

Risks for animal health related to the presence of ergot alkaloids in feed

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Abstract

The European Commission requested EFSA to provide an update of the 2012 Scientific Opinion of the Panel on Contaminants in the Food Chain (CONTAM) on the risks for animal health related to the presence of ergot alkaloids (EAs) in feed. EAs are produced by several fungi of the *Claviceps* and *Epichloë* genera. This Opinion focussed on the 14 EAs produced by *C. purpurea* (ergocristine, ergotamine, ergocornine, α - and β -ergocryptine, ergometrine, ergosine and their corresponding 'inine' epimers). Effects observed with EAs from *C. africana* (mainly dihydroergosine) and *Epichloë* (ergovaline/-inine) were also evaluated. There is limited information on toxicokinetics in food and non-food producing animals. However, transfer from feed to food of animal origin is negligible. The major effects of EAs are related to vasoconstriction and are exaggerated during extreme temperatures. In addition, EAs cause a decrease in prolactin, resulting in a reduced milk production. Based on the sum of the EAs, the Panel considered the following as Reference Points (RPs) in complete feed for adverse animal health effects: for pigs and piglets 0.6 mg/kg, for chickens for fattening and hens 2.1 and 3.7 mg/kg, respectively, for ducks 0.2 mg/kg, bovines 0.1 mg/kg and sheep 0.3 mg/kg. A total of 19,023 analytical results on EAs (only from *C. purpurea*) in feed materials and compound feeds were available for the exposure assessment (1580 samples). Dietary exposure was assessed using two feeding scenarios (model diets and compound feeds). Risk characterisation was done for the animals for which an RP could be identified. The CONTAM Panel considers that, based on exposure from model diets, the presence of EAs in feed raises a health concern in piglets, pigs for fattening, sows and bovines, while for chickens for fattening, laying hens, ducks, ovines and caprines, the health concern related to EAs in feed is low.

KEYWORDS

animal health risk assessment, ergot alkaloid, exposure, feed, toxicity

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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 related to the presence of ergot alkaloids (EAs) in feed. The previous assessment relating to the presence of EAs in animal feed was published by EFSA in 2012, with no observed adverse effect levels (NOAELs) established for pigs and poultry only. Information more recently provided to the Commission concluded that the Reference Points (RPs) established by EFSA in the abovementioned Opinion should be amended, based on references to studies performed since 2012. The 2012 Opinion was used as starting point, and new scientific information, which has become available on the risks for animal health related to EAs in feed, was incorporated in the current assessment. Information from the 2017 EFSA's Scientific Report on human and animal dietary exposure to ergot alkaloids was also used for this Opinion but new data on occurrence were added and used to update the exposure assessment.

EAs are a group of more than 50 different compounds produced by fungal species of the *Claviceps* and *Epichloë* genera (family Clavicipitaceae). The most relevant *Claviceps* species infecting crops are *C. purpurea* (ubiquitous, which infects grasses and cereals, such as rye, wheat, triticale), *C. africana* (infection of sorghum) and *C. fusiformis* (infection limited to pearl millet). *Epichloë* (formerly known as *Neotyphodium* or *Acremonium* spp.) species infect a range of grasses from the Pooideae family.

Sclerotia from *C. purpurea* are the major cause of food and feed contamination with EAs in Europe. The main alkaloids in ergots from *C. purpurea* are ergocristine, ergotamine, ergocornine, α - and β -ergocryptine, ergometrine, ergosine and their corresponding 'inine' epimers. In this Opinion, T-EAs refers to the sum of these 14 EAs produced by *C. purpurea*. Dihydroergosine has been reported as the main toxic alkaloid in the sclerotia from *C. africana* in Australia. *Epichloë* species can produce several classes of fungal toxins, the most relevant for livestock being the ergot alkaloids and the indole-diterpene lolitrem B. The main EAs produced by *Epichloë* species are ergovaline and its epimer ergovalinine.

