress as indicated by the low levels of SOD, GPx and TAS in different biological samples of OTA-treated chickens for fattening.

Khan et al. (2018) assessed the total circulating IgY and IgA serum levels in chicks, consuming a diet with doses between 0.1 mg OTA/kg and 1.1 mg OTA/kg diet for 14 or 21 days. Pure OTA was resuspended in ethanol (1 mg/10 mL), the suspension was used to add OTA to the diets. The authors also evaluated other immunological parameters (weight of thymus, bursa of Fabricius and spleen, and leukocyte profiles) at day 21. Significantly decreased IgY serum levels were observed in all OTA-treated groups. In the low-dose group, IgA levels were decreased on day 21, but not on day 14. The size of thymus and bursa of Fabricius was decreased in all OTA-treated groups, whereas reduced spleen size and altered leukocyte profiles were detected only in the high-dose group. The authors concluded that chronic exposure to OTA, even at 0.1 mg OTA/kg diet, affects IgY and IgA production in chicks.

El Cafsi et al. (2020) studied the effect of OTA on the intestinal membrane phospholipids content in chicken. Thirty broiler chicks (1-day-old male Hubbard chicks) were randomly allocated to two groups. Birds of the OTA group were intraperitoneally injected with 0.05 mg/kg BW daily for 21 days. Control birds received an equivalent volume of sodium bicarbonate (NaHCO3 0.1 M). After 21 days, all birds were necropsied and the intestine collected for histological and biochemical examination (lipid extraction and characterisation). OTA-treated birds showed in duodenum, jejunum and ileum blunting villosities and crypts with irregular shapes. In OTA birds, lipid content of duodenum, jejunum and ileum was significantly higher than in the control birds, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) significantly lower in all intestinal segments (with the exception of PC, which was higher in the ileum), phosphatidylinositol was higher in the ileum only and phosphatidylserine in all intestinal segments. The authors relate the growth depression of chickens, also observed in their experiment, with the modifications found in the phospholipid bilayer of the intestinal membrane after OTA intraperitoneal administration.

An increase in liver and kidney weight, decrease in thymus weight, associated with lesions in liver and immunosuppression all support the conclusion that 0.1 mg OTA/kg feed is a LOAEL for growing

chickens. Depression of zootechnical performance was found by two studies, but not confirmed by others.

Hens

Niemiec et al. (2005) studied the effect of OTA fed to a parent generation on the progeny. The experiment was conducted on three flocks of broiler breeders (Cobb, Hubbard and Ross). Each flock was divided into an experimental and control group of 30 hens and 4 roosters. The birds in the experimental group were fed for 4 weeks with a feed contaminated with 0.5 mg OTA/kg, while the feed for the control group was not contaminated. Infected wheat grain was used as OTA source (analysis: 98 mg OTA/kg). The hatched healthy chickens from both groups were reared to day 49 of life. On day 49, five males and five females from each group were slaughtered and carcass analysis was performed. Feeding OTA contaminated feed to the parent generation had a negative influence on the body weight, carcass yield and meat percentage of the offspring.

Szeleszczuk et al. (2007) studied the effect of different doses of OTA (2 and 4 mg/kg; a seed culture was used as OTA source (137 mg OTA/kg) and added to feed for 30-week old Rhode Island Red layer hens for a period of 5 weeks) on selected parameters of cellular and humoral immunity in the hens and humoral immunity in their progeny. In chickens at the 2nd week of life, also a histopathological examination of liver, kidneys, heart as well as of thymus, bursa of Fabricius and spleen was made. Feeding OTA contaminated diets resulted in serious impairment of the hen's immunological system. The wing web index (difference between the wing web swelling of the phytohemagglutinin (PHA)-injected and the phosphate buffered saline (PBS)-injected wings) in the control group was equal to 1.82 while in the birds exposed to 4 mg OTA/kg only 0.68. Also, the serological response after administration of SRBC and Brucella abortus antigen was lower in hens which received OTA. The serological tests and the histopathological study conducted in progeny of the high maternal OTA group showed lower titres after administration of non-specific and specific antigens; the presence of necrotic changes in bursa of Fabricius was also found. At the same time, body weight and weight of lymphatic organs of the chickens in the above-mentioned group at the 6th week of life was lower as compared to the control group. The results obtained indicate (i) a transfer of OTA into the egg and (ii) the possibility of trans-ovary transmission on immunosuppressive properties of OTA.

Hassan et al. (2010) divided 84 White Leghorn (layer breed) breeder hens into seven groups with 12 hens each. The hens were fed diets with no OTA or contaminated (by adding a seed culture to the diets) with 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg OTA/kg, respectively, for 21 days. OTA was obtained by acetonitrile-water extraction of a seed culture. Significant decrease in feed intake, body weight and egg mass production was found in the OTA exposed groups compared to control hens. Among different groups, diarrhoea, unthriftiness and reduced water intake increased with increase in dietary OTA levels. Enlargement and haemorrhages on liver and kidney were more severe in birds fed higher dietary OTA levels. Relative liver weight, serum ALT, urea, creatinine and total protein levels were significantly higher in OTA-treated groups. At study end, significant differences of the lowest OTA dose tested to the control values were seen for egg mass production, feed to egg ratio and serum ALT. Most significant OTA effects were seen from a dose of 3.0 mg OTA/kg upwards.

Hassan et al. (2012c) divided 84 White Leghorn breeder hens into seven groups with 12 hens each. The hens were fed diets with no OTA and contaminated (analytically confirmed) with 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg OTA/kg, respectively, for 21 days. These hens were artificially inseminated with semen obtained from healthy roosters kept on OTA free feed. Egg production and egg quality parameters were recorded. Fertile eggs obtained from each group were set for incubation on weekly basis. At the end of the experiment, hens in each group were euthanised to determine gross and microscopic lesions in different organs. OTA residues were analysed in liver, kidneys and breast muscles (immunoaffinity column elution and HPLC Fluorescent detection techniques). Feeding OTA contaminated diets resulted in a significant decrease in egg mass and egg quality parameters for all OTA concentrations. Liver and kidneys showed characteristic lesions of ochratoxicosis (gross lesions, histopathological lesions including structure of liver and kidney). Embryonic mortality was higher, while hatchability of the chicks was lower in the groups fed higher doses of OTA. The lowest concentration tested (0.1 mg OTA/Kg) had a significant effect in week 2 and 3 on lowering egg production and deteriorating feed to egg ratio. At study end, serum concentration of ALT and urea were significantly increased, whereas creatinine, total proteins, albumin and globulin were impaired only at higher OTA levels.

The depression of zootechnical performance (reduced egg production and deteriorated feed to egg ratio) observed in two studies (Hassan et al., 2010, 2012a) supports the conclusion that 0.1 mg OTA/kg feed is a LOAEL for hens.

Ducks

No relevant studies were made available for ducks.

Turkeys

Dwivedi and Burns (1985) studied the effects of OTA, particularly on the immune system, in two groups of 27 turkeys (British United Turkeys) that were fed diets contaminated with 0 and 4 mg OTA/kg, respectively, for 4 and 10 weeks. OTA resulted in a significant retardation of growth in both males and females. OTA caused a regression of the thymus and bursa of Fabricius and lymphoid depletion in these and other lymphoid organs. Analysis of covariance revealed an enlargement of the kidneys at 10 weeks of age in OTA-treated birds.

A study on the individual and combined effects of several mycotoxins given orally to turkeys was published by Kubena et al. (1997). In a second experiment, 1-day-old female turkeys (Nicholas Large Whites) were fed among others diets without and with OTA (3 mg/kg) for 3 weeks. There were four replicates of six turkeys per dietary treatment. At study end, body weight in the OTA group was significantly reduced by 8% compared to the 0 mg OTA group. In the OTA group, feed to gain ratio was adversely affected as also relative liver weight was significantly increased. The authors conclude that 3 mg OTA/kg feed is not tolerated by young turkeys, when administrated for 3 weeks.

Koynarsky et al. (2007) designed a 2×2 factorial study on 1-week old turkeys (BUT-9) with *Eimeria adenoeides* inoculation and OTA contaminated feed (2 mg/kg). Groups size was 10 animals (no replicates), study duration was 7 days. The CONTAM Panel concluded that the small number of animals, the short duration and the use of one (high) OTA dose only do not allow the use of the study to derive RP.

Slizewska et al. (2020) evaluated the effects of newly elaborated symbiotic preparations (probiotics) on the performance and the intestinal microbiota in turkeys Big-6 fed ochratoxin A contaminated feed. The study was conducted on 140 turkeys Big-6 (females), randomly divided into seven groups (20 animals per cage). The animals were reared for 15 weeks from the first day of their life. The feed was naturally contaminated by the addition of OTA-infected wheat. The OTA concentration in feed ranged from 198.6 to 462.0 μg/kg (starter 0–3 week: 0.199 mg/kg, starter 4–6 week: 0.252 mg/kg, grower, 7–9 week: 0.331 mg/kg, grower 10–12 week: 0.397 mg/kg and finisher 13–15 week: 0.462 mg/kg). At study end, it was found that OTA had an adverse effect on the body weight, the composition of the intestinal microbiota in turkeys. Although OTA in feed was analytically determined, a LOAEL or a NOAEL could not be derived since different dietary OTA concentrations were consecutively administered.

Based on the above studies, the CONTAM Panel is not in the position of deriving a RP for turkeys, due to the short duration of studies, the use of only one (high) dose, and inadequate study design.

Summary on poultry

Among the 17 studies on growing chickens, seven examined the potential adverse effects at an analytically confirmed dietary level of 0.1 mg/kg. The predominant adverse effects at this dose were an increase in weight of liver and kidney, a decrease in thymus weight, associated with lesions in liver and kidney and, and immunosuppression. A depression of zootechnical performance was found by two studies, but not confirmed by others.

In hens, 0.1 mg OTA/kg feed, the lowest dose tested, given for 21 days, significantly reduced egg mass production and deteriorated feed to egg ratio.

The CONTAM Panel considers the concentration of 0.1 mg OTA/kg feed for growing chickens, including chickens for fattening, chickens reared for laying and chickens reared for breeding, as well as hens as a LOAEL.

The CONTAM Panel considers 0.03 mg OTA/kg feed as reference point (RP) for growing chicken and hens, derived by applying an UF of 3 to the LOAEL.[18] Due to lack of data it is not possible to set a RP for ducks or other poultry species.

---

[18] The use of an UF of 3 is in line with EFSA's 'Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data' (EFSA Scientific Committee, 2012) and ECHA's 'Guidance on information requirements and chemical safety assessment Chapter R.8' (ECHA, 2012).

Table 4 summarises the new studies on adverse effects on poultry which have become available since the 2004 Opinion, arranged by animal category.

Table 4: New studies on adverse effects on poultry which have become available since the 2004 Opinion (EFSA CONTAM Panel, 2004)

N[$]/group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	NOAEL/ LOAEL/Lowest effect concentration (mg/kg feed)	Reference
Growing chickens				
Chickens for fattening – Pathology – immunology				
184 one-day broiler chicks, 2 groups	Control, 2. Inoculated on day 14 with Ecoli 078 (2 subgroups)	Changes in kidneys and liver. Immunosuppressive action favouring secondary infections	n/a	Kumar et al. (2004)
3 flocks of 60 broiler hens Cobb, Hubbard and Ross/2 groups (30 hens and 4 roosters)	Control, 0.5, for 4 weeks	Impact on body weight, carcass yield and meat percentage of offspring	Effects at 0.5	Niemiec et al. (2005)
80 one-day-old Ross chickens/3 groups	0, 0.4 and 0.8 for 5 weeks	Decrease humoral immune response and cell-mediated immunity. Decreased BW, FI, FGR, relative thymus weight. Increased in relative gizzard weight	LOAEL at 0.4	Elaroussi et al. (2006)
80 one-day-old Ross chickens/3 groups	Control, 0.4 and 0.8 for 5 weeks	Increased relative weights of kidney and liver. Increased GOT, GPT, uric acid and creatinine in blood. Decreased bursa Fabricius weight, serum Ab titres against Newcastle disease virus, total serum proteins, albumin, globulin. Degenerative changes in kidney, bursa and mononuclear cell infiltration in liver	LOAEL at 0.4	Elaroussi et al. (2008)
60 on-day-old Vencob chicks/6 groups	0, 1 and 2	Reduction in BW and FI, total serum protein and albumin levels at both levels	Effects at 1	Sakthivelan and Sudhakar Rao (2010)
230 Plymouth Rock chicks/3groups (5 male + 5 female per group)	Control, 5, for 2 years	Degenerative changes in liver and kidneys, in lymphoid organs, oedematous and degenerative changes in the brain, muscular haemorrhages and fatty changes in the bone marrow	n/a	Stoev (2010)
350 one-day-old male White Leghorn chicks/ 5 groups	0.1, 0.5, 1.0 and 1.5 for 21 days	decrease in FI and BWG. Severe diarrhoea, dullness, depression, increased water intake and ruffled feathers. Increased kidney, gizzard and liver weight. Gross lesions in liver and kidneys with degenerative and necrotic changes	LOAEL of 0.1	Hassan et al. (2012c)

N[$]/group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	NOAEL/ LOAEL/Lowest effect concentration (mg/kg feed)	Reference
350 one-day-old chicks/ 5 groups	Control, 0.1, 0.5, 1.0 and 1.5 for 21 days	Decreased in relative weight of the bursa of Fabricius and spleen. Reduced phagocytic function of reticuloendothelial system. Lower total antibody SRBC titres	LOAEL of 0.1	Hassan et al. (2012b)
48 Hybro-chickens for fattening/4 groups	Control, 0.5, 1.0 and 1.5 for 14 days	Dose-dependent cumulative effects on the blood serum protein status	n/a	Nedeljković et al. (2013)
36 one-day-old Chickens for fattening/2 groups	Control, 0.1 for 35 days	Moderate degenerative lesions in the liver, spleen and bursa of Fabricius	Effects at 0.1	Pozzo et al. (2013a,b)
40 Ross 308 chickens for fattening/2 groups	Control, 0.050* for up to 21 days. By gavage	Enlarged kidneys with degeneration and gout deposits	n/a	Solcan et al. (2013a)
200 one-day-old chickens	0, 0.1, 0.2, 0.3 and 0.4 for 6 weeks	Reduced BGW, FGR, weights of liver, kidneys, spleen and Bursa of Fabricius at 0.2 mg OTA/kg feed	NOAEL of 0.1	Singh et al. (2015)
60 one-day-old broiler chicks/4 groups	Control, 1.6, 3.2 and 6.4 for 10 days	Decrease in the SOD, GPx and TAS levels (oxidative stress)	Effects at 1.6	Hameed et al. (2017)
42 one-day Cobb chicks/7groups	Control, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 for 21 days	Decreased IgY and IgA serum levels	Effects at 0.1	Khan et al. (2018)
30 one-day-old male Hubbard chicks/2 groups	Control, 0.05* for 21 days (i.p. injection)	Blunting villosities in duodenum, jejunum and ileum and crypts with irregular shapes. Increased lipid content of duodenum, jejunum and ileum	n/a	El Cafsi et al. (2020)
Hens				
150 Rhode Island Red hens/3 groups	0, 2, 4	Impairment of immunological system (wing web index)	n/a	Szeleszczuk et al. (2007)
84 White Leghorn breeder hens/in 7 groups	Control, 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0, for 21 days	Decrease in feed intake, body weight and egg mass production. Impact on egg mass production, feed to egg ratio and serum ALT at all doses	LOAEL of 3.0	Hassan et al. (2010)
84 White Leghorn breeder hens/in 7 groups	Control, 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0, for 21 days	Lesions in liver and kidneys. Lower egg production and deteriorating feed to egg ratio at 0.1 mg OTA/kg feed	LOAEL of 0.1	Hassan et al. (2012a)

$: Including the number of poultry in the control group.

*: In these studies, the doses were expressed in mg/kg bw per day and administered by gavage or i.v., therefore the CONTAM Panel decided not to convert these doses.

3.1.4.3. Ruminants

The effects of OTA were assessed in calves (pre-ruminating and ruminating), in ewes and in male sheep. Sreemannarayana et al. (1988) investigated the effects of a single oral OTA dose of 1 or 4 mg OTA/kg body weight in pre-ruminating calves. Overall, from 1 mg OTA/kg bw dosed calves, one out of two individuals died, and all animals given the higher dose also died. With an i.v. dose of 0.25 mg/kg bw, also one out of two calves died. In male ruminating calves, Sreemannarayana et al. (1988) observed that a dose of 2 mg/kg bw did not result in clinical

adverse effects. These results suggest that the rumen microbiota contributes to protecting the young ruminant up to 2 mg OTA/kg bw.

Several studies assessed the effects of OTA in castrated adult male sheep. Höhler et al. (1999) exposed three groups of four animals to either 0, 2 or 5 mg OTA/kg concentrate feed for 28 days. These authors did not report any adverse effects on the sheep whatever the dose tested. Blank et al. (2003) exposed four groups of three animals to 0, 0.387, 0.774 and 1.161 mg OTA/kg bw for 29 days. Even at the highest dose, no adverse effects were found in this target species. The same research group (Blank et al., 2004) demonstrated the absence of effects of a dose of 0.014 mg/kg bw given for 29 days in castrated male sheep. Blank and Wolfram (2009), as part of a toxicokinetics study, did not observe adverse effects of a single OTA dose of 0.027 mg/kg bw. These studies reveal that OTA up to 5 mg OTA/kg concentrate or 1.161 mg/kg bw, corresponding to 3.5 mg OTA/kg complete feed (calculated having taken into account a 70% of the daily ratio as specified in the paper), did not show clinical effects on adult sheep. No data were available for other ruminant species.

One study was related to lactating ewes. Boudra et al. (2013) studied the effects of 0, 0.005 and 0.030 mg OTA/kg bw for 24 days in three groups of two lactating ewes. At both exposure doses, no measurable adverse effects were seen either on general health or on milk production. No studies on goats are currently available.

In Holstein lactating cows, the effects of a 28 day exposure to 0.005, 0.050 or 0.1 mg OTA/kg dry matter feed were assessed on body weight, feed intake and milk yield. None of the parameters tested were affected by OTA during the experimental period.

To summarise on ruminants, it is not possible to derive a RP, due to lack of information. Nevertheless, these studies demonstrate the protective function of the ruminal microbiota up to 3.5 mg OTA/kg complete feed in sheep.

3.1.4.4. Solipeds

No experimental data on OTA toxicity in horses was reported in the 2004 EFSA opinion. Although it has been suggested that, like other monogastric species, solipeds might be more susceptible to OTA than ruminants (Gross et al., 2015), no relevant reports documenting adverse effects of the mycotoxin in those species were retrieved from recent literature.

3.1.4.5. Rabbits

Four rabbit studies were aimed to evaluate the effects of OTA either on reproductive parameters in does or on nutritional and metabolic disorders in growing rabbits. Only one study was aimed at assessing the teratogenic effects of OTA in feed. Wangikar et al. (2005) administered, by gastric intubation from day 6 to 18 of gestation, three dose levels of OTA (0.025, 0.05 and 0.1 mg/kg bw, confirmed by analysis) in three groups of five gestating does. The reproductive performances such as number of live fetuses, fetal weights, gross and skeletal anomalies, of treated does were compared to control does. The results indicated that doses as low as 0.050 mg/kg bw caused anomalies in fetuses. No adverse effects were observed with the lowest tested dose.

Kumar et al. (2007) investigated the renal ultrastructural alterations in rabbits (16) fed a diet containing OTA (0.75 mg OTA/kg feed) for 60 days. Focal thickening of the glomerular basement membrane and degeneration of endothelial cells were the prominent alterations in the glomeruli in OTA-treated animals.

Mir and Dwivedi (2010) and El-Deep et al. (2020) assessed the effects of OTA in feed of weaned rabbits. Mir and Dwivedi (2010) studied the effects of high OTA concentrations ((1 or 2 mg/kg feed for 56 days) on serum biochemical parameters. Total protein and albumin as well as chloride concentration were decreased while creatine and urea concentration were increased, in exposed rabbits compared to control animals. El-Deep et al. (2020) investigated the effects of low levels of OTA in feed (0.03 mg/kg feed for 56 days) on zootechnical performances, oxidative stress, haemato-immunological and intestinal morphometric changes in growing rabbits. The tested dose impaired feed intake and feed conversion ratio as well as intestinal morphology which led to nutritional disorders. Blood haematology and chemistry were also adversely affected by OTA. Thus, the dose of 0.03 mg/kg feed decreased the growing performances of weaned rabbits.

Summary on rabbits

The CONTAM Panel considers the concentration of 0.03 mg OTA/kg feed for rabbits as a LOAEL.

The CONTAM Panel considers 0.01 mg OTA/kg feed as reference point (RP) rabbits, derived by applying an UF of 3 to the LOAEL.[19]

3.1.4.6. Fish

The effects of OTA were assessed in various juvenile fish species including grass carp (*Ctenopharyngodon Idella*), sea bass (*Dicentrarchus labrax L.*), catfish (*Ictalurus punctatus*), salmon (*S. salar*) and tambaqui (*Colossoma macropomum*). The salmon species appeared to be the most resistant species to OTA. Bernhoft et al. (2018) tested several OTA doses (0, 0.18, 0.34, 0.72, 1.49 and 2 mg/kg feed, confirmed by analysis) for 56 days on performance and health indices. Up to the highest dose tested (2 mg OTA/kg feed) no measurable effects on the target species were observed.

Two studies were conducted in juvenile grass carps. Liu et al. (2020) assessed the effects of various doses (0, 0.406, 0.795, 1.209, 1.612 and 2.003 mg OTA/kg feed, confirmed by analysis) for 60 days on growth performances and intestinal apical junctional complex in seven groups of 180 fish. From 1.209 mg OTA/kg feed, significant depression of feed efficiency, weight gain and growth rate were observed as well as oxidative damage and increased intestinal permeability. Zhao et al. (2022) also studied the effects of increasing doses of OTA (0, 0.4, 0.8, 1.2, 1.6, 2 and 2.4 mg/kg feed, confirmed by analysis) administered for 28 days on oxidative damage, apoptosis and immunosuppression in seven groups of 180 juvenile fish. In the same way as Liu et al. (2020), Zhao et al. (2022) showed adverse effects (decrease lymphocytes, necrotising renal parenchyma cells) from the dose of 1.2 mg OTA/kg.

Two further studies were performed in juvenile catfish by Manning et al. (2003) and Zahran et al. (2016). Manning et al (2003) evaluated the effects of various OTA doses (0, 0.5–1-2-4-or 8 mg/kg feed, not confirmed by analysis) for 56 days in juvenile catfish (six groups of 30 fish). From dose 2 mg OTA/kg feed, significant adverse effects on zootechnical performances and hepatopancreatic tissue were noted. Zahran et al. (2016) tested the effects on zootechnical performances of three OTA doses (2, 4 and 8 mg OTA/kg feed, not confirmed by analysis) in 7 months old channel catfish (four groups of 45 fish) for 56 days. The zootechnical performances were impaired in each OTA treatment group.

El-Sayed et al. (2009) estimated the 96 h LC50 of OTA by administration of various OTA doses (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4 mg/ kg body weight) for 4 days by oral gavage in juvenile sea bass (10 fish per group). These authors defined a 96 h LC 50 at 0,277 mg/kg bw or 9.23 mg/kg diet. Baldissera et al. (2020) assessed the effects on growing performance and hepatic purinergic signalling of three doses of OTA (0.8, 1.6 and 2.4 mg/kg feed) administered for 14 days in juvenile tambaqui. From dose 1.6 mg/kg feed, OTA reduced growth performance and impairs hepatic purinergic signalling.

The CONTAM Panel considered the concentration of 0.8 mg OTA/kg feed and of 0.5 mg OTA/kg feed for juvenile grass carps and juvenile catfish, respectively, as a NOAEL. The CONTAM Panel considered 0.5 mg OTA/kg feed as reference point (RP) for herbivorous fish.

3.1.4.7. Dogs

The data used in the 2004 Opinion showed that when young Beagle dogs were given 0.1 or 0.2 mg OTA/kg BW/day (corresponding approximately to 2–4 mg/OTA/kg feed, respectively) for a short period (14 days), no clinical signs and changes in the renal function were observed. However, kidney tubular necrosis and ultrastructural changes in proximal tubules were demonstrated at these dose levels. Furthermore, in these dogs also necrosis of lymphoid tissue in the thymus and tonsils was observed. Although dogs seem to be particularly sensitive to nephrotoxic effects, no new experimental data on OTA adverse effects in dogs was retrieved from recent literature. In an epidemiological assessment, Meucci et al. (2017), observed a higher incidence of OTA detection in blood samples of dogs affected by chronic kidney disease versus healthy dogs (96% vs. 56%) and higher median value of OTA plasma concentration (0.008 vs. 0.144 ng/mL). In the absence of new data, the CONTAM Panel cannot derive a RP for dogs also in consideration of the lifelong exposure of this species to OTA contaminated feed.

---

[19] The use of an UF of 3 is in line with EFSA's 'Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data' (EFSA Scientific Committee, 2012) and ECHA's 'Guidance on information requirements and chemical safety assessment Chapter R.8' (ECHA, 2012).

3.1.4.8. Cats

No experimental data on OTA toxicity in cats was reported in 2004 Opinion. As no new data is available, the CONTAM Panel is not in the position to derive a RP for cats.

3.1.4.9. Farmed mink

No experimental data on OTA toxicity in farmed mink was reported in the 2004 Opinion. Little is known on the adverse effects of OTA in farmed mink. In the only available study, Bursian et al. (2004) fed mink with a diet containing 0, 2.5, 5 or 10 mg OTA/kg with an intended exposure of 14 days. Animals exposed to 5 or 10 mg/kg were removed from the experiment after 8 days and euthanatised because of feed refusal. Reduced feed intake, reduced spleen (2.5 and 10 mg OTA/kg feed) and liver (2.5 mg OTA/kg feed) weight, and concentration-related kidney lesions were reported. Because of the short duration of the treatment and the use of high concentrations of the toxin, this study was not considered for deriving a RP for OTA in mink.

Table 5 summarises the Reference Points as identified by the CONTAM Panel for the various animal species.

Table 5: Reference Points for adverse animal health effects identified by the CONTAM Panel for the various animal species

Species	Adverse effect observed	Lowest OTA concentrations associated with the observed adverse effect mg OTA/kg feed	RP for adverse animal health effects mg OTA/kg feed	Reference
Growing pigs and piglets	Reduced BW, FI, FGR	0.025	0.01	Malagutti et al. (2005)
Ruminants (cattle, sheep and goats)	N/A	N/A	N/A	N/A
Growing rabbits and gestating does	Decrease FI and alteration of F/G ratio and intestinal morphology	0.03	0.01	El-Deep et al. (2020)
Herbivore fish (carp and catfish)	Depression of F/G, weight gain, SGR, increased intestinal permeability. Alteration of hepatopancreatic tissue	1 (LOAEL)	0.5 (NOAEL in study)	Liu et al. (2020), Zhao et al. (2022), Manning et al. (2003), Zahran et al. (2016)
Salmon	No measurable adverse effects on performance	n/a	n/a	Bernhoft et al. (2018)
Solipeds (horses, donkeys)	No data	No data	No data	No data
Growing chicken and hens	Increased liver and kidney weight. Decreased thymus weight and lesions in liver and kidney. Immunosuppression	0.1	0.03	Hassan et al. (2012a,b), Pozzo et al. (2013a,b), Khan et al. (2018)
Turkeys, ducks and minor poultry species	Insufficient data for turkeys and ducks, no data for other species	N/A	N/A	N/A
Dogs	Insufficient data	N/A	N/A	N/A
Cats	No data	No data	No data	No data
Farmed mink	Insufficient data	N/A	N/A	N/A

3.2. Feed occurrence data

3.2.1. Occurrence data submitted to EFSA

On November 2022, a total of 10,757 samples with analytical data on OTA in feed were extracted from the EFSA Data Warehouse covering the last 10 sampling years, from 2012 to 2021 (see Annex B for the raw data). A thorough analysis of the occurrence dataset was carried out to prepare the data for the dietary exposure assessment.

As commented in Section 2.1.2, the EFSA guideline 'Use of LOQ cut-off values for dietary exposure to chemical contaminants' (EFSA, 2018) was used to try to reduce the impact of the left-censored data (80% of the total) on the LB-UB estimations. The assessment of the reported LOQs identified two different cut-off values based on the 95th percentile of their distribution. For analytical methods with fluorescence detection, the LOQ selected as cut-off value was 2 μg/kg (ww), while for methods with mass spectrometry detection the cut-off value was 5 μg/kg (ww). By using these cut-offs, 208 samples were excluded, only one with quantified values. Two samples that were reported as analysed by HPLC without information on the detection method and a LOQ of 1,000 μg/kg were also excluded. Additionally other 561 samples were also excluded; a brief description of these samples is provided below:

— 201 samples with missing information (e.g. expression of results not provided, moisture content for forage absent, no analytical results reported, pooled samples without further details, etc.).
— 198 samples reported as 'Suspect sampling'.[20]
— 139 samples collected outside EU countries.
— 23 sample wrongly reported as feed.

After the exclusion of these samples, the final dataset consisted of 9,988 samples. Figure 4 shows the distribution of the samples by sampling country.[21] Samples collected in Bulgaria and France represented around 30% of the total (~ 15% each). Other sampling countries with a relatively high number of samples collected were Czechia, Denmark, Hungary and Belgium (> 10%).
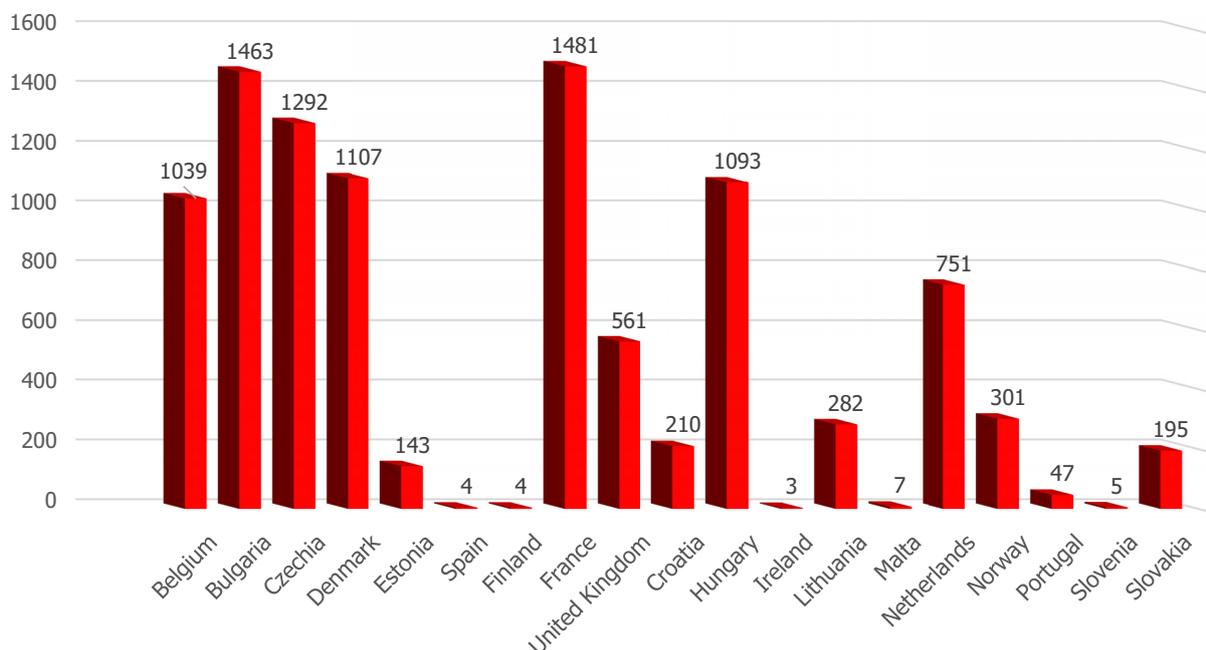
Figure 4: Number of samples by sampling country

Figure 5 shows the different sampling years in the final dataset, from 2012 to 2021. Overall, the feed sampled in the last 5 years represented around 60% of the total (n = 6,105), with almost 40% of the samples collected in the period 2019–2021 (n = 3,918). The lowest number of samples were collected in 2012 (n = 318, 3.2%).

---

[20] Samples analysed to confirm or reject a suspicion of non-conformity, i.e. not random sampling.
[21] Data from the United Kingdom refer to samples submitted before 1 January 2021.
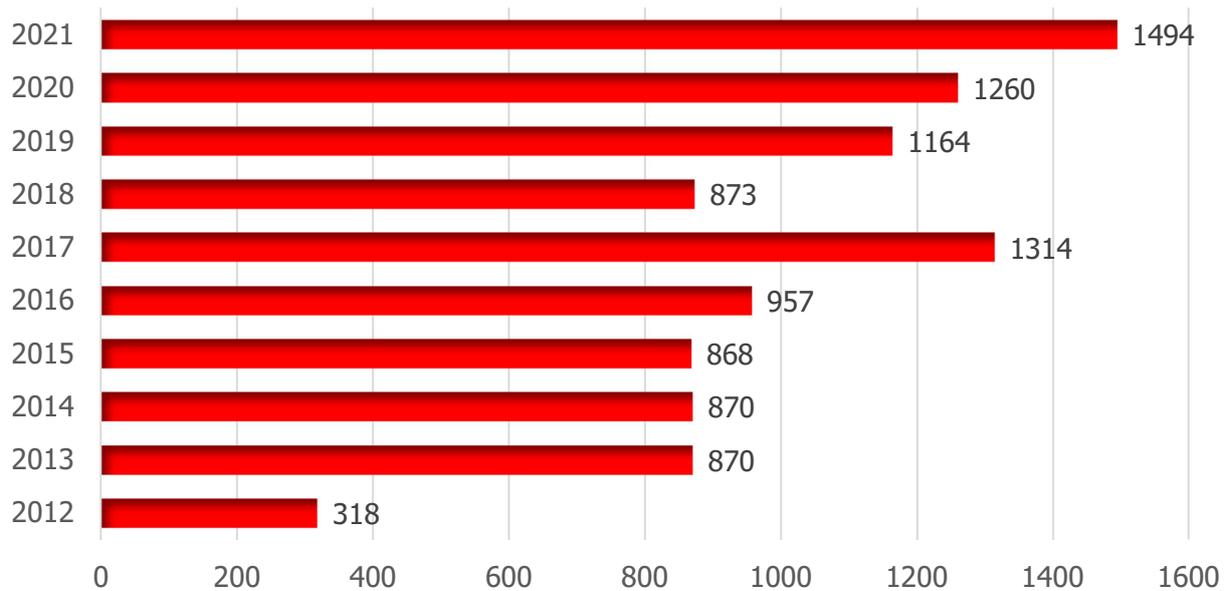
Figure 5: Number of samples by sampling year

Analytical methods

Three main analytical methods were reported for the analysis of OTA in feed samples, liquid chromatography with fluorescence detection (HPLC-FLD, n = 4,440), liquid chromatography either with mass spectrometry (MS) or MS/MS detection (n = 4,295) and enzyme-linked immunosorbent assay (ELISA, n = 1,109). For ELISA, the reported sensitivity ranged between 0.003 and 6 μg/kg (ww), for HPLC-FD between 0.05 and 2 μg/kg (ww) and for MS methods between 0.2 and 5 μg/kg (ww). Other analytical methods also reported for OTA analysis were liquid chromatography with electrochemical detection (LC-ECD, n = 31) and gas chromatography with MS detection (GC–MS, n = 14).

Figure 6 shows the percentage of non-detected, non-quantified and quantified results for OTA analysis in feed samples (Feed level 1). The left-censored data accounted for 79% of the final dataset.
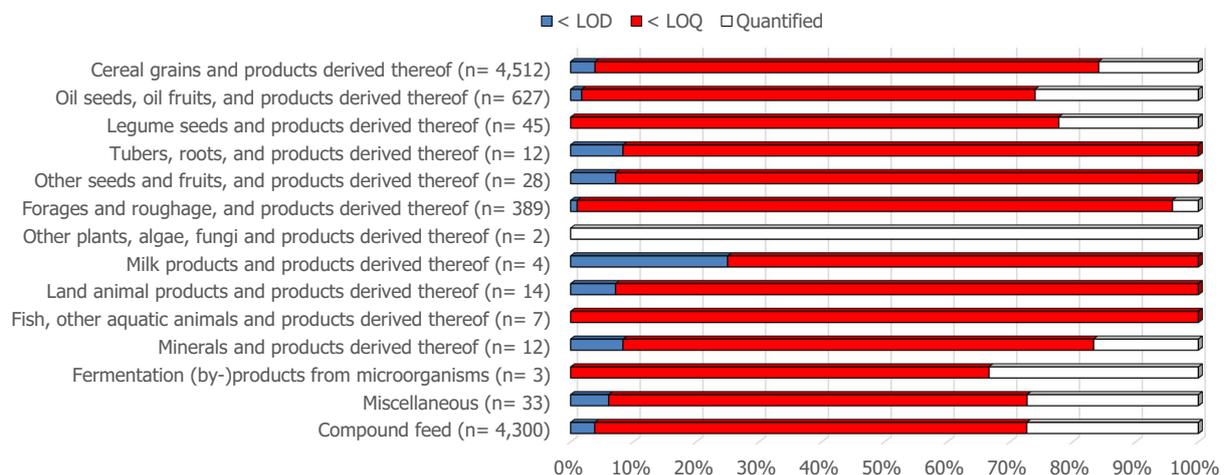
Figure 6: Percentage of quantified, non-quantified and non-detected analytical results (Feed level 1)

The main feed categories in the final dataset (n = 9,988) were 'Cereal grains and products derived thereof' (n = 4,512), 'Compound feed' (n = 4,300) and 'Oil seeds, oil fruits and products derived thereof' (n = 627). Additionally, 389 samples of 'Forages and roughage, and products derived thereof (feed)' were also available.

The analytical results in the final dataset were initially expressed either as whole weight or 88% dry matter; whenever the moisture content was reported or assumptions could be done in the absence of moisture (e.g. 88% DM for 'Cereal grains and products derived thereof' and selected compound feeds),[22] OTA concentrations were converted into dry matter to be used for the exposure estimations. Table 6 shows an overview of the OTA concentrations at Feed level 1 expressed in dry matter (n = 9,184 samples). Two additional tables are shown in Annexes C and D with OTA concentrations at more detailed feed classification. Annex C contains all the samples described in Table 6 expressed in dry matter, while Annex D lists the samples of 'Compound feed' and other feed materials except forage expressed in whole weight. The number of samples across some of the feed categories differ among the two annexes because in few cases the moisture content was not reported (and no assumption on the moisture could be done) precluding the conversion of the analytical results to either whole weight or dry matter.

Table 6: OTA concentrations in feed samples (Feed level 1); all values expressed in µg/kg (dry matter)

	N	%LC	µg/kg dry matter							
			Mean		Median		75th percentile		95th percentile	
			LB	UB	LB	UB	LB	UB	LB	UB
Cereal grains and products derived thereof	4,512	84	2.5	3.9	0.0	1.1	0.0	2.3	6.9	6.9
Oil seeds, oil fruits and products derived thereof	627	74	1.2	2.4	0.0	1.3	0.6	2.5	6.4	6.4
Legume seeds and products derived thereof	45	78	6.3	7.7	0.0	0.6	0.0	5.7	–	–
Tubers, roots and products derived thereof	4	100	0.0	3.1	–	–	–	–	–	–
Other seeds and fruits, and products derived thereof	28	100	0.0	1.6	0.0	0.6	0.0	1.7	–	–
Milk products and products derived thereof	1	100	0.0	0.6	–	–	–	–	–	–
Land animal products and products derived thereof	5	100	0.0	1.0	0.0	1.0	–	–	–	–
Fish, other aquatic animals and products derived thereof	3	100	0.0	2.4	–	–	–	–	–	–
Minerals and products derived thereof	10	80	2.9	3.4	0.0	0.6	–	–	–	–
Miscellaneous[a]	19	63	0.8	1.6	0.0	1.0	1.0	2.3	–	–
Compound feed	3,541	70	1.6	2.4	0.0	1.1	0.5	1.9	5.7	5.8
Forages and roughage, and products derived thereof	389	96	0.9	4.4	0.0	2.3	0.0	5.4	0.0	14.8

Percentiles were provided when they were considered as statistically reliable (α = 0.05) based on the number of samples reported (median = minimum five samples, 75th percentile = minimum 11 samples, and 95th percentile = minimum 59 samples).

(a): The category 'Miscellaneous' refers to feed materials containing animal by-products that fulfil the requirements of Regulation (EC) No 1069/2009 and Regulation (EU) No 142/2011 and may be subject to restrictions in use according to Regulation (EC) No 999/2001 (as described in Commission Regulation 2022/1104).

In preparation for the exposure estimations, different feed categories were grouped based on the type of feed and the OTA levels reported. Furthermore, when for a particular feed category all the samples were reported as left-censored data and there were no evidence reported in the literature that OTA might be present, that feed category was excluded from the exposure estimations. These excluded feeds were replaced in the diets by other feeds under conservative dietary exposure scenarios (e.g. wheat middling replaced by wheat grains).

---

[22] In the absence of reported moisture content, a 88% dry matter content was assumed for the following feed materials: 'Cereal grains and products derived thereof (feed)', 'Oil seeds, oil fruits and products derived thereof (feed)', 'Legume seeds and products derived thereof (feed)' and 'Other seeds and fruits, and products derived thereof (feed)'. The uncertainty associated to this approach is discussed in Section 3.5.

Table 7 shows all the feed categories that were used to estimate dietary exposure in the animal species and categories considered in this scientific opinion; mean OTA levels are provided together with the most reliable percentiles (μg/kg dry matter, LB-UB). The most reliable percentiles were provided when they were considered as statistically reliable ($\alpha = 0.05$) based on the number of samples reported (median = minimum five samples, 75th percentile = minimum 11 samples and 95th percentile = minimum 59 samples).

All samples of 'Forages and roughage, and products derived thereof (feed)' (n = 389) were merged into one single category except those reported as 'Maize silage' (n = 160) that were kept separated, and those reported as unspecified (n = 6) that were not used. The merged category contained a total of 223 samples from 12 different feed categories,[23] with OTA mean and 95th percentile concentrations (LB-UB, dry matter) of 0.7–3.9 μg/kg and 0–13.5 μg/kg, respectively. Seven samples reported as 'Lucerne meal (alfalfa meal)' were also used separated from the merged category as ingredient of the diets for turkeys, hens and rabbits.

As regards 'Cereal grains and products derived thereof (feed)', the following feed materials were used for the exposure estimations: barley grain (n = 568), maize grain (n = 1,017), maize protein (n = 27), oat grains (n = 150), oat feed (n = 97), rice, broken (n = 30), wheat feed (n = 262) and wheat grains (n = 1,051). The highest mean OTA levels (LB-UB, dry matter) were reported for barley grain (4.9–6.3 μg/kg), followed by wheat feed (3.5–4.6 μg/kg) and maize grain (2.2–3.8 μg/kg). More details on mean LB-UB estimations as well as on the most reliable high percentiles to be used to estimate dietary exposure are shown in Table 7.

For the feed category 'Oil seeds, oil fruits and products derived thereof (feed)', within each specific oilseed the samples reported as 'expeller' and those as 'meal' were merged. Three new groups were created for rape seed (n = 99), soybean (n = 88) and sunflower seeds (n = 174). The highest levels under this feed category were reported for the merged group 'Sunflower (expeller + meal)' with mean OTA concentration of 2.7–3.9 μg/kg (LB-UB, dry matter). Details on mean LB-UB estimations as well as on the most reliable high percentiles to be used to estimate dietary exposure are also shown in Table 7.

For 'Compound feed', as with other feed groups, a dedicated grouping was carried out before estimating dietary exposure. Overall, the highest number of samples were reported for 'Laying hens/Complete feed' (n = 411), while the highest mean levels (LB-UB, dry matter) were found in 'Calves (pre-ruminant)/Complete feed' (3.8–4.2 μg/kg). Details on mean LB-UB estimations as well as on the most reliable high percentiles to be used to estimate dietary exposure are also shown in Table 7.

Table 7: Feed samples as used for the estimation of dietary exposure to OTA (concentration expressed as μg/kg dry matter)

	N	%LC	Mean		Median		75th percentile		95th percentile	
			LB	UB	LB	UB	LB	UB	LB	UB
Cereal grains and products derived thereof										
Barley grain	568	88	4.9	6.3	0.0	0.6	0.0	2.3	10.5	10.5
Maize grain	1,017	85	2.2	3.8	0.0	2.3	0.0	3.4	7.3	7.3
Maize protein	27	70	1.7	3.2	0.0	2.3	1.7	2.3	–	–
Oat grains	150	83	1.1	2.6	0.0	0.6	0.0	2.3	4.8	5.7
Oat feed	97	67	0.7	1.4	0.0	1.1	0.5	1.1	4.3	4.3
Rice, broken	30	97	0.1	2.2	0.0	2.3	0.0	2.3	–	–
Wheat grains	1,051	92	0.8	2.3	0.0	1.1	0.0	2.3	2.9	5.7
Wheat feed	262	79	3.5	4.6	0.0	2.0	0.0	2.3	4.0	5.0

---

[23] Cereals straw; Cereal straw treated; Forage meal (Grass meal)/(Green meal); Hay; Grass, herbs; legume plants, dried; Grass, herbs, legume plants (green forage); Lucerne (Alfalfa); Lucerne field dried (Alfalfa field dried); Lucerne, high temperature dried (Alfalfa, high temperature dried); Lucerne, extruded (Alfalfa, extruded); Lucerne meal (Alfalfa meal); Lucerne pomace (Alfalfa pomace).