Most EAs consist of a tetracyclic ergolene ring system that is substituted at C-8, where EAs are susceptible to epimerisation. The latter results in the formation of 8-S epimers which are indicated by the suffix 'inine', but it is a reversible process in which the 'ine' and 'inine' forms strive for thermodynamic equilibrium. During analysis, the ratio between the two forms can change, depending on the alkaloid and temperature, solvent in use, pH and other factors.

Liquid chromatography with tandem mass spectroscopy (LC-MS/MS) and liquid chromatography with high resolution mass spectrometry (LC-HRMS) have become the most often applied analytical techniques, because they combine high selectivity with high sensitivity, with reported limits of quantification (LOQ) for individual EAs typically ranging from 0.1 to 5 $\mu\text{g}/\text{kg}$. LC methods with fluorescence detection (FLD) are still occasionally used, with LOQs in a similar range. Variety is seen in methods of sample preparation and clean-up of extracts. Acidic or alkaline extraction in the presence of an organic modifier has been used, as well as quick easy cheap efficient robust safe (QuEChERS) approaches. For sample clean-up solid phase extraction (SPE) has been used, as well as dispersive PSA (primary secondary amine). Dedicated immunoaffinity columns are also commercially available. Analytical standards of the regulated EAs are available but no pure standards of β -ergocryptine and β -ergocryptinine.

Data on toxicokinetics of EAs are sparse. The available literature indicates that EAs are absorbed from the gastrointestinal tract and subjected to oxidative biotransformation by Cytochrome P450 3A (CPY3A) to form mono- and di-hydroxylated metabolites. Ruminal microflora may also play an important role, metabolising ergopeptines to lysergic acid. Urinary excretion is predominant but faecal excretion has been shown.

The transfer of intact EAs to tissues of chickens, laying hens, Pekin ducks or piglets is negligible. The same holds for the transfer of intact EAs to eggs in laying hens and milk in dairy cows. No information is available for EA metabolites.

Available studies on effects of EAs in food- and non-food producing animals were considered in the assessment. Serum prolactin levels are a sensitive biomarker of effect, but, in the absence of adverse effects, the decrease in prolactin level by itself was not considered as a critical endpoint.

In pigs, effects were observed in terms of increase of relative weight of heart and spleen, reduced body weight gain and reduced feed intake and growth performance. In sows, milk production was reduced, nevertheless sows do not appear to be more sensitive than other pigs.

In poultry, feed intake and body weight gain were the most sensitive endpoints being reduced by dietary EAs in chicken for fattening and ducks. In laying hens, significantly reduced laying rate, daily egg mass, feed to egg mass ratio, relative eggshell weight, egg yolk colour and nitrogen and crude fat retention of the body were observed.

In bovines, the experimental studies failed to show any effect. However, several case reports suggest that lower levels than applied in the experimental studies may cause effects (decreased feed intake, poor weight gain, hyperthermia, loss of tail switches and tips, early lameness and swelling of the feet, rough haircoat, reduced milk production, death), probably aggravated by weather conditions. In sheep, reduced body weight gain and effects on the carcass characteristics were identified.

Mares were reported to be rather sensitive to ergovaline with various effects like delayed parturition, agalactia (associated with altered prolactin levels) and incidentally neurotoxic symptoms.

In rabbits, an association between tail necrosis and mycotoxins in feed was reported in one study, however, the interpretation is hampered by the simultaneous occurrence of other mycotoxins (DON, T2 toxin, zearalenone) in the feed.

No information is available regarding adverse effects of EAs in fish and non-food producing animals.

The modes of action (MoAs) of EAs-induced effects are complicated due to the highly variable individual animal response to the exposure to EAs. For a large part this is due to the complex plant-fungus-animal-microbe-environmental interaction that results in changing alkaloid concentrations, availability and distribution of various isomeric forms throughout the animal. The primary modes of action of the EAs, whether of ergot or tall fescue endophytic origin, involve vasoconstriction and/or hypoprolactinemia due to inhibited prolactin secretion. The vasoconstriction can lead to gangrenous ergotism (e.g. fescue foot) resulting in loss of extremities such as hooves, ear tips and tail switches. Hyperthermic syndrome relating to EAs is usually observed under heat stress conditions and develops due to impairment of an animal's abilities to thermoregulate. Horses seem to be less susceptible to the decrease in blood flow to the extremities than sheep and cattle. Effects on reproduction and milk production are also observed and more severely in horses than in ruminants. It is unclear what causes the rough hair coat in bovines, but this may contribute to the hyperthermia. Several *in vitro* studies evaluated toxic potencies of a number of EAs, showing that some EAs are more potent than others. However, data are too limited to derive relative potency factors and they were considered equipotent in the risk assessment.