	N	%LC	μg/kg dry matter							
			Mean		Median		75th percentile		95th percentile	
			LB	UB	LB	UB	LB	UB	LB	UB
Oil seeds, oil fruits and products derived thereof										
Rape seed	21	100	0.0	1.5	0.0	0.6	0.0	2.3	–	–
Rape seed (expeller + meal)	99	98	0.04	1.5	0.0	0.6	0.0	2.5	0.0	5.7
Soybean (expeller + meal)	88	85	0.9	1.9	0.0	0.6	0.0	0.6	2.3	5.7
Soybean hulls	13	69	0.2	0.7	0.0	0.6	0.6	0.7	–	–
Soybean, protein concentrate	25	92	0.1	0.9	0.0	0.6	0.0	0.6	–	–
Sunflower (expeller + meal)	174	45	2.7	3.6	1.0	2.3	3.0	4.3	10.9	10.9
Legume seeds and products derived thereof										
Carobs	2	50	9.1	12.0	–	–	–	–	–	–
Horse beans	26	73	10.0	10.6	0.0	0.6	2.4	2.8	–	–
Pea flour	4	75	0.7	1.3	–	–	–	–	–	–
Forages and roughage, and products derived thereof										
Grass meal, hay and alfalfa[a]	223	96	0.7	3.9	0.0	1.1	0.0	5.4	0.0	13.5
Lucerne meal (Alfalfa meal)[b]	7	57	10.0	12.5	0.0	5.5	–	–	–	–
Maize silage	160	98	0.7	4.9	0.0	2.3	0.0	2.3	0.0	16.2
Compound feed										
Calves (pre-ruminant)/ Complete feed	9	56	3.8	4.2	0.0	1.1	–	–	–	–
Piglets (weaning diets)/ Complete feed	280	73	1.9	2.7	0.0	1.3	1.0	2.3	9.8	9.8
Ducks/Complete feed	37	70	0.7	1.7	0.0	1.1	0.4	1.7	–	–
Dairy cows/ Complementary feed	98	54	1.0	1.4	0.0	0.6	0.8	1.2	5.7	6.8
Horses/Complementary feed	16	75	2.0	3.1	0.0	1.1	0.6	1.7	–	–
Growing/Fattening pigs/ Complete feed	352	68	1.3	1.9	0.0	1.1	0.4	1.7	5.1	5.1
Sows/Complete feed	36	67	0.6	1.2	0.0	1.1	0.8	2.0	3.6	3.6
Fattening chickens (broilers)/Complete feed	354	75	0.6	1.6	0.0	1.1	0.1	2.3	3.4	4.5
Laying hens/Complete feed	411	79	0.8	2.0	0.0	1.1	0.0	1.7	3.5	5.7
Turkeys/Complete feed	55	71	1.3	2.2	0.0	1.1	0.2	2.3	–	–
Rabbits/Complete feed	44	75	1.8	3.1	0.0	1.1	0.4	2.6	–	–
Salmon and trout/ Complete feed	262	97	0.1	0.9	0.0	1.1	0.0	1.1	0.0	1.1
Pet food, cats/Complete feed	55	96	0.04	0.7	0.0	0.5	0.0	0.9	–	–
Pet food, dogs/ Complete feed	10	70	2.1	3.3	0.0	1.1	–	–	–	–

| | N | %LC | μg/kg dry matter | | | | | | | |
| | | | Mean | | Median | | 75th percentile | | 95th percentile | |
			LB	UB	LB	UB	LB	UB	LB	UB
Fattening calves and fattening cattle/ Complementary feed	81	51	2.0	2.1	0.0	0.3	0.9	1.1	8.5	8.5

Percentiles were provided when they were considered as statistically reliable ($\alpha = 0.05$) based on the number of samples reported (median = minimum five samples, 75th percentile = minimum 11 samples, and 95th percentile = minimum 59 samples).

(a): Ad hoc feed category with all samples reported for 'Forages and roughage, and products derived thereof' except samples of maize silage and six samples reported as unspecified 'Forages and roughage, and products derived thereof' were not included.

(b): Seven samples reported as Lucerne meal (alfalfa meal) were also used separated for the diets of turkeys, hens and rabbits.

3.2.2. Previously reported occurrence data

Many articles have been published since the last EFSA Opinion on OTA in feed was published in 2004. In Table B.1/Appendix B is an overview of the results from feed sampled within the EU but also covering feed imported from outside the EU. Results are only included if information on number of samples, number of positive samples, the analytical method as well as the LOQ or LOD are given in the article or could be retrieved from other information in the article, e.g. number of samples and % positive are given. The table is ordered first, according for which animal species the feed is intended for and then year of publication. For several articles either results for feed for several animal species are given or the animal species are not stated, and all these articles are merged in the same category. The results are shown in different ways and the same information on the results is not given in all articles. it is therefore not possible to compare concentrations with each other within or across the different kind of feed.

In general, LC-FLD and LC–MS/MS are the most used methods with LOQs between 0.04 and 20 μg/kg for LC-FLD and 0.2–8 μg/kg for LC–MS/MS. ELISA has also been used for analysis with LOQs between 1 and 2 μg/kg. It is clear from the table that it is possible to find feed without measurable amounts of OTA in many different kinds of feeds e.g. compound feed for pigs and dairy cows, silage, forage and mixed feed for different animal species (Driehuis et al., 2008, Martins et al., 2008, Panasiuk et al., 2019, Arroyo-Manzanares et al., 2019, Tenbrik et al., 2020, Penagos-Taberes et al., 2021) even though it should be noted that in Martins et al. (2008) the LOQ is 20 μg/kg. In most cases there has been several positive findings of OTA in the feed but even though the concentrations are given in fresh weight is also seems that most samples have concentrations very well below the guidance values for OTA in feed. Some samples, were however, highly contaminated as in Stoev et al. (2009) where 50 out of 50 samples from chicken and pig farms with nephropathy problems were positive and with concentrations between 189 and 376 μg/kg.

In one study OTB has been analysed in maize and found in 4 out of 204 samples between 2 and 8 μg/kg (Kos et al., 2020). Processed animal protein was analysed in one study in fish feed and OTA was found in 2 of 19 samples with a concentration of 0.4 μg/kg in both samples (Nácher-Mestre et al., 2015).

3.3. Dietary exposure assessment

The dietary exposure assessment was performed using different scenarios, based on the available occurrence data. In particular, one exposure scenario was used considering consumption of compound feeds (complete and/or complementary), and one scenario considering feed materials and model diets, as described in Section 2.5. In addition, for ruminants and horses, forages were added to allow the daily ration at the recommended proportion for each ruminant category.

Among the different compound feeds which were submitted to EFSA's database and met the quality criteria detailed in previous sections, the following were used:

— Complete feeds were used for pets as it is common practice for them to be fed complete feed by the owners.

— Complete feeds were, generally, not used for ruminants, horses and rabbits as not in line with the standard feeding systems.
— Complete feeds were, however, used for ruminants only when information on their composition and/or information on DM content allowed to identify them as the only source of feed to the animals. Complete feed was used specifically for calves (non-ruminant) as a milk replacer.
— Complementary feeds (plus forages) were used for ruminants and horses, as complementary feeds are normally used mixed with forages for these animal species.

With regard to the feed materials, the exposure assessment was performed making use of the flexibility in the composition of the model diets as explained in Section 2.2, and using substitutions of feed materials within the groups detailed in Appendix E. In particular, maize protein was used in lieu of potato protein (no reported samples), wheat protein (no reported samples) and wheat gluten (only four reported samples); wheat was used in lieu of wheat middlings (only eight reported samples); maize was used in lieu of maize gluten feed (no reported samples), as indicated in Appendix A.2.

In terms of forages, these were added to the diets for ruminants and horses in the quantity indicated, as percentage of the daily diet (see Appendix A). For horses, goats and sheep, the occurrence data were grouped to create a group including grass meal, hay and alfalfa and added to their diets in the respective proportion (see Table 7). Maize silage as individual ingredient was used for cows/cattle, while for turkeys, hens and rabbits the ingredient lucerne meal (or alfalfa) was used in the diets.

The detailed results of the animal dietary exposure, summarised below, are tabulated in Appendix A. Within Appendix C the estimated dietary exposure levels are expressed also in ng/kg bw per day for each animal species.

Bovines

Dietary exposure to OTA in dairy cows varied between 1.8 (LB) and 3.8 (UB) μg/kg feed DM using the mean occurrence scenario, and between 2.7 and 8.9 μg/kg feed DM in the high exposure scenario when using model diet composed of feed materials and forages. Considering compound feeds, the exposure to OTA in dairy cows by complementary feed and forages varied between 0.9 (LB) and 2.5 (UB) μg/kg feed DM in the mean occurrence scenario, and between 4.0 and 9.6 μg/kg feed DM in the high exposure scenario.

Dietary exposure to OTA in Cattle for fattening varied between 1.1 (LB) and 4.7 (UB) μg/kg feed DM using the mean occurrence scenario, and between 1.1 and 14.3 μg/kg feed DM in the high exposure scenario when using feed materials and forages. Considering complementary feed and forages, the exposure to OTA in Cattle for fattening varied between 1.0 (LB) and 4.4 (UB) μg/kg feed DM in the mean occurrence scenario, and between 1.7 and 14.7 μg/kg feed DM in the high exposure scenario.

Exposure to OTA estimated for Veal calves was only possible using the complete feed reported to EFSA resulting in an exposure of 3.8 (LB) and 4.2 (UB) μg/kg feed DM for both mean and high exposure scenarios.

Caprines

OTA dietary exposure in dairy goats varied between 1.7 (LB) and 3.4 (UB) μg/kg feed DM in the mean exposure scenario, and between 3.7 (LB) and 8.1 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.

In kids for fattening exposure to OTA varied between 1.2 (LB) and 3.6 (UB) μg/kg feed DM, and between 1.9 and 10.5 μg/kg feed DM in the mean and high occurrence scenario, when using feed materials and forages.

Ovines

Dietary exposure to OTA in dairy sheep varied between 1.4 (LB) and 3.8 (UB) μg/kg feed DM in the mean exposure scenario, and between 1.3 (LB) and 10.6 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.

In lambs for fattening exposure to OTA varied between 1.4 (LB) and 3.6 (UB) μg/kg feed DM in the mean exposure scenario, and between 2.4 (LB) and 9.9 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.

Horses

Dietary exposure to OTA in horses varied between 1.2 (LB) and 3.8 (UB) μg/kg feed DM using the mean occurrence scenario, and between 1.0 and 11.3 μg/kg feed DM in the high exposure scenario when using the model diet composed of feed materials and forages. Considering compound feeds, the exposure to OTA in horses by complementary feed and forages varied between 1.1 (LB) and 3.7 (UB) μg/kg feed DM in the mean occurrence scenario, and between 0.1 and 10.6 μg/kg feed DM in the high exposure scenario.

Pigs

OTA dietary exposure in weaned piglets varied between 1.6 (LB) and 2.8 (UB) μg/kg feed DM in the mean occurrence scenario, and between 4.0 (LB) and 6.2 (UB) μg/kg feed DM in the high exposure scenario, when using a model diet with individual feed materials. OTA exposure in the complete feed scenario varied between 1.9 (LB) and 2.7 (UB) μg/kg feed DM in the mean occurrence scenario, and was 9.8 (LB and UB) μg/kg feed DM in the high exposure scenario.

Dietary exposure to OTA in pigs for fattening varied between 1.8 (LB) and 3.0 (UB) μg/kg feed DM in the mean occurrence scenario, and between 4.1 (LB) and 6.1 (UB) μg/kg feed DM in the high exposure scenario in the model diet composed with individual feed materials. With the complete feed, the exposure varied between 1.3 (LB) and 1.9 (UB) μg/kg feed DM in the mean occurrence scenario and was 5.1 (LB and UB) μg/kg feed DM in the high exposure scenario.

In lactating sows exposure to OTA varied between 1.6 (LB) and 2.8 (UB) μg/kg feed DM using the mean occurrence scenario, and between 3.5 (LB) and 5.6 (UB) μg/kg feed DM in the high exposure scenario, when using feed materials. OTA exposure in the complete feed scenario varied between 0.6 (LB) and 1.2 (UB) μg/kg feed DM in the mean occurrence scenario, and was 2.3 (LB and UB) μg/kg feed DM in the high exposure scenario.

Poultry

Dietary exposure to OTA in chicken for fattening varied between 1.2 (LB) and 2.6 (UB) μg/kg feed DM using the mean occurrence scenario, and between 4.1 and 5.7 μg/kg feed DM in the high exposure scenario when using feed materials. Considering complete feeds, the exposure to OTA in chicken for fattening varied between 0.6 (LB) and 1.6 (UB) μg/kg feed DM using the mean occurrence scenario, and between 3.4 (LB) and 4.5 (UB) μg/kg feed DM in the high exposure scenario.

OTA dietary exposure in laying hens varied between 1.2 (LB) and 2.5 (UB) μg/kg feed DM in the mean occurrence scenario, and between 3.2 and 5.0 μg/kg feed DM in the high exposure scenario when using feed materials. With complete feed, dietary exposure to OTA varied between 0.8 (LB) and 2.0 (UB) μg/kg feed DM in the mean occurrence scenario, and between 3.5 (LB) and 5.7 (UB) μg/kg feed DM in the high exposure scenario.

Dietary exposure to OTA in turkey varied between 3.1 (LB) and 4.4 (UB) μg/kg feed DM in the mean occurrence scenario, and between 4.9 and 6.8 μg/kg feed DM in the high exposure scenario when using feed materials. Considering complete feed, dietary exposure to OTA varied between 1.3 (LB) and 2.2 (UB) μg/kg feed DM in the mean occurrence scenario, and between 1.9 (LB) and 2.8 (UB) μg/kg feed DM in the high exposure scenario.

For ducks for fattening dietary exposure to OTA varied between 2.7 (LB) and 4.1 (UB) μg/kg feed DM in the mean occurrence scenario, and between 4.3 and 6.4 μg/kg feed DM in the high exposure scenario when using feed materials. With complete feed, dietary exposure to OTA in ducks for fattening varied between 0.7 (LB) and 1.7 (UB) μg/kg feed DM in the mean occurrence scenario, and between 3.2 (LB) and 3.9 (UB) μg/kg feed DM in the high exposure scenario.

Rabbits

Dietary exposure to OTA in rabbits for fattening varied between 2.8 (LB) and 4.0 (UB) μg/kg feed DM in the mean occurrence scenario, and between 3.2 and 5.0 μg/kg feed DM in the high exposure scenario when using feed materials. When using complete feed, the exposure to OTA in rabbits varied between 1.8 (LB) and 3.1 (UB) μg/kg feed DM in the mean occurrence scenario, and between 2.8 and 5.7 μg/kg feed DM in the high exposure scenario.

Fish

In salmon dietary exposure to OTA varied between 0.2 (LB) and 0.5 (UB) μg/kg feed DM in the mean and high occurrence scenario. With complete feed, the dietary exposure to OTA in salmon varied between 0.1 (LB) and 0.9 (UB) μg/kg feed DM and between 0.1 and 1.1 in the mean and high occurrence scenarios, respectively.

Non-food producing animals

Dietary exposure to OTA in dogs varied between 1.2 (LB) and 2.2 (UB) μg/kg feed DM using the mean occurrence scenario, and between 2.4 and 3.2 μg/kg feed DM in the high exposure scenario when using feed materials. Considering complete feed, the exposure to OTA in dogs varied between 2.1 (LB) and 3.3 (UB) μg/kg feed DM in the mean and high occurrence scenario.

OTA dietary exposure in dogs fed a vegetarian diet varied between 0.7 (LB) and 1.9 (UB) μg/kg feed DM using the mean occurrence scenario, and between 1.1 and 2.3 μg/kg feed DM in the high exposure scenario when using feed materials.

In cats the dietary exposure to OTA varied between 1.0 (LB) and 1.9 (UB) μg/kg feed DM in the mean and between 1.2 (LB) and 2.2 (UB) in the high occurrence scenario, respectively. With complete feed, dietary exposure to OTA in cats varied between 0.0 (LB) and 0.7 (UB) μg/kg feed DM in the mean occurrence scenario, and between 0.0 (LB) and 1.1 (UB) μg/kg feed DM in the high exposure scenario.

3.4. Risk characterisation

For risk characterisation, the CONTAM Panel took into account the dietary exposure assessment of OTA using recent analytical results on the occurrence of OTA in feed reported to EFSA (Section 2.1) and the diet composition and consumption of food producing and non-food producing animals described in Sections 2.2 and Appendix A. The estimates of exposure to OTA are presented in Section 3.3 and Appendices A and C.

The CONTAM Panel characterised the food producing and non-food producing animal health risk associated with dietary exposure to OTA by comparing the estimated Mean LB/UB and estimated High LB/UB exposures against the identified reference points (RPs) for adverse animal health effects, for the different feeding scenarios relevant for each animal species (e.g. feed materials, complete feeds). The exposure was expressed as a percentage of the RP for adverse animal health effects: a percentage below 100 was considered a low risk. The comparison was performed in μg OTA/kg complete feed (88% DM), following conversion of the exposure level into 88% DM, to be in line with the identified RPs, and are summarised in Tables 8–13.

3.4.1. Poultry

Table 8: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for chicken for fattening

Chicken for fattening	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	1.1	2.3	3.6	5.0	4	8	12	17
Compound feed	0.5	1.4	3.0	4.0	2	5	10	13
	LOAEL/Adverse effect concentration (μg/kg feed): 100				Reference Point (μg/kg feed): 30			

*: Expressed as Complete feed (88% DM).

For chickens for fattening, the estimated exposure to the OTA at the UB mean and UB high were 8% and 17% of the RP, respectively, for the model diet scenario and 10% and 13% of the RP, respectively, for the compound feed scenario, indicating a low risk for adverse health effects.

Table 9: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for hens

Hens	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	1.1	2.2	3.1	4.8	4	7	10	16
Compound feed	0.7	1.7	3.1	5.0	2	6	10	17
	LOAEL/Adverse effect concentration (μg/kg feed): 100				Reference Point (μg/kg feed): 30			

*: Expressed as Complete feed (88% DM).

For hens, the estimated exposure to the OTA at the UB mean and UB high were 9% and 15% of the RP, respectively, for the model diet scenario and 10% and 17% of the RP, respectively, for the compound feed scenario, indicating a low risk for adverse health effects.

3.4.2. Pigs

Table 10: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for piglets (weaned)

Piglets (weaned)	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	1.4	2.5	3.5	5.5	14	25	35	55
Compound feed	1.7	2.4	8.6	8.6	17	24	86	86
	LOAEL/ Adverse effect concentration (μg/kg feed): 25				Reference Point (μg/kg feed): 10			

*: Expressed as Complete feed (88% DM).

For weaned piglets, the estimated exposure to the OTA at the UB mean and UB high were 35% and 55% of the RP, respectively, for the model diet scenario and 86% of the RP for both UB mean and high, for the compound feed scenario. CONTAM Panel considers that this indicates a low risk for adverse health effects.

Table 11: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for pigs for fattening

Pigs for fattening	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Compound feed	1.6	2.7	3.6	5.4	16	17	36	54
Model diet	1.1	1.7	4.5	4.5	11	17	45	45
	LOAEL/Adverse effect concentration (μg/kg feed): 25				Reference Point (μg/kg feed): 10			

*: Expressed as Complete feed (88% DM).

For pigs for fattening, the estimated exposure to the OTA at the UB mean and UB high were 36% and 54% of the RP, respectively, for the model diet scenario and 45% of the RP for both UB mean and high, for the compound feed scenario, indicating a low risk for adverse health effects.

Table 12: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for sows, lactating

Lactating sows	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	1.4	2.4	3.1	4.9	14	24	31	49
Compound feed	0.6	1.1	2.0	2.0	6	11	20	20
	LOAEL/ Adverse effect concentration (μg/kg feed): 25				Reference Point (μg/kg feed): 10			

*: Expressed as Complete feed (88% DM).

For lactating sows, the estimated exposure to the OTA at the UB mean and UB high were 24% and 49% of the RP, respectively, for the model diet scenario and 11 and 20% for UB mean and high, respectively, for the compound feed scenario, indicating a low risk for adverse health effects.

3.4.3. Rabbits

Table 13: Comparison of estimated OTA Mean/High exposure levels (from model diet and compound feed) and RP for rabbits

Rabbits	μg OTA/kg feed*				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	2.5	3.5	4.6	5.6	25	35	46	56
Compound feed	1.6	2.7	2.5	5.0	16	27	25	50
	LOAEL/Adverse effect concentration (μg/kg feed): 30				Reference Point (μg/kg feed): 10			

*: Expressed as Complete feed (88% DM).

For rabbits, the estimated exposure to the OTA at the UB mean and UB high were 28% and 55% of the RP, respectively, for the model diet scenario; and 25% and 50% of the RP, respectively, for the compound feed scenario, indicating a low risk for adverse health effects.

3.5. Uncertainty analysis

The evaluation of the inherent uncertainties in the present assessment was performed following the guidance of the Scientific Committee related to uncertainties in dietary exposure assessment (EFSA, 2007), the report on 'Characterising and Communicating Uncertainty in Exposure Assessment' (WHO/IPCS, 2008), the new guidance on uncertainties of the EFSA Scientific Committee (EFSA Scientific Committee, 2018) and the guidance on communication of uncertainty in scientific assessments (EFSA, 2019). The uncertainties mentioned cover the studies and data used in this Opinion that have an influence on the risk characterisation. For some animal species including ruminants, solipeds and non-food producing animals, it has not been possible to derive an RP due to lack of relevant data or no data at all; therefore, this uncertainty analysis does not consider these animal species.

Sources of uncertainties related to hazard identification, hazard characterisation and exposure assessment of OTA for animal health were listed and discussed. The complete list is presented in Appendix F, together with 'low' and 'high' scenarios describing the Panel's qualitative evaluation of their potential impact on the assessment. This section below includes the most important uncertainties identified.

3.5.1. Uncertainty on TK/ADME

For some animal species, uncertainty is generated by the short duration of the TK studies and the lack of analysis in the target tissues (e.g. pigs). This could potentially lead to an under- or over-estimation of the risk characterised in this Opinion.

3.5.2. Uncertainty on the studies used for evaluation of the adverse effect in food producing and non-food producing animals

For some animal species/categories, only one dose besides the control is used. In other studies there are effects at all dose levels; in some studies a large interval between doses was used. Except for poultry, in general there are only a few studies that could be used to derive a RP directly.

Doses were not confirmed by analysis in all studies. This could result in an under- or over-estimation of the doses used in those studies.

3.5.3. Uncertainty on occurrence

A number of uncertainties were identified linked to the occurrence data, those with the highest impact on the assessment are listed below:

- Lack of information on recovery/samples reported as not corrected for recovery – this could lead to an under- or an over-estimation of the level of OTA in the samples;
- Assumptions made for the dry matter content when information was not available – this could lead to an under- or over-estimation of the level of OTA in the samples;
- Possible missing data from EU countries not submitting data (representativeness) – this could lead to an under- or over-estimation of the level of OTA in the samples;
- High amount of left-censored data. When using the LB approach, OTA levels in the feed samples might have been underestimated while overestimated at the UB approach.

3.5.4. Uncertainty on exposure

The exposure was calculated using model diets, feed intake and body weights, which are considered as a standardised procedure for the purposes of uncertainty analysis. In addition, the model diets, feed intakes and body weights were updated to allow for current feeding practices. This, together with the used of different exposure scenarios (e.g. compound feeds) goes towards reduction of uncertainty.

3.5.5. Overall uncertainty

Consensus probabilities were obtained by expert judgement as described in the EFSA Uncertainty Guidance. For the animal species for which it was possible to characterise the risk (poultry (hens and chickens), pigs and rabbits), the CONTAM Panel considers that the risk related to OTA in feed for adverse effects is very likely (95–99% certain) to be low.

4. Conclusions

OTA is produced by various fungi of the genera *Aspergillus* and *Penicillium*, e.g. *A. ochraceus*, *A. carbonarius* and *P. verrucosum*. OTA in food and feed is analysed by LC–MS, LC–MS/MS and HPLC methods.

Toxicokinetics

Data are available to a greater or lesser extent for cattle, sheep, goat, pigs, rabbits, poultry and fish species; little is reported for horses and donkeys, while no studies were retrieved for dogs, cats and farmed mink.

- In most species, OTA is in general rapidly and extensively absorbed in the gastro-intestinal tract.
- The main metabolic biotransformation is the microbial hydrolytic cleavage yielding phenylalanine and OTalpha, which is considered a detoxification pathway. Minor metabolic pathways include dechlorination, the CYP-dependent generation of OH-derivatives in liver and the subsequent formation of phase II metabolites.
- OTA is accumulated mainly in the kidneys and is excreted both through the urinary and faecal routes.
- In cattle, sheep and goats, rumen microbiota is mainly responsible for the extensive OTA conversion to OTalpha, which may be affected by diet composition and rumen pH.
- Sheep have a lower plasma protein binding and a shorter elimination half-life compared to cattle and goats.

- In monogastric species like pigs and in pre-ruminant calves OTA may accumulate due to limited metabolism to OTalpha.
- In pigs, TK is characterised by a very extensive protein binding and a long elimination half-life.
- In horses placental transfer has been demonstrated.
- In donkeys, OTA is rapidly and extensively absorbed and only partially excreted via urine and faeces; no further information is available on the metabolic fate of OTA.
- In rabbits OTA is rapidly absorbed, extensively distributed and rapidly eliminated.
- Poultry species appear to eliminate OTA faster than monogastric mammalian species.
- Turkeys have the highest plasma protein binding compared to broiler chickens, laying hens, breeder, roosters and ducks.
- In fish (salmon, carp and rainbow trout) a very low oral bioavailability and lower plasma protein binding has been observed compared to the other animal species.

Transfer

- Only negligible concentrations of OTA and OTalpha were found in tissues and milk from ruminants and donkeys.
- In poultry, transfer to eggs is negligible and only occurs when OTA intake is very high.
- In processed animal products and cheese, contamination can be due to OTA formed during the process and storage.
- The highest amount of transfer is seen in pigs, especially to the kidneys.
- Only trace amounts of OTA are transferred to edible tissues of fish.

Toxicity

- For ruminants, solipeds, farmed mink, salmonids as well as cats, dogs or other companion animals, there are either no studies at all or studies that cannot be used for setting an RP.
- In pigs, effects such as a decrease in body weight were seen at the concentration level of 0.025 mg/kg feed. The CONTAM Panel considers 0.01 mg OTA/kg feed as reference point (RP) for adverse animal health effect for pigs, derived by applying an UF of 3 to the LOAEL and rounding from 0.008 to 0.01.
- In hens and growing chickens, including chickens for fattening, chickens reared for laying and chickens reared for breeding, a LOAEL could be set at 0.1 mg/kg feed. Several adverse effects were seen at this concentration including increased liver and kidney weight. The CONTAM Panel considers 0.03 mg OTA/kg feed as reference point (RP) for growing chicken and hens, derived by applying an UF of 3 to the LOAEL.
- For other poultry species such as turkey and ducks no information is available to derive an RP.
- In rabbits, a decrease in the growing performances was identified following exposure to OTA. The CONTAM Panel considers 0.01 mg OTA/kg feed as reference point (RP) for rabbits, derived by applying an UF of 3 to the LOAEL.
- For herbivore fish a NOAEL of 0.5 mg/kg feed was derived by the CONTAM Panel, considering effects on zootechnical performances and hepatopancreatic tissue, significant depression of feed efficiency, weight gain and growth rate.

Mode of action

- Kidneys are the target organs in most species for toxic effects with alterations in structure and functions.
- In chicken carcinogenic effects of OTA were shown with kidneys and liver as target organs.
- The mechanisms behind the carcinogenic effects in target species has not been completely clarified.

Occurrence

- A total of 10,757 analytical results on OTA in feed were initially extracted from the EFSA Database (sampling years 2012–2021). After the assessment and cleaning of the data, 9,988 analytical results were available in the final dataset.
- Samples collected in Bulgaria and France represent around 30% of the total data (~ 15% each). Other sampling countries with a relatively high number of samples collected were Czechia, Denmark, Hungary and Belgium (> 10%). Overall, around 60% of the feed materials were sampled between 2017 and 2021.

- Two main analytical methods were reported for the analysis of OTA in feed samples: liquid chromatography with fluorescence detection (HPLC-FD, n = 4,440) and liquid chromatography either with mass spectrometry (MS) or MS/MS detection (n = 4,295).
- OTA concentrations were converted to be based on dry matter before being used for the dietary exposure estimations. Based on the information reported to EFSA and the assumptions taken on moisture content, data on a total of 9,184 samples were made available.
- Highest OTA levels (LB-UB, dry weight) were reported for 'Horse beans' (10.0–10.6 μg/kg, n = 26) and 'Lucerne meal (10.0–12.5 μg/kg, n = 7). Among cereal grains, highest levels were in 'Barley grain (4.9–6.3 μg/kg, n = 568) and among the samples of compound feed for 'Calves (pre-ruminant)/Complete feed' (n = 3.8–4.2 μg/kg, n = 9).

Dietary exposure

- Exposure was calculated for all animal species included in the exposure scenarios.
- Exposure was performed using two different scenarios based on either model diets composed of feed materials or compound feed (complete and/or complementary). Forages were also included for ruminants and horses.
- Exposure was performed using either a mean or a high exposure scenario. The high scenario was performed for the 95% percentile of occurrence or lower depending on the number of occurrence data.
- In dairy cows, the exposure to OTA varied between 0.9 (LB) and 3.8 (UB) μg/kg feed DM using the two mean occurrence scenarios, and between 3.2 and 9.6 μg/kg feed DM in the two high exposure scenarios.
- In cattle for fattening, the exposure to OTA varied between 1.0 (LB) and 4.7 (UB) μg/kg feed DM using the two mean occurrence scenarios, and between 1.6 and 14.7 μg/kg feed DM in the two high exposure scenarios.
- In veal calves, it was only possible to calculate the exposure using the complete feed scenario resulting in an exposure of 3.8 (LB) and 4.2 (UB) μg/kg feed DM for both mean and high exposure scenarios.
- In dairy goats, the exposure varied between 1.7 (LB) and 3.4 (UB) μg/kg feed DM in the mean exposure scenario, and between 3.9 (LB) and 8.1 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.
- In goat kids for fattening, exposure to OTA varied between 1.2 (LB) and 3.6 (UB) μg/kg feed DM, and between 2.3 and 10.5 μg/kg feed DM in the mean and high occurrence scenario, when using feed materials and forages.
- In dairy sheep, exposure to OTA varied between 1.4 (LB) and 3.8 (UB) μg/kg feed DM in the mean exposure scenario, and between 2.1 (LB) and 10.8 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.
- In lambs for fattening, exposure to OTA varied between 1.4 (LB) and 3.6 (UB) μg/kg feed DM in the mean exposure scenario, and between 2.4 (LB) and 10.0 (UB) μg/kg feed DM in the high exposure scenario when using feed materials and forages.
- In horses, exposure varied between 1.1 (LB) and 3.8 (UB) μg/kg feed DM using the two mean occurrence scenarios and between 0.1 and 11.3 μg/kg feed DM in the two high exposure scenarios.
- In weaned piglets, exposure to OTA varied between 1.6 (LB) and 2.8 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 4.0 (LB) and 9.8 (UB) μg/kg feed DM in the two high exposure scenarios.
- In pigs, for fattening exposure to OTA varied between 1.3 (LB) and 3.0 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 4.1 (LB) and 6.1 (UB) μg/kg feed DM in the two high exposure scenarios.
- In lactating sows, exposure to OTA varied between 0.6 (LB) and 2.8 (UB) μg/kg feed DM using the two mean occurrence scenario, and between 2.3 (LB) and 5.6 (UB) μg/kg feed DM in the two high exposure scenarios.
- In chicken for fattening, exposure to OTA varied between 0.6 (LB) and 2.6 (UB) μg/kg feed DM using the two mean occurrence scenarios, and between 3.4 and 5.7 μg/kg feed DM in the two high exposure scenarios.
- In laying hens, exposure to OTA varied between 0.8 (LB) and 2.5 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 3.5 and 5.7 μg/kg feed DM in the two high exposure scenarios.

- In turkeys, exposure to OTA varied between 1.3 (LB) and 4.4 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 1.9 and 7.5 μg/kg feed DM in the two high exposure scenarios.
- In ducks for fattening, dietary exposure to OTA varied between 0.7 (LB) and 4.1 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 3.2 and 7.0 μg/kg feed DM in the two high exposure scenarios.
- In rabbits for fattening, exposure to OTA varied between 1.8 (LB) and 4.0 (UB) μg/kg feed DM in the two mean occurrence scenarios, and between 2.8 and 6.4 μg/kg feed DM in the two high exposure scenarios.
- In salmon, dietary exposure to OTA varied between 0.1 (LB) and 0.9 (UB) μg/kg feed DM in the mean and high occurrence scenario. With complete feed, the dietary exposure to OTA in salmon varied between 0.1 (LB) and 0.9 (UB) μg/kg feed DM and between 0.1 and 1.1 in the mean and high occurrence scenarios, respectively.
- In dogs, the exposure to OTA varied between 1.1 (LB) and 3.3 (UB) μg/kg feed DM using the mean occurrence scenarios, and between 2.1 and 3.3 μg/kg feed DM in the high exposure scenarios.
- OTA dietary exposure in dogs fed a vegetarian diet varied between 0.7 (LB) and 1.8 (UB) μg/kg feed DM using the mean occurrence scenario, and between 1.1 and 2.4 μg/kg feed DM in the high exposure scenario when using feed materials.
- In cats, the dietary exposure to OTA varied between 0.0 (LB) and 1.8 (UB) μg/kg feed DM in the mean exposure scenarios and between 0.0 (LB) and 2.4 (UB) in the high exposure scenarios, respectively.

Risk characterisation

- To compare with the RPs, the dietary exposure was based converted to 88% DM. The exposure was expressed as a percentage of the RP for adverse animal health effects: a percentage below 100 was considered a low risk.
- For weaned piglets, the exposure amounted to 14–86% of the RP, to 6–49% of the RP for growing pigs while for sows the exposure amounted to 11–54% of the RP. The intervals range between the lowest LB and highest UB for two exposure scenarios.
- For chickens for fattening and laying hens, the exposure amounted to 2–17% of the RP, between the lowest LB and highest UB for two exposure scenarios.
- For rabbits, the exposure amounted to 16–55% of the RP, between the lowest LB and highest UB for two exposure scenarios.
- The CONTAM Panel considers, with 95–99% certainty, that for pigs, chickens for fattening, hens and rabbits the risk related to OTA in feed for adverse health effects is low.

5.   Recommendations

- Further information is needed on OTA TK particularly in solipeds, dogs, cats and farmed mink.
- Further data are required on the adverse effects of OTA and its metabolites (e.g. in solipeds, sows, dogs, cats and farmed mink).
- When submitting OTA occurrence data to EFSA, it is recommended to provide the adequate information on the feed samples analysed. This refers to reporting at least information on the expression of results and the moisture content (if the results are expressed in whole weight), and sufficient details on the samples analysed (e.g. target animals for the complete/complementary compound feed).
- The use of sensitive methods for the analysis of OTA in feed materials is recommended to reduce the uncertainties linked to the LB-UB estimations.

References

Adunphatcharaphon S, Elliott CT, Sooksimuang T, Charlermroj R, Petchkongkaew A and Karoonuthaisiri N, 2022. The evolution of multiplex detection of mycotoxins using immunoassay platform technologies. Journal of Hazardous Materials, 432, 128706. https://doi.org/10.1016/j.jhazmat.2022.128706

Alexa E, Dehelean CA, Poiana MA, Radulov I, Cimpean AM, Bordean DM, Tulcan C and Pop G, 2013. The occurrence of mycotoxins in wheat from western Romania and histopathological impact as effect of feed intake. Chemistry Central Journal, 7(1), 99. https://doi.org/10.1186/1752-153X-7-99

Almeida I, Martins HM, Santos S, Costa JM and Bernardo F, 2011. Co-occurrence of mycotoxins in swine feed produced in Portugal. Mycotoxin Research, 27, 177–181. https://doi.org/10.1007/s12550-011-0093-8

Altafini A, Armorini S, Zaghini A, Sardi L and Roncada P, 2017. Tissue distribution of ochratoxin A in pigs after administration of two-levels contaminated diets. World Mycotoxin Journal, 10, 263–272. https://doi.org/10.3920/WMJ2016.2152

Aoudia N, Callu P, Grosjean F and Larondelle Y, 2009. Effectiveness of mycotoxin sequestration activity of micronized wheat fibres on distribution of ochratoxin A in plasma, liver and kidney of piglets fed a naturally contaminated diet. Food and Chemical Toxicology, 47, 1485–1489. https://doi.org/10.1016/j.fct.2009.03.033

Armorini S, Al-Qudah KM, Altafini A, Zaghini A and Roncada P, 2015. Biliary ochratoxin A as a biomarker of ochratoxin exposure in laying hens: an experimental study after administration of contaminated diets. Research in Veterinary Science, 100, 265–270. https://doi.org/10.1016/j.rvsc.2015.03.004

Arroyo-Manzanares N, Rodríguez-Estévez V, Arenas-Fernández P, García-Campaña AM and Gámiz-Gracia L, 2019. Occurrence of Mycotoxins in Swine Feeding from Spain. Toxins (Basel), 11, 342. https://doi.org/10.3390/toxins11060342

Aukema HM, House JD, Bankovic-Calic N and Ogborn MR, 2004. Increased renal fibrosis and expression of renal phosphatidylinositol 4-kinase-beta and phospholipase C(gamma1) proteins in piglets exposed to ochratoxin-A. Nephron Physiology, 96, 19–25. https://doi.org/10.1159/000075576

Awuchi CG, Ondari EN, Ogbonna CU, Upadhyay AK, Baran K, Okpala COR, Korzeniowska M and Guiné RPF, 2021. Mycotoxins affecting animals, foods, humans, and plants: types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies-A revisit. Foods, 10, 1279. https://doi.org/10.3390/foods10061279

Baldissera MD, Souza CF, da Silva JA, Barroso DC, Glória EM, Baldisserotto B and Val AL, 2020. Dietary exposure to ochratoxin A reduces growth performance and impairs hepatic purinergic signaling in tambaqui (Colossoma macropomum). Fish Physiology and Biochemistry, 46, 2055–2064. https://doi.org/10.1007/s10695-020-00854-0

Bernardini C, Grilli E, Duvigneau JC, Zannoni A, Tugnoli B, Gentilini F, Bertuzzi T, Spinozzi S, Camborata C, Bacci ML, Piva A and Forni M, 2014. Cellular stress marker alteration and inflammatory response in pigs fed with an ochratoxin contaminated diet. Research in Veterinary Science, 97, 244–250. https://doi.org/10.1016/j.rvsc.2014.07.018

Bernhoft A, Høgåsen HR, Rosenlund G, Ivanova L, Berntssen MHG, Alexander J, Eriksen GS and Kruse Fæste C, 2017. Tissue distribution and elimination of deoxynivalenol and ochratoxin A in dietary-exposed Atlantic salmon (*Salmo salar*). Food Additives and Contaminants Part A, 34, 1211–1224. https://doi.org/10.1080/19440049.2017.1321149

Bernhoft A, Høgåsen HR, Rosenlund G, Moldal T, Grove S, Berntssen MHG, Thoresen SI and Alexander J, 2018. Effects of dietary deoxynivalenol or ochratoxin A on performance and selected health indices in Atlantic salmon (*Salmo salar*). Food and Chemical Toxicology, 121, 374–386. https://doi.org/10.1016/j.fct.2018.08.079

Bi X, Luo L, Li L, Liu X, Chen B and You T, 2020. A FRET-based aptasensor for ochratoxin A detection using graphitic carbon nitride quantum dots and CoOOH nanosheets as donor-acceptor pair. Talanta, 218, 121159. https://doi.org/10.1016/j.talanta.2020.121159

Bi S, Xu J, Yang X, Zhang P, Lian K and Ma L, 2022. An HPLC-MS/MS method using a multitoxin clean up column for analysis of seven mycotoxins in aquafeeds. Journal of AOAC International, 105, 107–114. https://doi.org/10.1093/jaoacint/qsab101

Bittner A, Cramer B, Harrer H and Humpf HU, 2015. Structure elucidation and in vitro cytotoxicity of ochratoxin a amide, a new degradation product of ochratoxin A. Mycotoxin Research, 31, 83–90.

Blank R and Wolffram S, 2009. Effects of live yeast cell supplementation to high concentrate diets on the toxicokinetics of ochratoxin A in sheep. Food Additives & Contaminants: Part A, 26, 119–126. https://doi.org/10.1080/02652030802320600

Blank R, Rolfs JP, Südekum KH, Frohlich AA, Marquardt RR and Wolffram S, 2003. Effects of chronic ingestion of ochratoxin a on blood levels and excretion of the mycotoxin in sheep. Journal of Agricultural and Food Chemistry, 5, 6899–6905. https://doi.org/10.1021/jf034547j

Blank R, Rolfs J, Südekum K, Frohlich AA, Marquardt RR and Wolffram S, 2004. Effect of roughage:concentrate ratio in the diet on systemic availability and excretion of ochratoxin A in sheep. Journal of Animal and Feed Sciences, 13, 673–676. https://doi.org/10.22358/jafs/74087/2004

Blank R and Wolffram S, 2005. Effect of dietary sodiumbicarbonate supplementation on the toxicokinetics of ochratoxin A in pigs. MycotoxinResearch, 21(2), 147–149. https://doi.org/10.1007/BF02954441

Böhm J, Koinig L, Razzazi-Fazeli E, Blajet-Kosicka A, Twaruzek M, Grajewski J and Lang C, 2010. Survey and risk assessment of the mycotoxins deoxynivalenol, zearalenone, fumonisins, ochratoxin A, and aflatoxins in commercial dry dog food. Mycotoxin Research, 26, 147–153. https://doi.org/10.1007/s12550-010-0049-4

Boudra H, Saivin S, Buffiere C and Morgavi DP, 2013. Short communication: toxicokinetics of ochratoxin A in dairy ewes and carryover to milk following a single or long-term ingestion of contaminated feed. Journal of Dairy Science, 96, 6690–6696. https://doi.org/10.3168/jds.2013-6707

Boudra H, Rouillé B, Lyan B and Morgavi DP, 2015. Presence of mycotoxins in sugar beet pulp silage collected in France. Animal Feed Science and Technology, 205, 131–135. https://doi.org/10.1016/j.anifeedsci.2015.04.010

Bryła M, Damaziak K, Twarużek M, Waśkiewicz A, Stępień Ł, Roszko M, Pierzgalski A, Soszczyńska E, Łukasiewicz-Mierzejewska M, Chmiel M and Wójcik W, 2023. Toxico-pathological effects of ochratoxin A and its

diastereoisomer under in ovo conditions and in vitro evaluation of the toxicity of these toxins against the embryo Gallus gallus fibroblast cell line. Poulty Science, 102, 102413. https://doi.org/10.1016/j.psj.2022.102413

Bursian SJ, Mitchell RR, Yamini B, Fitzgerald SD, Murphy PA, Fernandez G, Rottinghaus GE, Moran L, Leefers K and Choi I, 2004. Efficacy of a commercial mycotoxin binder in alleviating effects of ochratoxin A, fumonisin B1, moniliformin and zearalenone in adult mink. Veterinary and Human Toxicology, 46, 122–129.