The CONTAM Panel considered as reference point (RP) for adverse animal health effects for EAs from *C. purpurea* (in mg T-EAs/kg complete feed): 0.6 for piglets and pigs for fattening, 2.1 for chickens for fattening, 3.7 for laying hens, 0.2 for ducks, 0.1 for bovines and 0.3 for sheep.

For EAs from *C. africana* the CONTAM Panel considered an RP for adverse animal health effects of 0.5 mg EAs/kg complete feed for sows and 0.4 for bovines. For ergovaline/–inine (*Epichloë*) an RP of 0.1 mg EAs/kg complete feed for bovines and 0.2 mg EAs/kg complete feed for sheep was derived. For horses, a review states that they may show effects at levels as low as 0.05–0.1 mg/kg. However, this statement appears to be based on expert knowledge and it was not possible to retrieve the original underlying studies.

A total of 22,866 analytical results on EAs in feed were initially extracted from the EFSA Database (sampling years 2013–2022). Eight countries submitted data to EFSA; around 45% of the samples were collected in United Kingdom, followed by Czech Republic (~25%). After assessment, data cleaning and conversion based on dry matter (DM), data on a total of 19,023 analytical results were made available. The large majority of data comprised 14 EAs produced by *C. purpurea*. No data were submitted on EAs from *C. africana* and *E. coenophiala*. At Feed Level 1, most of the samples with analytical data on EAs were 'Cereal grains and products derived thereof' ($n = 1216$, 77%), followed by 'Compound feed' ($n = 249$) and 'Forages and roughage, and products derived thereof' ($n = 92$). Only 28% of the analytical results were quantified and for around 50% of the samples ($n = 791$) all analytical results were left-censored. The three most abundant EAs were ergotamine, ergosine and ergocristine; the three together represent on average 59% of the total EA concentration in the feed samples. Among the samples of 'Cereal grains and products derived thereof (feed)', the highest mean EA levels were reported for 'Rye bran' ($n = 12$) with EA concentrations (LB-UB, dry matter) of 307–336 $\mu\text{g}/\text{kg}$. Relatively high values were reported for 'Triticale grains' ($n = 59$) with the highest 95th percentile EA concentration reported among the different feed samples (1411–1423 $\mu\text{g}/\text{kg}$, dry matter, LB-UB).

Dietary exposure was assessed using two scenarios of exposure, one based on the consumption of complete feeds and complementary feed for ruminants supplemented by forages, and another one based on the consumption of model diets composed of feed materials such as cereals and oil seeds, again including forages for ruminants and horses. Exposure assessment was performed using either a mean or a high exposure scenario (using the highest reliable percentile based on the number of samples available). The outcomes of both scenarios, the compound feed and the model diets, were compared.

Risk characterisation was performed for those animal species for which an RP could be identified. Since EAs produced by *C. africana* and the endophyte *E. coenophiala* appear to be of less relevance in the EU and no occurrence data were provided, the focus was on EAs produced by *C. purpurea*. 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 above 100 was considered a risk.

For weaned piglets, the exposure range (Mean LB to High UB) was 17%–105% of the RP, for growing pigs 17%–100% of the RP while for sows it was 19%–105% of the RP derived for pigs. When considering the EA levels in the compound feeds, the estimated exposure (Mean LB and High UB) was 13% and 40% of the RP, respectively, for all pig categories. This was based on a relatively small amount of data.