Cai Y, McLaughlin M and Zhang K, 2020. Advancing the FDA/office of regulatory affairs mycotoxin program: new analytical method approaches to addressing needs and challenges. Journal of AOAC International, 103, 705–709. https://doi.org/10.1093/jaocint/qsz007

Chen X, Gao D, Sun F, Li Z, Wang Y, Qiu C, He K and Wang J, 2022. Nanomaterial-based aptamer biosensors for ochratoxin A detection: a review. Analytical and Bioanalytical Chemistry, 414, 2953–2969. https://doi.org/10.1007/s00216-022-03960-5

Chu FS, 1971. Interaction of ochratoxin A with bovine serum albumin. Archives of Biochemistry and Biophysics, 147, 359–366. https://doi.org/10.1016/0003-9861(71)90391-2

Cramer B, Königs M and Humpf HU, 2008. Identification and in vitro cytotoxicity of ochratoxin A degradation products formed during coffee roasting. Journal of Agricultural Food Chemistry, 56, 5673–5681. https://doi.org/10.1021/jf801296z

Denli M, Blandon JC, Guynot ME, Salado S and Perez JF, 2008. Efficacy of a new ochratoxin-binding agent (OcraTox) to counteract the deleterious effects of ochratoxin A in laying hens. Poultry Science, 87, 2266–2272. https://doi.org/10.3382/ps.2008-00024

Devreese M, Croubels S, De Baere S, Gehring R and Antonissen G, 2018. Comparative toxicokinetics and plasma protein binding of ochratoxin A in four avian species. Journal of Agricultural and Food Chemistry, 66, 2129–2135. https://doi.org/10.1021/acs.jafc.7b06048

Driehuis F, Spanjer MC, Scholten JM and te Giffel MC, 2008. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. Journal of Dairy Science, 91, 4261–4271. https://doi.org/10.3168/jds.2008-1093

Dwivedi P and Burns RB, 1985. Immunosuppressive effects of ochratoxin A in young turkeys. Avian Pathology, 14 (2), 213–225. https://doi.org/10.1080/03079458508436223

ECHA (European Chemicals Agency), 2012. Guidance on information requirements and chemical safety assessment Chapter R.8. Characterisation of dose [concentration]-response for human health. 2nd edn, Helsinki: 195 p.

EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed. EFSA Journal 2010;8(1):1457, 54 pp. https://doi.org/10.2903/j.efsa.2010.1457

EFSA (European Food Safety Authority), 2010b. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557.

EFSA (European Food Safety Authority), Arcella D and Gómez Ruiz JA, 2018. Technical report on use of cut-off values on the limits of quantification reported in datasets used to estimate dietary exposure to chemical contaminants. EFSA Supporting Publication 2018;15(7):EN-1452, 11 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1452

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

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

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk, D, Bodin, L, Chipman, JK, del Mazo, J, Grasl-Kraupp, B, Hogstrand, C, Hoogenboom, L, Leblanc, J-C, Nebbia, CS, Nielsen, E, Ntzani, E, Petersen, A, Sand, S, Schwerdtle, T, Vleminckx, C, Wallace, H, Alexander, J, Dall'Asta, C, Mally, A, Metzler, M, Binaglia, M, Horváth, Z, Steinkellner, H and Bignami, M, 2020. Scientific Opinion on the risk assessment of ochratoxin A in food. EFSA Journal 2020;18(5):6113, 150 pp. https://doi.org/10.2903/j.efsa.2020.6113

EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2011. Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA Journal 2011;9 (12):2481, 187 pp. https://doi.org/10.2903/j.efsa.2011.2481

EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798, 158 pp. https://doi.org/10.2903/j.efsa.2012.2798

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2004. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A (OTA) as undesirable substance in animal feed, EFSA Journal 2004;2(10):101, 36 pp. https://doi.org/10.2903/j.efsa.2004.101

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

El Cafsi I, Bjeoui S, Rabeh I, Nechi S, Chelbi E, El Cafsi M and Ghram A, 2020. Effects of ochratoxin A on membrane phospholipids of the intestine of broiler chickens, practical consequences. Animal, 14, 933–941. https://doi.org/10.1017/S1751731119002593

Elaroussi MA, Mohamed FR, El Barkouky EM, Atta AM, Abdou AM and Hatab MH, 2006. Experimental ochratoxicosis in broiler chickens. Avian Pathology, 35, 263–269. https://doi.org/10.1080/03079450600817115

Elaroussi MA, Mohamed FR, Elgendy MS, El Barkouky EM, Abdou AM and Hatab MH, 2008. Ochratoxicosis in broiler chickens: functional and histological changes in target organs. International Journal of Poultry Science, 7, 414–422. https://doi.org/10.3923/ijps.2008.414.422

El-Deep MH, Amber KA, Elgendy S, Dawood MAO, Elwakeel EM and Paray BA, 2020. Oxidative stress, hemato-immunological, and intestinal morphometry changes induced by ochratoxin A in APRI rabbits and the protective role of probiotics. Environmental Science and Pollution Research International, 27, 35439–35448. https://doi.org/10.1007/s11356-020-09837-3

El-Sayed YS, Khalil RH and Saad TT, 2009. Acute toxicity of ochratoxin-A in marine water-reared sea bass (Dicentrarchus labrax L.). Chemosphere, 75, 878–882. https://doi.org/10.1016/j.chemosphere.2009.01.049

European Food Safety Authority, 2007. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

Ferrufino-Guardia EV, Tangni EK, Larondelle Y and Ponchaut S, 2000. Transfer of ochratoxin A during lactation: exposure of suckling via the milk of rabbit does fed a naturally-contaminated feed. Food Additives and Contaminants, 17, 167–175. https://doi.org/10.1080/026520300283522

Fuchs R, Appelgren L-E and Hult K, 1986. Distribution of [14]Cochratoxin A in the rainbow trout (Salmo gairdneri). Acta Pharmacologica et Toxicologica, 59, 220–227. https://doi.org/10.1111/j.1600-0773.1986.tb00158.x

Gan F, Hou L, Zhou Y, Liu Y, Huang D, Chen X and Huang K, 2017. Effects of ochratoxin A on ER stress, MAPK signaling pathway and autophagy of kidney and spleen in pigs. Environmental Toxicology, 32(10), 2277–2286. https://doi.org/10.1002/tox.22443

Galtier P, Alvinerie M and Charpenteau JL, 1981. The pharmacokinetic profiles of ochratoxin A in pigs, rabbits and chickens. Food and Cosmetics Toxicology, 19, 735–738. https://doi.org/10.1016/0015-6264(81)90528-9

Ganesan AR, Balasubramanian B, Park S, Jha R, Andretta I, Bakare AG and Kim IH, 2021. Ochratoxin A: carryover from animal feed into livestock and the mitigation strategies. Animal Nutrition, 7, 56–63. https://doi.org/10.1016/j.aninu.2020.06.006

Ganesan AR, Mohan K, Karthick Rajan D, Pillay AA, Palanisami T, Sathishkumar P and Conterno L, 2022. Distribution, toxicity, interactive effects, and detection of ochratoxin and deoxynivalenol in food: a review. Food Chemistry, 378, 131978. https://doi.org/10.1016/j.foodchem.2021.131978

Gao Y, Wei J, Li X, Hu Q, Qian J, Hao N and Wang K, 2022. Region separation type bio-photoelectrode based all-solid-state self-powered aptasensor for ochratoxin A and aflatoxin B1 detection. Sensors and Actuators B: Chemical, 364, 131897. https://doi.org/10.1016/j.snb.2022.131897

Gazzotti T, Biagi G, Pagliuca G, Pinna C, Scardilli M, Grandi M and Zaghini G, 2015. Occurrence of mycotoxins in extruded commercial dog food. Animal Feed Science and Technology, 202, 81–89. https://doi.org/10.1016/j.anifeedsci.2015.02.004

Gonçalves RA, Schatzmayr D, Hofstetter U and Santos GA, 2017. Occurrence of mycotoxins in aquaculture: preliminary overview of Asian and European plant ingredients and finished feeds. World Mycotoxin Journal, 10, 183–194. https://doi.org/10.3920/WMJ2016.2111

Gonçalves RA, Naehrer K and Santos GA, 2018. Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: a real risk to aquaculture? Reviews in Aquaculture, 10, 263–280. https://doi.org/10.1111/raq.12159

González-Jartín JM, Alfonso A, Sainz MJ, Vieytes MR and Botana LM, 2021. Multi-detection method for mycotoxins with a modified QuEChERS extraction in feed and development of a simple detoxification procedure. Animal Feed Science and Technology, 272, 114745. https://doi.org/10.1016/j.anifeedsci.2020.114745

Grandi M, Vecchiato CG, Biagi G, Zironi E, Tondo MT, Pagliuca G, Palmonari A, Pinna C, Zaghini G and Gazzotti T, 2019. Occurrence of mycotoxins in extruded commercial cat food. ACS Omega, 4(9), 14004–14012. https://doi.org/10.1021/acsomega.9b01702

Grajewski J, Twarużek M and Kosicki R, 2012. High levels of ochratoxin A in blood serum and kidneys of wild boars Sus scrofa in Poland. Wildlife Biology, 18, 272–279. https://doi.org/10.2981/11-059

Griessler K, Rodrigues I, Handl J and Hofstetter U, 2010. Occurrence of mycotoxins in Southern Europe. World Mycotoxin Journal, 3(3), 301–309. https://doi.org/10.3920/WMJ2009.1198

Gross M, Bauer JI, Wegner S, Friedrich K, Machnik M and Usleber E, 2015. Mycotoxicoses in horses: toxins, occurrence in feed, and the search for markers of exposure In: Applied equine nutrition and training. Wageningen: Publisher Wageningen Academic Publishers, pp. 43–58.

Gruber-Dorninger C, Jenkins T and Schatzmayr G, 2019. Global mycotoxin occurrence in feed: a ten-year survey. Toxins (Basel), 11(7), 375. https://doi.org/10.3390/toxins11070375

Hagelberg S, Hult K and Fuchs R, 1989. Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. Journal of Applied Toxicology, 9, 91–96.

Hameed MR, Khan MZ, Khan A and Javed I, 2012. Ochratoxin induced pathological alterations in broiler chicks: effect of dose and duration. Pakistan Veterinary Journal, 8318, pp. 145–149.

Hameed MR, Khan MZ, Saleemi MK, Khan A, Akhtar M, Hassan ZU and Hussain Z, 2017. Study of ochratoxin A (OTA)-induced oxidative stress markers in broiler chicks. Toxin Reviews, 36, 270–274. https://doi.org/10.1080/15569543.2017.1303780

Hashimoto Y, Katsunuma Y, Nunokawa M, Minato H and Yonemochi C, 2015. Influence of repeated ochratoxin A ingestion on milk production and its carry-over into the milk, blood and tissues of lactating cows. Journal of Animal Science, 87, 541–546. https://doi.org/10.1111/asj.12466

Hassan ZU, Khan M, Khan A and Javed I, 2010. Pathological responses of white leghorn breeder hens kept on ochratoxin A contaminated feed. Pakistan Veterinary Journal, 30, 118–123.

Hassan ZU, Khan MZ, Khan A, Javed I and Noreen M, 2012a. In vivo and ex vivo phagocytic potential of macrophages from progeny of breeder hens kept on ochratoxin A (OTA)-contaminated diet. Journal of Immunotoxicology, 9, 64–71. https://doi.org/10.3109/1547691X.2011.635349

Hassan ZU, Khan MZ, Saleemi MK, Khan A, Javed I and Noreen M, 2012b. Immunological responses of male White Leghorn chicks kept on ochratoxin A (OTA)-contaminated feed. Journal of Immunotoxicology, 9, 56–63. https://doi.org/10.3109/1547691X.2011.627393

Hassan ZU, Khan MZ, Khan A, Javed I and Noreen M, 2012c. Ochratoxicosis in White Leghorn breeder hens: production and breeding performance. Journal of Immunotoxicology, 9, 64–71. https://doi.org/10.3109/1547691X.2011.635349

Heussner AH and Bingle LEH, 2015. Comparative ochratoxin toxicity: a review of the available data. Toxins, 7, 4253–4282.

Höhler D, Südekum KH, Wolffram S, Frohlich AA and Marquardt RR, 1999. Metabolism and excretion of ochratoxin A fed to sheep. Journal of Animal Science, 77, 1217–1223. https://doi.org/10.2527/1999.7751217x

Ibáñez-Vea M, González-Peñas E, Lizarraga E and López de Cerain A, 2011. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in barley from a northern region of Spain. Food Chemistry, 132(1), 35–42. https://doi.org/10.1016/j.foodchem.2011.10.023

Jaimez J, Fente CA, Franco CM, Cepeda A and Vázquez BI, 2004. A survey of the fungal contamination and presence of ochratoxin A and zearalenone on Spanish feed and raw materials. Journal of the Science of Food and Agriculture, 84, 832–840. https://doi.org/10.1002/jsfa.1763

Jo HW, Park MK, Heo H, Jeon HJ, Choi SD, Lee SE and Moon JK, 2021. Simultaneous determination of 13 mycotoxins in feedstuffs using QuEChERS extraction. Applied Biological Chemistry, 64, 34. https://doi.org/10.1186/s13765-021-00602-9

Kang R, Qu H, Guo Y, Zhang M, Fu T, Huang S, Zhao L, Zhang J, Ji C and Ma Q, 2023. Toxicokinetics of a single oral dose of OTA on Dezhou male donkeys. Toxins (Basel), 15, 88. https://doi.org/10.3390/toxins15020088

Khan SA, Venancio EJ, Fernandes EV, Hirooka EY, Oba A, Flaiban KKMC and Itano EN, 2018. Low doses of ochratoxin-A decrease IgY and IgA production in broiler chicks. Toxins (Basel), 10, 316. https://doi.org/10.3390/toxins10080316

Kiessling KH, Pettersson H, Sandholm K and Olsen M, 1984. Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. Applied Environmental Microbiology, 47, 1070–1073. https://doi.org/10.1128/aem.47.5.1070-1073.1984

Kihal A, Rodríguez-Prado M and Calsamiglia S, 2022. The efficacy of mycotoxin binders to control mycotoxins in feeds and the potential risk of interactions with nutrient: a review. Journal of Animal Science, 100, skac328. https://doi.org/10.1093/jas/skac328

Konak UI, Yatmaz HA, Nilüfer Ş, Erkaymaz T and Certel M, 2021. Multiresidue method for the simultaneous analysis of antibiotics and mycotoxins in feeds by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Acta Alimentaria, 50, 74–82. https://doi.org/10.1556/066.2020.00159

Kos J, Janić Hajnal E, Malachová A, Steiner D, Stranska M, Krska R, Poschmaier B and Sulyok M, 2020. Mycotoxins in maize harvested in Republic of Serbia in the period 2012-2015. Part 1: regulated mycotoxins and its derivatives. Food Chemistry, 312, 126034. https://doi.org/10.1016/j.foodchem.2019.126034

Kosicki R, Błajet-Kosicka A, Grajewski J and Twarużek M, 2016. Multiannual mycotoxin survey in feed materials and feedingstuffs. Animal Feed Science and Technology, 215, 165–180. https://doi.org/10.1016/j.anifeedsci.2016.03.012

Koszegi T and Poor M, 2016. Ochratoxin A: molecular interactions, mechanisms of toxicity and prevention at the molecular level. Toxins, 8, 111. https://doi.org/10.3390/toxins8040111

Koynarsky V, Stoev SD, Grozeva N and Mirtcheva T, 2007. Experimental coccidiosis provoked by *Eimeria adenoeides* in Turkey poults given ochratoxin A. Veterinarski Arhiv, 77, 113–128.

Kubena LF, Edrington TS, Harvey RB, Buckley SA, Phillips TD, Rottinghaus GE and Casper HH, 1997. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poultry Science, 76, 1239–1247. https://doi.org/10.1093/ps/76.9.1239

Kühn I, Valenta H and Rohr K, 1995. Determination of ochratoxin A in bile of swine by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications, 668, 333–337. https://doi.org/10.1016/0378-4347(95)00076-u

Kumar A, Jindal N, Shukla CL, Asrani RK, Ledoux DR and Rottinghaus GE, 2004. Pathological changes in broiler chickens fed ochratoxin A and inoculated with *Escherichia coli*. Avian Pathology, 33, 413–417. https://doi.org/10.1080/03079450410001724021

Kumar M, Dwivedi P, Sharma AK, Singh ND and Patil RD, 2007. Ochratoxin A and citrinin nephrotoxicity in New Zealand White rabbits: an ultrastructural assessment. Mycopathologia, 163, 21–30. https://doi.org/10.1007/s11046-006-0079-9

Leeson S and Summers JD, 2008. Commercial Poultry Nutrition, 3rd Edition. Nottingham University Press, Thrumpton, Nottingham, pp. 371–377.

Li SJ, Ediage EN, De Saeger S, Van Waeyenberghe L, Garmyn A, Verlinden M, Ducatelle R, Croubels S, Haesebrouck F, Pasmans F and Martel A, 2013. Occurrence and pathology of mycotoxins in commercial parrot feeds. World Mycotoxin Journal, 6, 449–453. https://doi.org/10.3920/WMJ2013.1570

Liesener K, Curtui V, Dietrich R, Märtlbauer E and Usleber E, 2010. Mycotoxins in horse feed. Mycotoxin Research, 26, 23–30. https://doi.org/10.1007/s12550-009-0037-8

Lippolis V, Asif S, Pascale M, Cervellieri S, Mancini E, Peli A, De Amicis I, Robbe D and Minervini F, 2020. Natural occurrence of ochratoxin A in blood and milk samples from Jennies and their foals after delivery. Toxins, 12, 758. https://doi.org/10.3390/toxins12120758

Liu X, Wu P, Jiang WD, Liu Y, Jiang J, Kuang SY, Tang L, Zhou XQ and Feng L, 2020. effects of dietary ochratoxin a on growth performance and intestinal apical junctional complex of Juvenile Grass Carp (Ctenopharyngodon idella). Toxins (Basel), 13(1), 11. https://doi.org/10.3390/toxins13010011

Liu WC, Pushpparaj K, Meyyazhagan A, Arumugam VA, Pappuswamy M, Bhotla HK, Baskaran R, Issara U, Balasubramanian B and Mousavi Khaneghah A, 2022. Ochratoxin A as an alarming health threat for livestock and human: a review on molecular interactions, mechanism of toxicity, detection, detoxification, and dietary prophylaxis. Toxicon, 15, 59–75. https://doi.org/10.1016/j.toxicon.2022.04.012

Loh ZH, Ouwerkerk D, Klieve AV, Hungerford NL and Fletcher MT, 2020. Toxin degradation by Rumen microorganisms: a review. Toxins, 12, 664. https://doi.org/10.3390/toxins12100664

Mackay N, Marley E, Leeman D, Poplawski C and Donnelly C, 2022. Analysis of aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, zearalenone, HT-2, and T-2 toxins in animal feed by LC-MS/MS using cleanup witha multi-antibody immunoaffinity column. Journal of AOAC International, 105(5), 1330–1340. https://doi.org/10.1093/jaoacint/qsac035

Malagutti L, Zannotti M, Scampini A and Sciaraffia F, 2005. Effects of Ochratoxin A on heavy pig production. Animal Research, 54, 179–184. https://doi.org/10.1051/animres:2005019

Mannin BB, Ulloa RM, Li MH, Robinson EH and Rottinghaus GE, 2003. Ochratoxin A fed to channel catfish (*Ictalurus punctatus*) causes reduced growth and lesions of hepatopancreatic tissue. Aquqculture, 219, 739–750. https://doi.org/10.1016/S0044-8486(03)00033-4

Marin DE, Motiu M, Pistol GC, Gras MA, Israel-Roming F, Calin L, Stancu M and Taranu I, 2016. Diet contaminated with ochratoxin A at the highest level allowed by EU recommendation disturbs liver metabolism in weaned piglets. World Mycotoxin Journal, 9, 587–596. https://doi.org/10.3920/WMJ2015.1993

Marin DE, Pistol GC, Gras MA, Palade ML and Taranu I, 2017. Comparative effect of ochratoxin A on inflammation and oxidative stress parameters in gut and kidney of piglets. Regulatory Toxicology and Pharmacology, 89, 224–231. https://doi.org/10.1016/j.yrtph.2017.07.031

Marin DE, Pistol GC, Gras M, Palade M and Taranu I, 2018. A comparison between the effects of ochratoxin A and aristolochic acid on the inflammation and oxidative stress in the liver and kidney of weanling piglets. Naunyn-Schmiedeberg's Archives of Pharmacology, 391, 1147–1156. https://doi.org/10.1007/s00210-018-1538-9

Marin DE, Braicu C, Dumitrescu G, Pistol GC, Cojocneanu R, Neagoe IB and Taranu I, 2019. MicroRNA profiling in kidney in pigs fed ochratoxin A contaminated diet. Ecotoxicology and Environmental Safety, 184, 109637. https://doi.org/10.1016/j.ecoenv.2019.109637

Martins HM, Marques M, Almeida I, Guerra MM and Bernardo F, 2008. Mycotoxins in feedstuffs in Portugal: an overview. Mycotoxin Research, 24(1), 19–23. https://doi.org/10.1007/BF02985266

Martins HM, Almeida I, Camacho C, Costa JM and Bernardo F, 2012. A survey on the occurrence of ochratoxin A in feeds for swine and laying hens. Mycotoxin Research, 28(2), 107–110. https://doi.org/10.1007/s12550-011-0118-3

Meucci V, Luci G, Vanni M, Guidi G, Perondi F and Intorre L, 2017. Serum levels of ochratoxin A in dogs with chronic kidney disease (CKD): a retrospective study. Journal of Veterinary Science, 79(2), 440–447. https://doi.org/10.1292/jvms.16-0226

Mikula P, Blahova J, Honzlova A, Kalinova J, Macharackova P, Rosmus J, Svoboda Z and Svoboda M, 2020. Occurrence of mycotoxins in complete poultry feeds in The Czech Republic – Multiannual survey (2013–2018). Veterinární Medicína, 65, 487–494. https://doi.org/10.17221/109/2020-VETMED

Minervini F, Giannoccaro A, Nicassio M, Panzarini G and Lacalandra GM, 2013. First evidence of placental transfer of ochratoxin A in horses. Toxins (Basel), 5, 84–92. https://doi.org/10.3390/toxins5010084

Mir MS and Dwivedi P, 2010. Ochratoxin A-induced serum biochemical alterations in New Zealand white rabbits (*Oryctolagus cuniculus*). Turkish Journal of Veterinary and Animal Sciences, 34, 525–531. https://doi.org/10.3906/vet-0901-23

Mobashar M, Hummel J, Blank R and Südekum KH, 2010. Ochratoxin A in ruminants—A review on its degradation by gut microbes and effects on animals. Toxins (Basel), 2, 809–839. https://doi.org/10.3390/toxins2040809

Mobashar M, Blank R, Hummel J, Westphal A, Tholen E and Südekum KH, 2012. Ruminal ochratoxin A degradation—Contribution of the different microbial populations and influence of diet. Animal Feed Science and Technology, 171, 85–97. https://doi.org/10.1016/j.anifeedsci.2011.10.002

Monbaliu S, Van Poucke C, Detavernier C, Dumoulin F, Van De Velde M, Schoeters E, Van Dyck S, Averkieva O, Van Peteghem C and De Saeger S, 2010. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. Journal of Agricultural and Food Chemistry, 58, 66–71. https://doi.org/10.1021/jf903859z

Müller HM, Klara Müller K and Steingass H, 2001. Effect of feeding regime on the metabolism of ochratoxin a during the in vitro incubation in buffered rumen fluid from cows. Archiv für Tierernaehrung, 54, 265–279. https://doi.org/10.1080/17450390109381984

Nácher-Mestre J, Serrano R, Beltrán E, Pérez-Sánchez J, Silva J, Karalazos V, Félix Hernández F and Berntssen MHG, 2015. Occurrence and potential transfer of mycotoxins in gilthead sea bream and Atlantic salmon by use of novel alternative feed ingredients. Chemosphere, 128, 314–320. https://doi.org/10.1016/j.chemosphere.2015.02.021

Nakhjavan B, Ahmed NS and Khosravifard M, 2020. Development of an improved method of sample extraction and quantitation of multi-mycotoxin in feed by LC-MS/MS. Toxins, 12, 462. https://doi.org/10.3390/toxins12070462

Nedeljković-Trailović J, Trailović S, Dimitrijević M and Ilić V, 2013. Blood serum protein status in broilers fed with increasing concentrations of ochratoxin A. Acta veterinaria, 63, 77–88. https://doi.org/10.2298/AVB1301077N

Niemiec J, Stępińska M, Riedel J and Szeleszczuk P, 2005. The effect of feeding ochratoxin A-contaminated diet to broiler breeding flocks on the chickens performance. Journal of Animal and Feed Sciences, 14, 471–474. https://doi.org/10.22358/jafs/70709/2005

Nip WK and Chu FS, 1979. Fate of ochratoxin A in goats. Journal of Environmental Science and Health Part B, 14, 319–333. https://doi.org/10.1080/03601237909372131

Nochetto CB and Li H, 2022. A dilute-and-shoot UHPLC–MS/MS isotope dilution method for simultaneous determination and confirmation of 11 mycotoxins in dried distiller's grains with solubles. Journal of AOAC International, 105, 95–106. https://doi.org/10.1093/jaoacint/qsab111

NRC (National Research Council), 2006. Nutrient Requirements of Dogs and Cats. National Academies Press, Washington, DC. 424 p..

Nualkaw K, Poapolathep S, Zhang Z, Zhang Q, Giorgi M, Li P, Logrieco AF and Poapolathep A, 2020. Simultaneous determination of multiple mycotoxins in swine, poultry and dairy feeds using ultra high performance liquid chromatography-tandem mass spectrometry. Toxins, 12, 253. https://doi.org/10.3390/toxins12040253

Panasiuk L, Jedziniak P, Pietruszka K, Piatkowska M and Bocian L, 2018. Frequency and levels of regulated and emerging mycotoxins in silage in Poland. Mycotoxin Research, 35(1), 17–25. https://doi.org/10.1007/s12550-018-0327-0

Penagos-Tabares F, Khiaosa-ard R, Nagl V, Faas J, Jenkins T, Sulyok M and Zebeli Q, 2021. Mycotoxins, phytoestrogens and other secondary metabolites in Austrian pastures: occurrences, contamination levels and implications of geo-climatic factors. Toxins, 13, 460. https://doi.org/10.3390/toxins13070460

Pietruszka K, Piątkowska M and Jedziniak P, 2017. Occurrence of Ochratoxin a in Animal Tissues and Feeds in Poland in 2014-2016. Journal of Veterinary Research, 61(4), 483–487. https://doi.org/10.1515/jvetres-2017-0071

Pleadin J, Persi N, Mitak M, Terzic S, Milic D, Vulic A and Brstilo M, 2012. Biochemical changes in pig serum after ochratoxin A exposure. Bulletin of Environmental Contamination and Toxicology, 88, 1043–1047. https://doi.org/10.1007/s00128-012-0615-x

Pleadin J, Kudumija N, Kovačević D, Scortichini G, Milone S and Kmetič I, 2016. Comparison of ochratoxin A levels in edible pig tissues and in biological fluids after exposure to a contaminated diet. Mycotoxin Research, 32, 145–151. https://doi.org/10.1007/s12550-016-0249-7

Pozzo L, Cavallarin L, Nucera D, Antoniazzi S and Schiavone A, 2010. A survey of ochratoxin A contamination in feeds and sera from organic and standard swine farms in northwest Italy. Journal of the Science of Food and Agriculture, 90, 1467–1472. https://doi.org/10.1002/jsfa.3965

Pozzo L, Salamano G, Mellia E, Gennero MS, Doglione L, Cavallarin L, Tarantola M, Forneris G and Schiavone A, 2013a. Feeding a diet contaminated with ochratoxin A for chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 1. Effects on growth and slaughter performance, haematological and serum traits. Journal of Animal Physiology and Animal Nutrition (Berlin), 97(Suppl. 1), 13–22. https://doi.org/10.1111/jpn.12050

Pozzo L, Cavallarin L, Antoniazzi S, Guerre P, Biasibetti E, Capucchio MT and Schiavone A, 2013b. Feeding a diet contaminated with ochratoxin A for broiler chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 2. Effects on meat quality, oxidative stress, residues and histological traits. Journal of Animal Physiology and Animal Nutrition (Berlin), 97(Suppl. 1), 23–31. https://doi.org/10.1111/jpn.12051

Rao TP, Manasa Varra M and Kumar TV-S, 2018. Effect of dietary ochratoxin on body weight and biochemical changes in broiler chicks. The Pharma Innovation Journal, 7(4), 947–950.

Raja K, Saikumar G, Sharma R and Dwivedi P, 2008. OTA in swine: clinical and pathological changes following prolonged exposure to OTA. The Indian Journal of Animal Science, 78, 922–928.

Ringot D, Chango A, Schneider YJ and Larondelle Y, 2006. Toxicokinetics and toxicodynamics of ochratoxin A, an update. Chemico-Biological Interactions, 159, 18–46. https://doi.org/10.1016/j.cbi.2005.10.106

Rodrigues I and Naehrer K, 2012. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins (Basel), 4(9), 663–675. https://doi.org/10.3390/toxins4090663

Rokvić N, Aksentijević K, Kureljušić J, Vasiljević M, Todorović N, Zdravković N and Stojanac N, 2020. Occurrence and transfer of mycotoxins from ingredients to fish feed and fish meat of common carp (Cyprinus carpio) in Serbia. World Mycotoxin Journal, 13, 545–552. https://doi.org/10.3920/WMJ2020.2580

Sailaja N, Balachandran C, Murali Manohar B, Sridhar R, Kumanan K and Lakshman M, 2017. Ultrastructural changes in low grade ochratoxicosis in broiler chicken. Indian Veterinary Journal, 94, 79–80.

Sakthivelan SM and Sudhakar Rao GV, 2010. Effect of ochratoxin a on body weight, feed intake and feed conversion in broiler chicken. Veterinary Medicine International, 27, 590432. https://doi.org/10.4061/2010/590432

Schiavone A, Cavallero C, Girotto L, Pozzo L, Antoniazzi S and Cavallarin L, 2008. A survey on the occurrence of Ochratoxin A in feeds and sera collected in conventional and organic poultry farms in Northern Italy. Italien Journal of Animal Science, 7, 495–503. https://doi.org/10.4081/ijas.2008.495

Seo H, Jang S, Jo H, Kim H, Lee S, Yun H, Jeong M, Moon J, Na T and Cho H, 2021. Optimization of the QuEChERS-Based Analytical Method for Investigation of 11 Mycotoxin Residues in Feed Ingredients and Compound Feeds. Toxins, 13, 767. https://doi.org/10.3390/toxins13110767

Singh R, Mandal AB, Sharma M and Biswas A, 2015. Effect of varying levels of dietary ochratoxin A on the performance of broiler chickens. Indian Journal of Animal Sciences, 85, 296–300.

Śliżewska K, Markowiak-Kopeć P, Sip A, Lipiński K and Mazur-Kuśnirek M, 2020. The effect of using new synbiotics on the Turkey performance, the intestinal microbiota and the fecal enzymes activity in Turkeys fed ochratoxin A contaminated feed. Toxins (Basel), 12, 578. https://doi.org/10.3390/toxins12090578

Solcan C, Timofte D, Floristean VC, Carter SD and Solcan G, 2013a. Ultrastructural lesions and immunohistochemical analysis of Bcl-2 protein expression in the kidney of chickens with experimental ochratoxicosis. Acta Vet Hung, 61, 344–353. https://doi.org/10.1556/Avet.2013.021

Solcan C, Viorel F, Pavel G and Solcan G, 2013b. Induced malabsorbtion in chickens by experimental administration of ochratoxin A. Current Opinion in Biotechnology, 24, 105. https://doi.org/10.1016/j.copbio.2013.05.321

Sreemannarayana O, Frohlich AA, Vitti TG, Marquardt RR and Abramson D, 1988. Studies of the tolerance and disposition of ochratoxin A in young calves. Journal of Animal Science, 66, 1703–1711. https://doi.org/10.2527/jas1988.6671703x

Stoev DMF, Njobehb PB, Mosonikb JS and Steenkamp PA, 2009. Mycotoxic nephropathy in Bulgarian pigs and chickens: complex aetiology and similarity to Balkan endemic nephropathy. Food Additives and Contaminants, Part A: Chemistry Analysis Control Exposure & Risk Assessment, 27(1), 72–88.

Stoev SD, 2010. Studies on carcinogenic and toxic effects of ochratoxin A in chicks. Toxins (Basel), 2, 649–664. https://doi.org/10.3390/toxins2040649

Stormer FC, Storen O, Hansen CE, Pedersen JI and Aarsen AJ, 1983. Formation of (4R)- and (4S)-hydroxyochratoxin A and 10- ochratoxin A from ochratoxin A by rabbit liver microsomes. Applied and Environmental Microbiology, 45, 1183–1187. https://doi.org/10.1128/aem.45.4.1183-1187.1983

Sueck F, Hemp V, Specht J, Torres O, Cramer B and Humpf HU, 2019. Occurrence of the ochratoxin A degradation product 2'R-ochratoxin A in coffee and other food: an update. Toxins, 11, 329–338.

Svoboda M, Blahová J, Honzlová A, Kalinová J, Macharáčková P, Rosmus J, Mejzlík V, Kúkol P, Vlasáková V and Mikulková K, 2019. Multiannual occurrence of mycotoxins in feed ingredients and complete feeds for pigs in The Czech Republic. Acta Veterinaria, 88, 291–301. https://doi.org/10.2754/avb201988030291

Szeleszczuk P, Niemiec J, Karpińska E and Bielecki W, 2007. Influence of different levels of ochratoxin A in the diet on morphological changes in immunological system and humoral and cellular immunity in hens and their progeny. Poultry Archives, 71(1), 19–24.

Tabuc C, Marin D, Guerre P, Sesan T and Bailly JD, 2009. Molds and mycotoxin content of cereals in southeastern Romania. Journal of Food Protection, 72(3), 662–665. https://doi.org/10.4315/0362-028x-72.3.662

Tao Y, Xie S, Xu F, Liu A, Wang Y, Chen D, Pan Y, Huang L, Peng D, Wang X and Yuan Z, 2018. Ochratoxin A: toxicity, oxidative stress and metabolism. Food and Chemical Toxicology, 112, 320–331. https://doi.org/10.1016/j.fct.2018.01.002

Tenbrink KM, Schulz J and Kemper N, 2020. Mycotoxin contamination of selected organic enrichment materials used in pig farming. Agriculture, 10, 565. https://doi.org/10.3390/agriculture10110565

Tittlemier SA, Cramer B, Dall'Asta C, Iha MH, VMT L, Maragos C, Solfrizzo M, Stranska M, Stroka J and Sumarah M, 2020. Developments in mycotoxin analysis: an update for 2018–19. World Mycotoxin Journal, 13, 3–24. https://doi.org/10.3920/WMJ2019.2535

Tkaczyk A, Jedziniak P, Zielonka Ł, Dąbrowski M, Ochodzki P and Rudawska A, 2021. Biomarkers of deoxynivalenol, citrinin, ochratoxin A and zearalenone in pigs after exposure to naturally contaminated feed close to guidance values. Toxins (Basel), 13, 750. https://doi.org/10.3390/toxins13110750

Tolosa J, Rodríguez-Carrasco Y, Mj R and Vila-Donat P, 2021. Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: a review. Food and Chemical Toxicology, 158, 112661. https://doi.org/10.1016/j.fct.2021.112661

Twarużek M, Skrzydlewski P, Kosicki R and Grajewski J, 2021. Mycotoxins survey in feed materials and feedingstuffs in years 2015-2020. Toxicon, 202, 27–39. https://doi.org/10.1016/j.toxicon.2021.09.005

Upadhaya S, Park M and Ha J, 2010. Mycotoxins and their biotransformation in the rumen: a review animal. Bioscience, 23, 1250–1260. https://doi.org/10.5713/ajas.2010.r.06

Upadhaya S, Yang L, Seo J, Kim M, Lee C, Lee CH and Ha JK, 2011. Effect of feed types on ochratoxin A disappearance in goat rumen fluid. Animal Bioscience, 24, 98–205. https://doi.org/10.5713/ajas.2011.10318

Upadhaya SD, Song JY, Park MA, Seo JK, Yang L, Lee CH, Cho KJ and Ha JK, 2012. Isolation, screening and identification of Swine gut microbiota with ochratoxin a biodegradation ability. Asian-Australasian Journal of Animal Sciences, 25, 114–121. https://doi.org/10.5713/ajas.2011.11104

Urbani D, Centonze D, Varchi G, Evangelisti M, Parenti MD and Del Rio A, 2023. Extensive literature searches on bromine and ochratoxin A in feed. EFSA Supporting Publication 2023;20(3):EN-7938, 24 pp. https://doi.org/10.2903/sp.efsa.2023.EN-7938

Vettorazzi A, Gonzales-Penas E and Lopez de della A, 2014. Ochratoxin A kinetics: a review of analytical methods and studies in rat model. Food and Chemical Toxicology, 72, 273–288.

Wang W, Zhai S, Xia Y, Wang H, Ruan D, Zhou T, Zhu Y, Zhang H, Zhang M, Ye H, Ren W and Yang L, 2019. Ochratoxin A induces liver inflammation: involvement of intestinal microbiota. Microbiome, 7, 151. https://doi.org/10.1186/s40168-019-0761-z

Wangikar PB, Dwivedi P, Sinha N, Sharma AK and Telang AG, 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. Toxicology, 215, 37–47. https://doi.org/10.1016/j.tox.2005.06.022

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2008. Uncertainty and data quality in exposure assessment. Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. International Programme on Chemical Safety (IPCS), IPCS harmonization project document no. 6. Available online: https://www.who.int/publications/i/item/9789241563765

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. International Programme on Chemical Safety (IPCS), Environmental Health Criteria 240 Available online: https://www.who.int/publications/i/item/9789241572408

Xiao H, Marquardt RR, Frohlich AA, Phillips GD and Vitti TG, 1991a. Effect of a hay and a grain diet on the rate of hydrolysis of ochratoxin A in the rumen of sheep. Journal of Animal Science, 69, 3706–3714. https://doi.org/10.2527/1991.6993706x

Xiao H, Marquardt RR, Frohlich AA, Phillips GD and Vitti TG, 1991b. Effect of a hay and a grain diet on the bioavailability of ochratoxin A in the rumen of sheep. Journal of Animal Science, 69, 3715–3723. https://doi.org/10.2527/1991.6993715x

Xiao H, Marquardt RR, Abramson RR and Frohlich AA, 1996. Metabolites of ochratoxins in rat urine and in a culture of Aspergillus ochraceus. Applied and Environmental Microbiology, 62, 648–655. https://doi.org/10.1128/aem.62.2.648-655.1996

Yang S, Zhang H, De Saeger S, De Boevre M, Sun F, Zhang S, Cao X and Wang Z, 2015. In vitro and in vivo metabolism of ochratoxin A: a comparative study using ultra-performance liquid chromatography-quadrupole/time-of-flight hybrid mass spectrometry. Analytical and Bioanalytical Chemistry, 407, 3579–3589. https://doi.org/10.1007/s00216-015-8570-0

Zahran E, Manning B, Seo JK and Noga EJ, 2016. The effect of Ochratoxin A on antimicrobial polypeptide expression and resistance to water mold infection in channel catfish (*Ictalurus punctatus*). Fish & Shellfish Immunology, 57, 60–67. https://doi.org/10.1016/j.fsi.2016.08.034

Zapaśnik A, Bryła M, Waśkiewicz A, Ksieniewicz-Woźniak E and Podolska G, 2022. Ochratoxin A and 2'R-Ochratoxin A in selected foodstuffs and dietary risk assessment. Molecules, 27, 188. https://doi.org/10.3390/molecules27010188

Zepnik H, Pahler A, Schauer U and Dekant W, 2001. Ochratoxin A-induced tumor formation: is there a role of reactive ochratoxin A metabolites? Toxicological Sciences, 59, 59–67. https://doi.org/10.1093/toxsci/59.1.59

Zhai S, Zhu Y, Feng P, Li M, Wang W, Yang L and Yang Y, 2021. Ochratoxin A: its impact on poultry gut health and microbiota, an overview. Poultry Science, 100, 101037. https://doi.org/10.1016/j.psj.2021.101037

Zhang Z, Fan Z, Nie D, Zhao Z and Han Z, 2019. Analysis of the carry-over of Ochratoxin A from feed to milk, blood, urine, and different tissues of dairy cows based on the establishment of a reliable LC-MS/MS Method. Molecules, 24, 2823. https://doi.org/10.3390/molecules24152823

Zhang B, Liu W, Liu Z, Fu X and Du D, 2022. Establishment of a chemiluminescence immunoassay combined with immunomagnetic beads for rapid analysis of Ochratoxin A. Journal of AOAC International, 105, 346–351. https://doi.org/10.1093/jaoacint/qsab104

Zhao P, Liu X, Jiang WD, Wu P, Liu Y, Jiang J, Zhang L, Mi HF, Kuang SY, Tang L, Zhou XQ and Feng L, 2022. The multiple biotoxicity integrated study in grass carp (Ctenopharyngodon della) caused by Ochratoxin A: oxidative damage, apoptosis and immunosuppression. Journal of Hazardous Materials, 436, 129268. https://doi.org/10.1016/j.jhazmat.2022.129268

Zachariasova M, Dzuman Z, Veprikova Z, Hajkova K, Jiru M, Vaclavikova M, Zachariasova A, Pospichalova M, Florian M and Hajslova J, 2014. Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. Animal Feed Science and Technology, 193, 124–140. https://doi.org/10.1016/j.anifeedsci.2014.02.007

Abbreviations

A/G	Albumine/Globuline
Ab	antibody
ADME	Absorption, Distribution, Metabolism, Excretion and Toxicology
AUC	area under the curve
BMDL	benchmark dose (lower confidence limit)
bw	body weight
CF	crude fat
C_{max}	maximum concentration
CONTAM	Panel on Contaminants in the Food Chain
CP	crude protein
CYP	cytochrome P450 enzymes
DM	dry matter
DPI	day post-infection
ECHA	European chemical agency
ELISA	enzyme-linked immunosorbent assay
ELS	extensive literature search
FGR	feed to gain ratio
GOT	glutamic oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
GPx	glutathione peroxidase
HBGV	health-based guidance value
HPLC	high pressure liquid chromatography
IPCS	International Programme on Chemical Safety
i.v.	intravenous
k_a	first-order rate constant for the absorption of the drug in the central compartment
LC	lethal Concentration
LC-FLD	Liquid Chromatography – fluorescence detector
LC–MS	Liquid Chromatography – Mass Spectroscopy
LD_{50}	median Lethal Dose
LDH	lactate dehydrogenase
LOAEL	Lowest Observed Adverse Effect Level
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	Limit of quantification
MOE	margin of exposure
MS	Mass Spectroscopy
NADPH	nicotinamide adenine dinucleotide phosphate
NDV	Newcastle disease virus
NO	nitric oxide
NOEL	no adverse effect level
NOAEL	no observed adverse effect level
OTA	ochratoxin A
OTalpha	ochratoxin alpha
OTB	ochratoxin B (dechlorinated metabolite of OTA)
PBS	phosphate buffered saline
PCV	packed cell volume
PHA	phytohemagglutinin
Q-TOF	quadrupole time-of-flight

QuEChERS	Quick Easy Cheap Efficient Robust Safe
RP	Reference point
SOD	superoxide dismutase
SRBC	sheep red blood cells
$t_{1/2}$	half-life (the time taken a drug to decrease by half compared to the highest concentration)
$T_{1/2el}$	elimination half-life
TAC	total antioxidant capacity
TAS	total antioxidant status
TK	Toxicokinetics
TLC	thin layer chromatography
t_{max}	time to reach C_{max}
UF	uncertainty factor
UPLC	ultra-high performance liquid chromatography
Vd	volume of distribution
WG	weight gain
WHO	World Health Organization
ww	wet weight

Appendix A – Intakes and composition of diets used to estimate animal exposure to OTA. Estimanted OTA concentrations

The feed intake and the diet composition used to estimate the exposure to OTA of the animal species considered in this report were derived from information extensively described by the CONTAM Panel in previous Scientific Opinions on the risks for animal and public health (EFSA CONTAM Panel, 2011, 2012) and modified by the CONTAM Panel in May 2023 in line with current with common practices and published guidelines. In particular, the amendments aimed at a harmonisation between CONTAM Panel and the FEEDAP Panel when dealing with compounds in feed. The estimated feed intakes are based on published guidelines on nutrition and feeding (NRC, 2006; Leeson and Summers, 2008; EFSA FEEDAP Panel, 2017).

Diets, feed intakes and body weights for the various animal species and categories are summarised in this Appendix.

In addition, the diets for food producing and non-food producing animals also include the calculated lower-bound (LB) and upper-bound (UB) mean and high concentrations for OTA, based on the LB and UB mean and high (P90–P95) concentrations in the feedingstuff reported in Table 7.

A.1. Feed intake

A.1.1. Cattle, sheep, goats and horses

Table A.1: Default values for live weight and feed intake of ruminants and horses

	Live weight (kg)	Feed intake (kg/day)		Reference
		Dry matter (DM)	Complete feed* (CF)	
Cattle				
Dairy cows	650	20.0	22.7	EFSA (2017)
Cattle for fattening	400	8.0	9.2	EFSA (2017)
Veal calves	100	1.89	2.0**	EFSA (2017)
Small ruminants				
Dairy sheep/goat	60.0	1.20	1.36	EFSA (2017)
Lambs for fattening	20.0	1.10	1.25	NRC (2006)
Horses				
All categories	400	8.00	9.1	EFSA (2017)

*: 88% dry matter.
**: Milk replacer (94.5% dry matter).