For chickens for fattening and laying hens the exposure range (Mean LB to High UB) was 1%–4% and 1%–3% of the RP, respectively. Similar results were obtained from the compound feed. For ducks, the exposure range (Mean LB to High UB) was 13%–66% of the RP. No occurrence data were available for a comparison with compound feed for ducks.

For dairy cows the exposure range (Mean LB to High UB) was 25%–133% of the RP and 20%–156% of the RP for cattle for fattening. Similar results were obtained from the compound feed, with the exception of High UB exposure for dairy cows which amounted to 98% of the RP.

For dairy sheep the exposure range (Mean LB to High UB) was 8%–52% of the RP, for lambs for fattening 9%–54% of the RP. For dairy goats and kids for fattening the exposure range (Mean LB to High UB) was 11%–51% and 9%–53% of the RP derived for sheep. No occurrence data were available for a comparison with compound feed.

Uncertainty analysis was performed for the assessment. The uncertainties were identified, prioritised by the experts based on their potential influence on the risk assessment output and consensus probabilities obtained by expert judgement.

The CONTAM Panel considers that the presence of EAs in feed raises a health concern with 66%–90% certainty in bovines and 90%–95% certainty in pigs (piglets, pigs for fattening and sows). The CONTAM Panel considers, with 90%–95%

certainty, that for chickens for fattening, laying hens, ducks, ovines and caprines, the health concern related to EAs in feed is low.

To reduce the uncertainties in the assessment, further consolidated data to assign relative potency factors to the individual EAs and their epimers are needed. Further information is needed on toxicokinetics of EAs in food producing and non-food producing animals. There is a need for non-animal studies to support the assessment of adverse effects of EAs in food producing and non-food producing animals. There should be more studies on the consequences of the decreased prolactin levels observed in various species in terms of potential adverse effects, including the degree of decrease leading to such effects. Occurrence data on EAs in forage (*C. purpurea*) and sorghum (*C. africana*), and on ergovaline/–inine (*Epichloë*) in forage and feed are needed. The EA occurrence data submitted to EFSA should contain the adequate information on the feed samples analysed, including the moisture content, the target animals and the type of compound feed (complete/complementary). Sensitive methods for the analysis should be used to reduce the uncertainties linked to the LB-UB estimations (e.g. LOQ of 1 µg/kg for individual EAs).

1 | INTRODUCTION

1.1 | Background and terms of reference as provided by the requestor

Background

In 2012, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks related to the presence of ergot alkaloids in food and feed. EFSA established for ergot alkaloids (EAs) a No Observed Adverse Effect Level (NOAEL) of 3.57 mg EAs/kg feed¹ for adverse effects in pigs and a NOAEL or 1.4 mg EAs/kg feed for poultry (EFSA CONTAM Panel, 2012).

Information was more recently provided to the Commission services concluding that the Reference Points (RPs) for adverse animal health effects for ergot alkaloids (EAs) in pigs and poultry established by EFSA in the abovementioned Opinion should be amended, based on references to studies performed since 2012.

The Commission has requested EFSA to assess newly available information that has become available since 2012 to verify if the Reference Points for adverse animal health effects established for EAs in pigs and poultry can be confirmed or need to be updated and if Reference Points for adverse animal health effects for other animal species can be established based on scientific information available since 2012.

In case the Reference Points (RPs) for pigs and poultry are updated, and RPs for other animal species derived, the risks to these animal species in relation to the presence of EAs in feed, will be assessed using the exposure assessment included in EFSA's 2017 Scientific Report on Human and animal dietary exposure to ergot alkaloids (EFSA, 2017).

Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002,² the European Commission asks the European Food Safety Authority to provide a scientific opinion on the risks for animal health related to the presence of ergot alkaloids in feed, taking into account:

- information submitted to the Commission, and
- the animal dietary exposure published by EFSA (2017).

The information on adverse effects of EAs on animal health submitted by the European Commission relates to a publication of Schwake-Anduschus et al. (2020).