A.1.2. Pigs, poultry, fish and rabbit

Table A.2: Default values for live weight and feed intake of pigs, poultry, fish and rabbits

	Live weight [kg]	Feed intake [kg/day]		Reference
		100% Dry matter (DM)	Complete feed (CF)	
Pigs				
Piglets (weaned)	20	0.88	1.0	EFSA (2017)
Pigs for fattening	60	2.20	2.5	EFSA (2017)
Sows, lactating	175	5.28	6.0	EFSA (2017)
Poultry				
Chickens for fattening	2.0	0.158	0.18	EFSA (2017)
Laying hens	2.0	0.106	0.12	EFSA (2017)
Turkeys for fattening	3.0	0.176	0.20	EFSA (2017)

	Live weight [kg]	Feed intake [kg/day]		Reference
		100% Dry matter (DM)	Complete feed (CF)	
Ducks, fattening	3.0	0.132	0.15	Leeson and Summers (2008)
Fish				
Salmonids	0.12	0.0021	0.0024	EFSA (2017)
Rabbits				
Rabbits for fattening	2.0	0.10	0.114	EFSA (2017)

A.1.3. Dogs and cats

Table A.3: Default values for live weight and feed intake of dogs and cats

	Live weight (kg)	Feed intake (kg/day)		Reference
		Dry matter (DM)	Complete feed (CF)	
Dogs	15	0.25	0.284	EFSA (2017)
Cats	3	0.06	0.068	EFSA (2017)

A.2. Diet composition and OTA concentration estimates

A.2.1. Cattle, sheep, goats and horses

Table A.5: Compositions of feed for bovines using feed materials, and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy cow	Cattle for fattening		Dairy cow	Cattle for fattening
Cereal grains and products derived thereof	55	60	Wheat	15	
			Wheat feed	10	10
			Barley	20	40
			Maize	10	10
Oil seeds, oil fruits and products derived thereof	26	22	Soybean meal	5	
			Rapeseed meal	20	20
			Vegetable oils and fats	1	2
Tubers, roots and products derived thereof	11	15	Sugar beet pulp	8	12
			Molasses	3	3
Legume seeds and products derived thereof	5		Horse beans	5	
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
Concentrate:Forages[a]				70:30	20:80
OTA[b]					
Mean lower bound (μg/kg DM)				1.8	1.1
Mean upper bound (μg/kg DM)				3.8	4.7
High lower bound (μg/kg DM)				3.2	1.6
High upper bound (μg/kg DM)				9.2	14.3

(a): The ratio of concentrate to forages on a dry matter basis defines the daily ration.
(b): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.6: Compositions of feed for caprines using feed materials, and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy goat	Kids for fattening		Dairy goat	Kids for fattening
Cereal grains and products derived thereof	70	70	Wheat feed	10	10
			Barley	25	20
			Oats	35	40
Oil seeds, oil fruits and products derived thereof	22	22	Soybean meal	10	10
			Rapeseed meal	10	10
			Vegetable oils and fats	2	2
Tubers, roots and products derived thereof	5	5	Sugar beet pulp	2	2
			Molasses	3	3
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
Concentrate:Forages[a]				35:65	40:60
OTA[b]					
Mean lower bound (μg/kg DM)			1.7		1.2
Mean upper bound (μg/kg DM)			3.4		3.6
High lower bound (μg/kg DM)			3.9		2.3
High upper bound (μg/kg DM)			8.1		10.5

(a): The ratio of concentrate to forages on a dry matter basis defines the daily ration.
(b): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.7: Compositions of feed for ovines using feed materials, and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy sheep	Lambs for fattening		Dairy sheep	Lambs for fattening
Cereal grains and products derived thereof	47	70	Wheat	14	20
			Wheat feed	15	10
			Barley	18	20
			Oats		20
Oil seeds, oil fruits and products derived thereof	20	20	Soybean meal	4	4
			Rapeseed meal	10	10
			Sunflower meal	5	5
			Vegetable oils and fats	1	1
Tubers, roots and products derived thereof	20	5	Sugar beet pulp	10	2
			Molasses	5	3
Legume seeds and products derived thereof	10	2	Beans	10	2
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives				0.5	0.5
Concentrate:Forages[a]				35:65	50:50
OTA[b]					
Mean lower bound (μg/kg DM)				1.4	1.4
Mean upper bound (μg/kg DM)				3.8	3.6
High lower bound (μg/kg DM)				2.1	2.8

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	Dairy sheep	Lambs for fattening		Dairy sheep	Lambs for fattening
High upper bound (μg/kg DM)				10.8	10.0

(a): The ratio of concentrate to forages on a dry matter basis defines the daily ration.
(b): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.8: Compositions of feed for horses using feed materials, and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	82	Oats	40
		Oat feed	12
		Wheat feed	30
Tubers, roots and products derived thereof	5	Molasses	5
Legume seeds and products derived thereof	10	Beans	10
Minerals and products derived thereof	2.5	Mineral salts	2.5
Feed additives	0.5	Premix	0.5
Concentrate:Forages[a]			25:75
OTA[b]			
Mean lower bound (μg/kg DM)			1.2
Mean upper bound (μg/kg DM)			3.8
High lower bound (μg/kg DM)			1.5
High upper bound (μg/kg DM)			11.3

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).
(b): The ratio of concentrate to forages on a dry matter basis defines the daily ration.

A.2.2. Pigs, poultry, fish and rabbit

Table A.9: Diet compositions for piglets. pigs for fattening and lactating sows and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet			Feed material	Composition (%)		
	Piglet	Pig	Sow		Piglet	Pig	Sow
Cereal grains and products derived thereof	68	77	75	Wheat	48	48	50
				Wheat feed		9	14
				Barley	20	20	11
Oil seeds, oil fruits and products derived thereof	26	16	18	Soybean meal	22	11	16
				Rapeseed meal	3	4	
				Vegetable oils and fats	1	1	2
Tubers, roots and products derived thereof	3	4	4	Sugar beet pulp			
				Molasses	3	4	4
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix	0.5	0.5	0.5
OTA[a]							
Mean lower bound (μg/kg DM)					1.6	1.8	1.6
Mean upper bound (μg/kg DM)					2.8	3.0	2.8
High lower bound (μg/kg DM)					4.0	4.1	3.5
High upper bound (μg/kg DM)					6.2	6.1	5.6

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.10: Diet compositions for chickens, turkeys and ducks for fattening and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet			Feed material	Composition (%)		
	Chickens	Turkeys	Ducks		Chickens	Turkeys	Ducks
	For fattening				For fattening		
Cereal grains and products derived thereof	75	65	65	Wheat	38	30	35
				Wheat feed	1		5
				Barley		35	25
				Maize	36		
Oil seeds, oil fruits and products derived thereof	20	20	20	Soybean meal	15	16	18
				Vegetable oils and fats	5	4	2
Tubers, roots and products derived thereof	2	2	3	Molasses	2	2	3
Forage dehydrated		10	9	Lucerne meal		10	9
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix	0.5	0.5	0.5
OTA[a]							
Mean lower bound (μg/kg)					1.2	3.1	2.7
Mean upper bound (μg/kg)					2.6	4.4	4.1
High lower bound (μg/kg)					4.1	5.9	5.2
High upper bound (μg/kg)					5.7	7.5	7.0

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.11: Diet compositions for laying hens and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	65	Maize	25
		Wheat	40
		Wheat middlings	0
Oil seeds, oil fruits and products derived thereof	20	Soybean meal	10
		Rapeseed	8
		Vegetable oils and fats	2
Forage dehydrated	3	Lucerne meal	3
Tubers, roots and products derived thereof	2	Molasses	2
Minerals and products derived thereof	9.5	Mineral salts	9.5
Feed additives	0.5	Premix	0.5
OTA[a]			
Mean lower bound (μg/kg DM)			1.2
Mean upper bound (μg/kg DM)			2.5
High lower bound (μg/kg DM)			3.5
High upper bound (μg/kg DM)			5.5

Ash corrected: High dietary calcium necessary for egg shell therefore major changes made.

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7). When high amount of LC data caused the highest reliable percentile to be lower than the mean, only the mean concentrations were used to calculate both exposures.

Table A.12: Diet compositions for salmons* and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Fish, other aquatic animals and products derived thereof	60	Fish meal	33
		Fish oil	23
		Fish ensiled	4
Oil seeds, oil fruits and by-products	27	Soybean protein	15
		Vegetable oil and fat	12
Cereal grains products derived thereof	10	Wheat gluten	10
Minerals and products derived thereof	3	Mineral salts	3
Feed additives		Premix	
OTA[a]			
Mean lower bound (μg/kg DM)			0.2
Mean upper bound (μg/kg DM)			0.5
High lower bound (μg/kg DM)			0.2
High upper bound (μg/kg DM)			0.5

*: Ellingsen H, Olaussen JO and Utne IB, 2009. Environmental analysis of the Norwegian fishery and aquaculture industry—A preliminary study focusing on farmed salmon. Marine Policy, 33, 479–488.

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.13: Diet compositions for rabbits for fattening and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Cereal grains and products derived thereof	25	Wheat	20
		Corn	5
		Wheat middlings	0
Forage dehydrated	20	Alfalfa meal	20
Oil seeds, oil fruits and products derived thereof	30	Sunflower meal	20
		Soybean meal	3
		Soya (bean) hulls	7
Tubers, roots and products derived thereof	20	Sugar beet pulp	18
		Molasses	2
Land animal products and products derived thereof	2	Fat	2
Minerals and products derived thereof	2.5	Mineral salts	2.5
Feed additives	0.5	Premix	0.5
OTA[a]			
Mean lower bound (μg/kg DM)			2.8
Mean upper bound (μg/kg DM)			4.0
High lower bound (μg/kg DM)			5.2
High upper bound (μg/kg DM)			6.4

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

A.2.3. Dogs and cats

Table A.14: Diet compositions for dogs and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet		Feed material	Composition (%)	
	With meat	Vegetarian		With meat	Vegetarian
Land animal products and products derived thereof	35		Animal by-products	24	
			Fish meal	5	
			Hydrolysed animal products[a]	1	
			Fat	5	
Cereal grains and products derived thereof	50	45	Rice	20	40
			Oats	10	
			Barley	10	
			Maize protein	10	5
Oil seeds, oil fruits and products derived thereof	4	20	Sunflower meal		5
			Soybean meal	1	10
			Vegetable oil and fat	3	5
Tubers, roots and products derived thereof	5	15	Sugar beet pulp	5	5
			Maize protein		10
Forages and roughage, and products derived thereof	3	2	Herbs	3	2
Legume seeds and products derived thereof		10	Peas		5
			Carobs		5
Milk products and products derived thereof		5	Milk protein powder		5
Minerals and products derived thereof	2.5	2.5	Mineral salts	2.5	2.5
Feed additives	0.5	0.5	Premix	0.5	0.5
OTA[b]					
Mean lower bound (μg/kg DM)				1.1	0.7
Mean upper bound (μg/kg DM)				2.2	1.8
High lower bound (μg/kg DM)				2.4	1.1
High upper bound (μg/kg DM)				3.3	2.4

(a): Includes poultry meal, lambs meal and fish meal.
(b): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

Table A.15: Diet compositions for cats and calculated mean and high lower-bound and upper-bound levels of OTA in these diets

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Land animal products and products derived thereof	40	Animal by-products*	35
		Hydrolysed animal products*	1
		Fat	4
Cereal grains and products derived thereof	30	Rice	10
		Wheat	10
		Maize protein	10
Oil seeds, oil fruits and products derived thereof	5	Soybean meal	5
Tubers, roots and products derived thereof	12	Sugar beet pulp	2
		Maize protein	10

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition (%)
Legume seeds and products derived thereof	10	Peas	5
		Carobs	5
Minerals and products derived thereof	2.5	Mineral salts	2.5
Feed additives	0.5	Premix	0.5
OTA[a]			
Mean lower bound (μg/kg DM)			1.0
Mean upper bound (μg/kg DM)			1.8
High lower bound (μg/kg DM)			1.2
High upper bound (μg/kg DM)			2.4

*: Includes poultry meal, lamb meal and fish meal.

(a): OTA concentration (DM) present in the diets calculated by using the mean or the high concentrations (the highest reliable percentile based on the number of samples available) reported for the individual feeds (Table 7).

A.3. OTA concentration estimates from compound feed (complementary or complete feeds)

Table A.16: OTA concentration in complete feed[b] (mean and high lower-bound and upper-bound levels) for all animal species

Animal species	OTA Concentrations μg/kg DM			
	Mean LB	Mean UB	High[a] LB	High UB
Pigs				
Piglets (weaned)	1.9	2.7	9.8	9.8
Pigs for fattening	1.3	1.9	5.1	5.1
Sows, lactating	0.6	1.2	2.3	2.3
Cattle				
Veal calves*	3.8	4.2	3.8**	4.2**
Poultry				
Chickens for fattening	0.6	1.6	3.4	4.5
Laying hens	0.8	2.0	3.5	5.7
Turkeys for fattening	1.3	2.2	1.9	2.8
Ducks for fattening	0.7	1.7	3.2	3.9
Fish				
Salmonids	0.1	0.9	0.1**	1.1
Rabbits				
Rabbits for fattening	1.8	3.1	2.8	5.7
Dogs	2.1	3.3	2.1**	3.3**
Cats	0.0	0.7	0.0	1.1

*: Milk replacer.

**: High amount of LC data caused the highest reliable percentile to be lower than the mean therefore only the mean was used to calculate both exposure scenarios.

(a): The highest reliable percentile based on the number of samples available.

(b): Concentrations in complete feeds are also reported in Table 7.

Table A.17: OTA concentration estimated from complementary feed and forages[a] (mean and high lower-bound and upper-bound levels) for all ruminants and horses

Animal species	OTA Concentrations μg/kg DM			
	Mean LB	Mean UB	High[34] LB	High UB
Cattle				
Dairy cows	0.9	2.5	4.2	9.6
Cattle for fattening	1.0	4.4	2.3	14.7
Small ruminants				
Dairy sheep/goat	n/a*	n/a	n/a	n/a
Lambs for fattening	n/a*	n/a	n/a	n/a
Horses	1.1	3.7	1.1	10.9

*: No data available.
(a): Forages included in the diets in the ratios indicated in the Section A.2.

Appendix B – New studies on occurrence data in feed within the EU published since the 2004 Opinion

Table B.1: New studies on occurrence data in feed within the EU published since the 2004 Opinion (EFSA CONTAM Panel, 2004)

Feed	Positive/analysed samples	Information on the concentration	Analytical method	Additional information	Reference
Pigs					
Grain samples and pig feed	2/82	Range: 22–33 μg/kg	LC–MS/MS; LOQ = 5 μg/kg	Sampled in Spain, Portugal and Czech Republic	Monbaliu et al. (2010)
Complete feeds for swine (organic and conventional)	Conventional: 22/22; Organic: 8/8	Conventional: 0.22–3.66 μg/kg DM; Organic: 0.43–38.4 μg/kg DM	LC-FLD; LOQ = 1 μg/kg	Sampled at pig farms in Italy	Pozzo et al. (2010)
Feed for fattening pigs including different grains, husks, vitamins and minerals	21/277	Range: 2–6.8 μg/kg	LC-FLD; LOQ = 2 μg/kg	Sampled in Portugal	Almeida et al. (2011)
Feed mixtures for fattening pigs	8/30	0.97–2.7 μg/kg 9; mean of positive = 1.53 μg/kg	ELISA; LOD =1.3 μg/kg	Sampled in Croatia	Pleadin et al. (2012)
Compound feed for piglets, sows, gilts, fattening pig + maize	0/228		LC–MS/MS; LOQ = 8.1 μg/kg	Sampled in Spain	Arroyo-Manzanares et al. (2019)
Feed ingredient and complete feed for pigs (including cereals as well feed for starters, gilts, growers and finisher pigs)	225/905. No. of positive are not given but the prevalence of positive in the different feed groups are between 13 and 88%	Mean: 0.60–9.35 μg/kg Max.: 2.38–168 μg/kg	LC-FLD; LOQ = 0.06–0.2 μg/kg	Sampled in Czech Republic. Analysed at different laboratories	Svoboda et al. (2019)
Organic enrichment materials (including straw, hay, maize sugar beet) for pig feed	0/21		LC–MS/MS; LOQ = 1.5 μg/kg	Sampled in Germany	Tenbrink et al., 2020
Cows, sheep and horses					
Pastures from dairy cow farms	0/18		LC–MS/MS, LOQ not stated but results included since they are new and LOQ is supposed to be comparable to other LC–MS/MS methods	Sampled in Austria	Penagos-Tabares et al. (2021)
Feed for dairy cows including silage, compound feed, forage and feed ingredients	0/169		LC–MS/MS; LOQ = 8 μg/kg	Sampled in the Netherlands	Driehuis et al. (2008)

Feed	Positive/analysed samples	Information on the concentration	Analytical method	Additional information	Reference
Feed for horses including cereals, muesli and mash	26/62	0.2–4 µg/kg	Enzyme immunoassay, LOQ = 0.2 µg/kg	Sampled in Germany	Liesener et al. (2010)
Poultry					
Feed for poultry (including complete feed and broiler starters, growers and finishers)	31/96	From LOQ up to 1.6 µg/kg	LC-FLD or LC–MS/MS; LOQ = 0.06–1 µg/kg	Sampled in Czech Republic Analysed at different laboratories	Mikula et al. (2020)
Complete feed for poultry (broiler, laying hens)	20/20	0.04–6.50 µg/kg	LC-FLD; LOQ not stated but all samples are positive so < 0.04 µg/kg	Sampled in Italy	Schiavone et al. (2008)
Fish					
Plants ingredients and animal proteins used in feed for fish	Plants: 11/19 Animal: 2/19	0.4–5.2 µg/kg 0.4 µg/kg in both	LC–MS/MS; LOQ = 0.2–1.7 µg/kg dependent on the matrix	Plant ingredients were from feed producers in Europe and most samples originated from Europe but also samples from China. Animal proteins were produced in Centra Europe	Nácher-Mestre et al. (2015)
Different plant proteins and finished aquaculture feed (fish and shrimp)	1129/1300	Mean: 1–128 µg/kg	ELISA for proteins, LOQ = 2 µg/kg; LC-FLD For finished feed LOQ = 0.2 µg/kg	Europe	Gonçalves et al. (2017)
Finish aquaculture feed	4/6	Mean = 1.53 µg/kg	LC-FLD, LOQ = 0.2 µg/kg	Sampled in Croatia and Portugal	Gonçalves et al. (2018)
Ingredients to fish feed and complete fish feed for common carp	24/27	2.65–103.9 µg/kg (88% DM)	LC–MS/MS; LOQ = 1.6 µg/kg	Sampled in Serbia	Rokvić et al. (2020)
Pets					
Parrot food	1/10	13 µg/kg	LC–MS/MS; LOQ = 12.60 µg/kg	Sampled in Belgium	Li et al. (2013)
Dry dog food	4/76	Max: 4.7 µg/kg	ELISA, LOQ =2 µg/kg	Sampled in Austria	Böhm et al. (2010)
Extruded dry dog food	39/48	Max: 41.1 µg/kg given as dry matter	LC–MS/MS; LOQ = 5 µg/kg	Sampled in Italy	Gazzotti et al. (2015)
Dry cat food	2/64	5.1–14 µg/kg	LC–MS; LOQ = 2 µg/kg	Sampled in Italy	Grandi et al. (2019)

Feed	Positive/analysed samples	Information on the concentration	Analytical method	Additional information	Reference
Different animal species					
Different kind of feed including feed for poultry, bovine, egg-laying hen, oil seed, grain, unspecified	27/91	Mean: 0.50–6.19 µg/kg; Range: 0.14–12.24 µg/kg	LC-FLD, LOD = 0.1 µg/kg	Sampled in Spain	Jaimez et al. (2004)
Feed samples from pig/ chicken farms	50/50	Mean: 189–376 µg/kg	LC-FLD; LOQ not stated but all samples are positive	Sampled in Bulgaria from pigs and chicken farms having nephropathy problems; samples from 25 farms for two years	Stoev et al. (2009)
Mixed feed for cattle, pigs, poultry, horses, aquaculture fisk, pets, laboratory rats	0/2215		LC-FLD; LOQ = 20 µg/kg	Sampled in Portugal	Martins et al. (2008)
Feed for pigs and laying hens	31/488 for pig feed; 12/186 for feed for laying hens	Max. = 130 µg/kg for swine feed and 10.9 µg/kg for feed for laying hens	LC-FLD, LOQ = 2 µg/kg	Sampled in Portugal in 2009–2010	Martins et al. (2012)
Animal species not specified					
Maize, wheat, oat, rye, soya, sunflower, colza, rice, triticale	11/86	Up to 81 µg/kg Mean: 0.59–1.71 µg/kg for the three years	ELISA, LOQ =1 µg/kg	Sampled in Romania 2008–2010	Tabuc et al. (2011)
Grain, finished feed and feed premixes	10/46	1–54 µg/kg	ELISA, LOQ = 2 µg/kg or LC-FLD, LOQ = 0.2 µg/kg	Sampled in Greed, Cyprus, Spain, Portugal and Italy	Griessler et al. (2010)
Cereals, silage, mixed feeds	387/714	Mean: 0.21–33 µg/kg; Max: 0.23–675 µg/kg	LC-FLD, LOQ = 0.20 µg/kg	Sampled in Poland	Grajewski et al. (2012)
Barley	71/123	Mean = 0.10 µg/kg; Max = 3.53 µg/kg	LC-FLD, LOQ = 0.0375–0.15 µg/kg	Sampled in Spain	Ibáñez-Vea et al. (2012)
Samples of maize, wheat, soybean meal, dried distiller's grains with solubles (DDGS)	Maize: 28/62; Wheat: 15/37; Soybean: 5/25; DDGS: 60/147	Max. for maize: 46 µg/kg; for wheat: 331 µg/kg; for soybean: 21 µg/kg; for DDGS: 30 µg/kg	ELISA, LOD = 2 µg/kg; LC-FLD, LOD = 0.2 µg/kg	Sampled in Europe	Rodrigues and Naehrer (2012)
Wheat	33/52	2.67–25.70	ELISA, LOQ = 4 µg/kg	Sampled at farms in Romania	Alexa et al. (2013)
Non-fermented and fermented feeds (samples are	10/343	Positive samples found in wheat, soya meal, distiller's grain from maize and wheat,	LC–MS/MS; LOQ = 0.5 or 1 µg/kg	Sampled in Czech Republic and UK 2008–2012. ½ LOQ	Zachariasova et al. (2014)

Feed	Positive/analysed samples	Information on the concentration	Analytical method	Additional information	Reference
divided into 18 different classes)		complex compound feed for pigs. Mean: 1–4 Max.: 20–56		used in the calculations of mean if results were < LOQ	
Sugar beet pulp silage	1/40	15	LC–MS/MS; LOQ not given	Sampled in France	Boudra et al. (2015)
Maize silage, cereals, complete feed	385/800	Maximum: 1.16–115	LC-FLD, LOQ = 0.40 µg/kg	Sampled in Poland 2011–2014	Kosicki et al. (2016)
Feed samples, not further described	27/300	Mean: 27.3; Range: 10–50	LC-FLD, LOQ = 0.3 µg/kg	Sampled in Poland	Pietruszka et al. (2017)
Finisher feed, grains, soybean, silage, maize DDGS, other feed	Northern: 159/1957; Central: 2503/21036; Southern: 748/3527; Eastern: 867/2382	Median of positive in each region: 1.9–3.6	ELISA, LC–MS/MS, LC-FLD; LOD = 0.2–1.9	Feed sampled in Europe during a period of 10 years; analysed at different laboratories	Grueber-Dorninger et al. (2019)
Grain and maize silage	0/120		LC–MS/MS, LOQ = 1.80 µg/kg for maize and 2.00 µg/kg for grass	Sampled in Poland	Panasiuk et al. (2019)
Maize	OTA:21/204 OTB:4/204	OTA: 0.5–318; OTB: 2–8	LC–MS/MS, LOD = 0.4 µg/kg for OTA and 1.6 µg/kg for OTB	Sampled in Serbia	Kos et al. (2020)
Maize, feed (not further defined), small grain, maize silage, TMR (total mixed ration)	626/3980	Range: 0.4–33.5	LC-FLD, LOQ = 0.4 µg/kg	Sampled in Poland, 2015–2020; mean given per feed and year; The highest value is a single sample with high concentration of 33.5 µg/kg in small grain in 2017	Twaruzek et al. (2021)

Appendix C – Exposure assessment of OTA for animals

C.1. Estimated intake of OTA using mean and high, LB and UB OTA concentrations in feedingstuffs

Table C.1: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 650-kg body weight lactating dairy cow and a 400-kg body weight Cattle for fattening using model diet[a] plus forages, and a 100-kg body weight Veal calf, using complete feeds

	Exposure											
	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day
	Mean		High		Mean		High		Mean		High	
	Dairy cow				Cattle for fattening				Veal calves			
Model diet plus forages												
LB	35	0.05	64	0.10	8.6	0.02	13.1	0.03	n/a	n/a	n/a	n/a
UB	77	0.12	183	0.28	38	0.09	114	0.29	n/a	n/a	n/a	n/a
Complementary feeds plus forages												
LB	17.6	0.03	83.8	0.13	7.7	0.02	18.0	0.05	n/a	n/a	n/a	n/a
UB	49.9	0.08	192.1	0.30	35.1	0.09	117.4	0.29	n/a	n/a	n/a	n/a
Complete feeds												
LB	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.2	0.07	7.2	0.07
UB	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.9	0.08	7.9	0.08

(a): As detailed in Appendix A.2.