1.1.1 | Interpretation of the Terms of Reference

The CONTAM Panel aims to derive RPs for adverse animal health effects expressed as the levels of ergot alkaloids in complete feed (including roughage in the case of ruminants and horses). The RPs would not result in effects considered adverse in food-producing and non-food producing animals. Therefore, the reference to EA doses in 'feed' needs to be understood as 'complete feed', as laid down in Reg (EC) 767/2009³ ('compound feed which, by reason of its composition, is sufficient for a daily ration').

The CONTAM Panel uses the terms food-producing and non-food producing animals, as laid down in Reg (EC) 767/2009,² rather than farm and companion animals.

This Opinion focuses on the EAs produced by *C. purpurea*, the most relevant source of EAs in Europe. In addition, in contrast with the previous Opinion (EFSA CONTAM Panel, 2012), the effects of other EAs, like ergovaline, produced by other fungi, were evaluated, since they can occur in European countries. However, for these EAs, no occurrence data were available in the EFSA database.

1.2 | Supporting information

1.2.1 | Chemistry

The chemistry, natural sources and analysis of ergot alkaloids have been described in detail in the 2012 EFSA Opinion on ergot alkaloids in food and feed (EFSA CONTAM Panel, 2012). The information relevant for this Opinion is summarised below.

¹There was an incongruence in the 2012 Opinion, where the NOAEL was reported as 0.15 mg EAs/kg bw in the body of the text while as mg EAs/kg feed in the conclusion. The correct units are mg EAs/kg bw therefore the correct RP is 3.57 mg EAs/kg feed.

²Regulation (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, p. 1–24.

³Regulation (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.

Chemistry

Ergot alkaloids are a group of more than 50 different compounds produced by fungal species of the *Claviceps* and *Epichloë* (synonym *Neotyphodium*, *Acremonium*) genera (family Clavicipitaceae) (Flieger et al., 1997). Next to the naturally occurring EAs, a number of semi-synthetic derivatives have been studied with respect to their pharmacological activity and medical applications. Most EAs consist of a tetracyclic ergolene ring system that is substituted at C-8. Based on the substitution on C-8, EAs can be classified into three main groups: (i) clavines and 6,7-secoergolenes, (ii) simple lysergic acid derivatives and (iii) ergopeptines (Figure 1, Appendix A). The most important EAs in this Opinion are presented in Table 1.

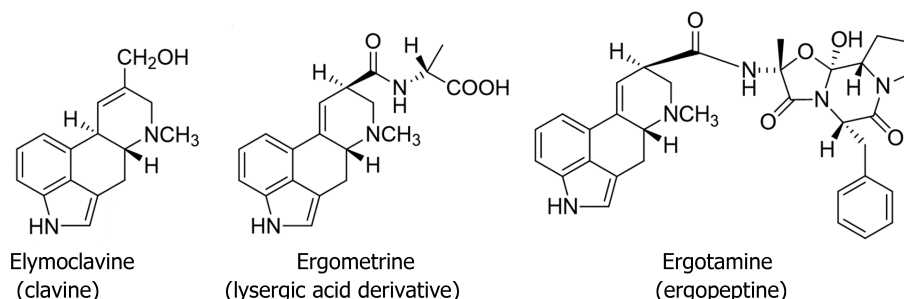


FIGURE 1 Structures exemplifying the three main groups of ergot alkaloids.

TABLE 1 Ergot alkaloids most relevant to this Opinion ('ine' forms only).

Ergot alkaloid	Structure	Ergot alkaloid	Structure
Ergotamine C ₃₃ H ₃₅ N ₅ O ₅ CAS: 113-15-5		Ergocristine C ₃₅ H ₃₉ N ₅ O ₅ CAS: 511-08-0	
α-Ergocryptine C ₃₂ H ₄₁ N ₅ O ₅ CAS: 511-09-1		β-Ergocryptine C ₃₂ H ₄₁ N ₅ O ₅ CAS: 20315-46-2	
Ergocornine C ₃₁ H ₃₉ N ₅ O ₅ CAS: 564-36-3		Ergosine C ₃₀ H ₃₇ N ₅ O ₅ CAS: 561-94-4	
Ergometrine C ₁₉ H ₂₃ N ₃ O ₂ CAS: 60-79-7		Ergovaline C ₂₉ H ₃₅ N ₅ O ₅ CAS: 2873-38-3	

An important chemical feature of EAs is their susceptibility to epimerisation at the C-8 position of the ergolene ring system (Figure 2). All EAs that possess a $\Delta^{9,10}$ -bond in their ring system can undergo this isomerisation. In naturally occurring EAs, the predominant stereochemistry at C-8 is the R-form and this is indicated by the suffix 'ine'. Epimerisation results in the formation of 8-S epimers which are indicated by the suffix 'inine'.

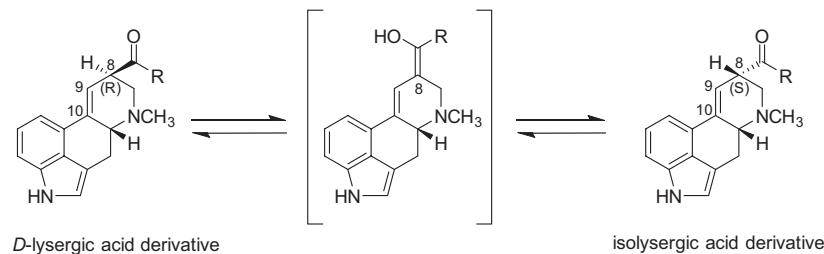


FIGURE 2 Epimerisation of $\Delta^{9,10}$ -ergolenes via enolisation at C-8 (*D*-lysergic acid (8-*R*))-derivative (suffix 'ine'), into the corresponding isolysergic acid (8-*S*))-derivative (suffix 'inine').

Also considering differences in toxic potencies, an issue is to what extent the ratio between the two forms can change during analysis. The effect of solvents on the epimerisation of EAs has been studied in detail. It was reported that epimerisation is promoted under acidic and alkaline conditions, which are often used during e.g. extraction of grain samples (Komarova & Tolkachev, 2001; Krska et al., 2008). The stability of EA standards in solvent was assessed by Hafner et al. (2008). A mixture of six 'ines' was dissolved in seven different solutions and kept at 3 different temperatures (-20°C , $+4^{\circ}\text{C}$, $+20^{\circ}\text{C}$) for up to 6 weeks of storage in the dark. For all solvents ergometrine showed the lowest rate of epimerisation (maximum of 11% in a mixture of methanol/dichloromethane, kept at 20°C for 6 weeks), while the highest rate of epimerisation was observed for ergosine (43% under the same conditions). Chloroform was the solvent providing the lowest rates of epimerisation ($< 6\%$). Acetonitrile, that is often recommended as solvent for EAs, resulted in a substantial epimerisation (25%) of ergotamine when stored at 20°C .

The epimerisation of α -ergocryptine and ergovaline in several solvents during storage for 51 days was studied by LC-UV (Smith & Shappell, 2002). Methanol and methanol/water (70/30) strongly promoted epimerisation of α -ergocryptine, while this was limited in acetonitrile, acetone and chloroform (less than 4% after 51 days at room temperature). Ergovaline was very prone to epimerisation in phosphate buffers and in fetal bovine serum (FBS) solutions. Epimerisation was complete in 2 h in 0.1 M phosphate buffer pH 7.5 at 37°C . In 9.1% FBS pH 7.5, it took about 12 h for complete epimerisation. Epimerisation was fast in phosphate buffers spanning a pH range from 5.4 to 9, but at pH 3, epimerisation was much slower, not having reached equilibrium after 72 h. It should be noted that the thermodynamic equilibrium between the two epimers is compound, solvent and pH dependent. The equilibrium of ergovaline/–inine in phosphate buffer pH 7.5 was reached at a ratio of 2:1, while in buffer pH 5.4 the ratio was 1:1.