Table C.2: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 60-kg body weight Dairy goat, by a 20-kg body weight lamb for fattening and 400-kg body weight horse, using model diet plus forages and complementary feeds plus forage

	Exposure											
	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day
	Mean		High		Mean		High		Mean		High	
	Dairy goat				Lamb for fattening				Horse			
Model diet plus forages												
LB	1.7	0.03	2.5	0.04	1.6	0.08	3.1	0.15	9.6	0.02	12	0.03
UB	4.5	0.08	13	0.22	3.9	0.20	11	0.55	31	0.08	90	0.23
Complementary feeds plus forages												
LB	–	–	–	–	–	–	–	–	8.5	0.02	8.5	0.02
UB	–	–	–	–	–	–	–	–	29.3	0.07	87.2	0.22

Table C.3: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 20-kg body weight weaned piglet, a 60-kg body weight pig for fattening and a 175-kg bodyweight lactating sow using model diets, and complete feeds

	Exposure											
	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day
	Mean		High		Mean		High		Mean		High	
	Weaned piglet				Pig for fattening				Lactating sow			
Model diet												
LB	1.4	0.07	3.5	0.18	3.9	0.06	9.0	0.15	8.2	0.05	19	0.11
UB	2.5	0.12	5.5	0.27	6.7	0.11	13.5	0.22	15	0.08	30	0.17
Complete feeds												
LB	1.7	0.08	8.6	0.4	2.9	**0.05**	11.2	**0.2**	3.4	**0.02**	12.0	**0.07**
UB	2.4	0.12	8.6	0.4	4.2	**0.07**	11.3	**0.2**	6.5	**0.04**	12.0	**0.07**

Table C.4: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 2-kg body chicken for fattening and a 2-kg body weight Laying hen, using model diets, and complete feeds

	Exposure							
	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day
	Mean		High		Mean		High	
	Chicken for fattening				Laying hen			
Model diet								
LB	0.20	0.10	0.65	0.33	0.13	0.07	0.37	0.19
UB	0.40	0.20	0.90	0.45	0.27	0.13	0.58	0.29
Complete feeds								
LB	0.09	0.04	0.54	0.27	0.09	0.04	0.38	0.19
UB	0.25	0.13	0.72	0.36	0.21	0.11	0.60	0.30

Table C.5: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 3 kg body weight turkey and a 3-kg body weight duck for fattening, using model diets, and complete feeds

	Exposure							
	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day	µg/day	µg/kg bw day
	Mean		High		Mean		High	
	Turkey				Duck for fattening			
Model diet								
LB	0.55	0.18	1.0	0.35	0.36	0.12	0.68	0.23
UB	0.78	0.26	1.3	0.44	0.53	0.18	0.92	0.31
Complete feeds								
LB	0.24	0.08	0.34	0.11	0.09	0.03	0.42	0.14
UB	0.39	0.13	0.50	0.17	0.22	0.07	0.51	0.17

Table C.6: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 2 kg body weight rabbit for fattening and a 0.12-kg body weight salmon, using model diets, and complete feeds

	Exposure							
	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day
	Mean		High		Mean		High	
	Rabbit for fattening				Salmon			
Model diet								
LB	0.28	0.14	0.52	0.26	0.00	0.00	0.00	0.00
UB	0.40	0.20	0.64	0.32	0.00	0.01	0.00	0.01
Complete feeds								
LB	0.18	0.09	0.28	0.14	0.00	0.00	0.00	0.00
UB	0.31	0.15	0.57	0.28	0.00	0.02	0.00	0.02

Table C.7: Estimated exposure to OTA using Mean LB/UB and High LB/UB by a 15 kg body weight dog and a 3 kg body weight cat, using model diets and complete feeds

	Exposure											
	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day	μg/day	μg/kg bw day
	Mean		High		Mean		High		Mean		High	
	Dog				Dog (vegetarian diet)				Cat			
Model diet												
LB	0.29	0.02	0.60	0.04	0.16	0.01	0.29	0.02	0.06	0.02	0.07	0.02
UB	0.54	0.04	0.83	0.16	0.46	0.03	0.61	0.04	0.11	0.04	0.14	0.05
Complete feeds												
LB	0.52	**0.03**	0.52	**0.03**	–	–	–	–	0.00	**0.00**	0.00	**0.00**
UB	0.84	**0.06**	0.84	**0.06**	–	–	–	–	0.04	**0.01**	0.07	**0.02**

Appendix D – TK parameters

Species/ category	Dose (mg/kg bw) (N)	Route of admin.	C_{max} (ng/mL)	T_{max} (h)	AUC (h·ng /mL)	$T_{1/2el}$ (h)	VdL/ kg	Bioavailability (%)	Reference
Sheep	0.027 (6♂ castrated)	Oral	14.4 ± 12.9	6.5 ± 1.9	290 ± 283	16.6 ± 0.7	–	–	Blank and Wolfram (2009)
	0.005 (3 ♀)	Oral	3.5 ± 1.4	7.0 ± 1.7	51 ± 32	15.3 ± 1.6	–	–	Boudra et al. (2013)
	0.030 (3♀)	Oral	7.2 ± 5.2	5.0 ± 1.7	98 ± 79	9.9 ± 4.2	–	–	
Donkey	2.5 (4 ♂)	Oral	10340 ± 2050	12 ± 0	656,200 ± 99,490	24.5 ± 2.5	0.15 ± 0.036	–	Kang et al. (2023)
Broiler chicken	0.25 (4 ♂)	i.v.	–	–	336 ± 79	24.0 ± 15.3	19.6 ± 11.7	–	Devreese et al. (2018)
	0.25 (4♀)	i.v.	–	–	277 ± 15	22.2 ± 19.7	19.8 ± 14.3	–	
	0.25 (4 ♂)	Oral	47.5 ± 18.1	4.6 ± 2.1	301 ± 43	14.1 ± 6.5	14.4 ± 5.9	93 ± 15	
	0.25 (4♀)	Oral	75.1 ± 48.0	1.4 ± 1.1	304 ± 38	8.2 ± 2.0	9.3 ± 2.8	110 ± 14	
Laying hens & roosters	2 (6)	Oral				4.2	2.1	40	Galtier et al. (1981)
	0.25 (4 ♂)	i.v.	–	–	234 ± 9	12.2 ± 7.6	16.2 ± 9.4	–	Devreese et al. (2018)
	0.25 (4♀)	i.v.	–	–	222 ± 14	14.2 ± 5.9	19.4 ± 6.4	–	
	0.25 (4 ♂)	Oral	55.5 ± 15.7	0.8 ± 1.1	199 ± 22	21.2 ± 2.9	30.8 ± 16.5	85 ± 5	
	0.25 (4♀)	Oral	49.2 ± 15.7	1.9 ± 1.1	217 ± 22	17.3 ± 3.9	30.0 ± 16.5	99 ± 12	
Turkey	0.25 (4 ♂)	i.v.	–	–	584 ± 85	18.2 ± 6.2	10.3 ± 4.3	–	Devreese et al. (2018)
	0.25 (4♀)	i.v.	–	–	693 ± 73	11.3 ± 4.9	4.8 ± 2.3	–	
	0.25 (4 ♂)	Oral	201.0 ± 97.1	0.8 ± 0.6	611 ± 89	9.9 ± 1.7	5.5 ± 0.9	110 ± 30	
	0.25 (4♀)	Oral	179.0 ± 44.7	0.8 ± 0.3	679 ± 73	15.5 ± 7.3	7.4 ± 3.6	88 ± 20	
Duck	0.25 (4 ♂)	i.v.	–	–	201 ± 17	17.0 ± 1.4	28.2 ± 9.4	–	Devreese et al. (2018)
	0.25 (4♀)	i.v.	–	–	185 ± 15	16.8 ± 7.5	29.3 ± 13.6	–	
	0.25 (4 ♂)	Oral	48.0 ± 18.9	0.3 ± 0.1	208 ± 21	35.1 ± 4.9	35.1 ± 4.9	104 ± 8	
	0.25 (4♀)	Oral	34.9 ± 4.6	0.3 ± 0.1	173 ± 24	39.0 ± 18.5	38.4 ± 5.9	94 ± 14	
Pig	0.5 (6)	Oral				88.8	0.04	66	Galtier et al. (1981)
Rabbit	2 (6♂)	Oral				8.3	0.5	56	Galtier et al. (1981)
Atlantic salmon	0.8 mg/kg feed (2 × 25)	Feed	–	–		–			Bernhoft et al. (2017)
	2.4 mg/kg feed (2 × 25)	Feed	0.3 µg/kg	2		1.2			

Appendix E – Groups of feed materials[24]

The list identifies which feed materials could be considered when the groups of feed materials are attributed to a compound feed for target animals.

Cereal grains and products derived thereof

Cereals: Barley, maize, oats, broken rice, rye, triticale, wheat.
By-products: From dry milling: middling's, feed, flakes, bran, hulls.
 From wet milling: starch, germ meal, gluten feed, gluten.
 From fermentation: DDG, DDGS, brewer's grains.

Oil seeds, oil fruits and products derived thereof

Oil seeds: Cotton seed, linseed, rape seed, soya beans, sunflower seed.
Main products: Expeller, solvent extracted meal, extruded/toasted beans, flakes.
By-products: Hulls, protein concentrate

Legume seeds and products derived thereof

Legumes seeds: Beans, lentils, sweet lupins, peas.
By-products: Protein/protein concentrate, germ, flakes, hulls.

Tubers, roots and products derived thereof

Sugar beet, potatoes.
By-products: Molasses, beet pulp, protein, inulin.

Other seeds and fruits, and products derived thereof

Acorn, almond, buckwheat, red clover seed, white clover seed.
By-products. Apple pulp, citrus pulp, grape pulp, middling's, bran/hulls, pectin.

Forages and roughage, and products derived thereof

Beet leaves, green silage, lucerne (alfalfa) meal.
By-products: Hay, straw, maize silage.

Other plants, algae, fungi and products derived thereof

Algae, seaweed, fungi.
By-products. Sugar cane molasses, cellulose.

Milk products and products derived thereof

Butter, buttermilk, skimmed milk powder, whey/whey powder, delactosed (and demineralised) whey, casein, whey protein, lactose, whey permeate.

Land animal products and products derived thereof

Animal by products, animal fat, blood meal, feather meal, gelatine, egg products, dried, Terrestrial invertebrates.

Fish, other aquatic animals and products derived thereof

Crustacea meal, fish meal, fish solubles, fish protein, fish oil, krill protein concentrate.

Minerals and products derived thereof

Products and co-products obtained by fermentation using microorganisms

Yeast (brewer's yeast), single cell protein (bacterial or fungal origin).

Miscellaneous

Products from the bakery and pasta industry, fruit syrup, dextrose, fructose, xylose, lactulose, Gluco/fructo-oligosaccharides, starch, dextrins, sorbitol, Fatty acids esterified with glycerol, soap stocks, glycerine, propylene glycol, chondrotitin sulphate.

---

[24] As per Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials.

Appendix F – Uncertainty tables

Table F.1: Hazard identification and characterisation

Main group	Sub-group	Overarching questions	Examples of sources of uncertainty in CONTAM opinions	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
Chemical composition and analytical methods	**Chemical composition**	**Is there uncertainty associated with the dose in the critical studies used in the risk assessment?**	Uncertainty in the applied dose (e.g. evaporation, feed or drinking water, dead volumes in syringe, calibration of the equipment used, feed or drinking water)	Not in all studies used it is stated if dose is confirmed by analysis	1
			The exact composition of the tested compounds (e.g congener pattern of technical mixtures used in toxicological studies do not resemble the profiles found in food or presence of impurities is based on limited information) and its characteristics (e.g. storage, processing etc) are based on limited information	OTA is the sole component	0
			Naturally contaminated materials resulting in co-exposure to other compounds (e.g. mycotoxins).	However then naturally contaminated materials were used and analysed only for OTA, they might have contained other compounds. If co-exposure is stated the study is not used for setting RP	1
	Analytical methods		Lack of certified reference materials, proficiency tests and method validation	Not relevant	0

Main group	Sub-group	Overarching questions	Examples of sources of uncertainty in CONTAM opinions	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
Hazard identification and characterisation	ADME	Is there uncertainty in any aspect of ADME in the various animal species?	Insufficient information on **absorption**	Sufficient information available	0
			Accumulation potential (e.g. duration of studies, sample size, sex, number of studies, direct measurements, biomarkers, metabolites)	Uncertainty due to short duration studies and not always with analysis in the target tissues (e.g. pigs)	2
			Metabolism	Most studies do not consider OTA metabolites	1
			Confounders (e.g. effects of other chemicals that may affect the ADME of the tested compounds)	Not relevant	
			Elimination	Mainly eliminated by faeces and urine and only very little through milk and eggs	1
			Little information on **transfer rate** to animal products	Information is available from previous Opinion (2020) and the literature	0
	Toxicity studies: critical endpoints and critical study design	Are there sources of uncertainties in the design of the studies?	Studies carried out only in one gender or certain age groups, duration of studies, sample size, direct measurements, biomarkers, dosing regime leading to uncertainties for additional endpoints. Unknown prior exposure when the study commenced (e.g. related to effects on progeny)	Some studies performed with one dose only and/or inappropriate dosing regime	3
			Focus on zootechnical parameter, which might not identify other endpoints	Some effects might be overlooked	2

Main group	Sub-group	Overarching questions	Examples of sources of uncertainty in CONTAM opinions	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
	Mixture group membership and interactions	Is there uncertainty on the extent and profile of effects due to co-exposure (e.g. metabolites, interaction of chemicals, combined effects)?	Interaction/combined effects between congeners or other substances	In certain tox studies, the exposure to OTA happens together with other mycotoxins. These studies have not been used to derive RP for animal heath	0
	Mode of action	Are there uncertainties on the MoA of the substance for the various animal species that could affect the conclusions of the risk assessment?	Uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect in animals	MoA is described in the 2020 opinion. For genotoxicity MoA is unknown	1
	Selection of reference point	What are the uncertainties in the use of NOAEL/ LOAEL due to lack of appropriate BMDL?	Dosing intervals, number of doses, etc.	In some studies only one dose level used. In some studies with more dose levels there is effect at all levels, nevertheless not suitable for a BMD modelling.	3
			Uncertainty factors used	Uncertainty factor of 3 used from LOAEL to NOAEL, as a default value	1

Table F.2: Elements of the CONTAM road map and relevance for the uncertainty analysis of the NAs in food draft Opinion – **OCCURRENCE AND EXPOSURE**

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
Occurrence data	Analytical measurements	**Is there uncertainty due to the performance of the analytical method? This may include identification, sensitivity and recovery**	Performance (e.g. specificity for the target compounds) of the analytical method (GC-ECD, GC–MS, etc.).	The analysis of OTA in feed relies on well-established methods, and reliable results are generally obtained as evidenced by the results from proficiency testing	0
			Analytical capability of the method - sensitivity (e.g. LOQ, LOD).	The available analytical methods produce data with suitably low LOQ/LOD to allow assessment for the scope of this Opinion	0
			Consideration of recovery (e.g. correction carried out or not)	At times the lack of information on recovery was identified, or samples were reported as not corrected for recovery	2
			Lack of certified reference materials and proficiency tests	Reference materials for OTA in feed are commercially available and proficiency tests are offered by the EURL for mycotoxins and plant toxins as well as by private providers	0
	Data reporting	**Is there uncertainty on whether there are errors in the reported occurrence data or linked to missing information?**	Potential errors in reporting the occurrence data (e.g. in the classification of the feed category, unit of measurement, parameter, moisture content, etc.) – unidentified errors (not apparent from the data provided), missing information in reporting the	For some feed samples, information is missing e.g. moisture content, correct classification of feed category. Some very high concentrations were observed. Some of these aspects could not be clarified by data providers	2

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
			occurrence data (e.g. analytical method, moisture content)		
		Is there uncertainty in the information on sampling strategy	Sampling strategy not fully random (e.g. risk based or based on screening methods),	Only random sampling included	0
		Is there uncertainty in the information on processing, e.g. processing prior to the analysis of the samples	Unclear whether and what kind of the treatment/processing has been applied prior to the analysis of the sample	In compound feeds, mycotoxin detoxifier could have been added. This would not be reported but possibly interfere with the recovery	1
		Is there uncertainty in the form of the feed material reported (compound feed/ complementary, etc.)	Certain compound/complementary feed materials were reported without clear information on target animal, whether the complete feed for certain animals met the daily ration and/or if the complementary feed for ruminants is intended to be given in a certain relation to forage	The data providers were contacted with the aim of clarifying the unclear aspects. Data were only used when these aspects could be clarified	0
		Other:	Moisture/dry matter content of samples was, at times, not reported	Where possible, assumptions made for the DM content (e.g. compound feed at 88% DM). For the remainder of the samples, the data providers were contacted with the aim of obtaining information on DM. For these samples, data were only used when DM information was available.	1

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
	Representativeness and completeness of the data	**Is there uncertainty in the occurrence data due to limited data availability**	Use of feed categories at high (often not enough specified) FoodEx/FoodEx2 level	The majority of the results were reported up to a level 3, which allowed a suitable use of the results.	1
			Low number of samples per feed category	A suitable number of samples is available for the large majority of relevant feed categories.	1
			Low number of reporting countries	30% of the samples were reported by two countries, other 4 countries contributed with approx. 10% individually to the total each. Possible missing data from EU countries not submitting data.	2
			Not optimal distribution of year of samplings (e.g. too many old data)	Samples are suitably distributed in the 10 year period taken in consideration, with 60% of the data reported in the most recent 5 years.	0
		Is there uncertainty in the occurrence data due to lack of data for potentially relevant feed categories?	Lack of data for potentially relevant feed categories	Suitable number samples is available for the most relevant feed categories	1
	Left censorship	**Is there uncertainty in the occurrence data due to extrapolation or use of models?**	High LOQ and LOD	n/a	n/a

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty 0 - U with negligible priority 1 – U with low priority 2 – U with medium priority 3 – U with high priority
		Is there uncertainty in the occurrence data due to left censorship and the substitution method	High percentage of left-censored data	High amount of LC data skewed the data distribution causing the highest reliable percentile to be lower than the mean for certain feed categories. In these cases, only the mean was used to calculate exposure	2
Animal diets	Representativeness of the data	**Is there uncertainty in the animal diets (e.g. feed materials)**	Unidentified errors in the animal diets	The samples are correctly classified into feed material categories and compound feeds.	0
			Body weight and feed intake of the animals	Body weight and feed intake were recently aligned to default values used in EFSA's FEEDAP guidance which are aimed at targeting the moment in the life of the animals, when the ratio feed intake/BW is maximised (providing a conservative approach)	1
Dietary Exposure estimates methodology		**Is there uncertainty linked to the methodology used for calculating the exposure?**	In the methodology deviating from standard procedures?	The approach is following a standardised procedure, (i) considering complete feed as the only source of exposure, (ii) designing model diets providing a conservative estimate, using predominantly the feed materials which are likely to be contaminated by OTA.	0

Annex A – Protocol for the development of the opinion

The protocol undertaken for the scientific development of this opinion is available under the Supporting Information section on the online version of the scientific output.

Annex B – Raw occurrence data

The occurrence data in feed extracted from EFSA Data Warehouse for the period from 2012 to 2021 is available at the on EFSA's Knowledge Junction Ccommunity on Zenodo at: (link: https://doi.org/10.5281/zenodo.10027826.)

Annex C – Occurrence samples in dry matter

OTA concentrations at Feed expressed in based on dry matter is available at theon EFSA's Knowledge Junction community on Zenodo at: (link: https://doi.org/10.5281/zenodo.10027826).

Annex D – Occurrence samples in whole weight

OTA concentrations at Feed expressed in whole weight is available at the on EFSA's Knowledge Junction community on Zenodo at :(link: https://doi.org/10.5281/zenodo.10027826).