The effects of pH, heating and UV light on the epimerisation of EAs in the presence of cereal matrix was studied by Schummer et al. (2020). Wheat, triticale, rye and fodder pellets were spiked with a mixture of 6 'ine' EAs and subjected for 1–3 h to dry or moist heat (100°C), UV light (302 nm) or kept for 2 h in the presence of buffers (pH 3, 7, 10). Substantial differences in epimerisation rate were observed. Low epimerisation levels were found under each of these conditions for ergotamine and ergosine ($\leq 12\%$ epimerisation), while ergocornine and ergocristine showed extensive epimerisation to the 'inine' epimers (up to 86% for ergocornine and up to 96% for ergocristine). Dry heating had the most pronounced effect on epimerisation, followed by heating under moist conditions. The addition of buffer had the smallest impact on the epimerisation, and no major differences were observed for the differences in pH, nor the matrix material used.

It can be concluded that epimerisation of EAs is a reversible process in which the 'ine' and 'inine' forms strive for thermodynamic equilibrium. The rate of epimerisation is strongly dependent on the alkaloid and temperature, followed by solvent, pH and other factors. Experiments indicate that substantial epimerisation can occur within minutes under physiological conditions. For these reasons, the two epimers should both be determined and the sum of the epimers should be used to characterise the contamination.

In this Opinion, T-EAs refers to the sum of 14 EAs produced by *C. purpurea* (see Sources below) which is the most common source of EAs in feed materials in Europe. In some papers, particularly in some case study reports, only the sum of five or six 'ines' is reported. This may result in an underestimation of the toxicologically relevant levels. In these cases, the Panel estimated the amount of the remaining EAs. For other EAs, like those produced by *C. africana* or *Epichloë*, the specific compounds are mentioned.

Sources

The most relevant *Claviceps* species infecting crops are *C. purpurea* (ubiquitous, which infects grasses and cereals, such as rye, wheat, triticale), *C. africana* (infection of sorghum) and *C. fusiformis* (infection limited to pearl millet) (EFSA CONTAM Panel, 2012; Miedaner & Geiger, 2015). *Epichloë* (formerly known as *Neotyphodium* or *Acremonium* spp.) species infect a range of grasses from the Pooideae family. Of particular relevance are *E. coenophiala* syn *N. coenophialum* (infects tall fescue grass) and *E. festucea* var. *lolii* syn *Neotyphodium lolii* (infects perennial ryegrass) (Caradus et al., 2022; Vikuk et al., 2019). The sources and effects of the latter toxins were not included in the previous Opinion and are now described in detail.

Claviceps

Claviceps purpurea

Sclerotia from *C. purpurea*, which are the major cause of food and feed contamination with EAs in Europe, contain lysergic acid derivatives and ergopeptines. The main alkaloids in ergots from *C. purpurea* are ergocristine, ergotamine, ergocornine, α - and β -ergocryptine, ergometrine, ergosine and their corresponding 'inine' epimers. The alkaloid composition and concentration are variable (Appelt & Ellner, 2009; Franzmann et al., 2010; Mainka et al., 2007a; Young & Chen, 1982). Based on data on EA content of sclerotia submitted to EFSA, indicative average concentrations could be estimated for sclerotia in various grain crops (EFSA, 2017). An average T-EA content of 2500–3000 mg/kg is found for *C. purpurea* sclerotia in wheat, barley and triticale, while for *C. purpurea* sclerotia in rye the content is around 1000 mg/kg. The T-EA content in rye ergot, however, fluctuates more strongly than in the other grain crops, and there are indications of the presence of non-EA-producing *Claviceps* strains as in a minor proportion of sclerotia EAs have been found to be absent (EFSA, 2017; Mulder et al., 2012). Ergotamine and ergocristine are generally the most abundant EAs, followed by ergosine, ergocryptine, ergocornine and ergometrine (EFSA CONTAM Panel, 2012; Miedaner & Geiger, 2015). The proportion of 'ines' is generally 70%–80% of the total EAs in fresh sclerotia (EFSA CONTAM Panel, 2012). In compound feeds the proportion of 'ines' is generally slightly lower, around 60%–70% of the total EAs.

Claviceps africana

Dihydroergosine was found as the main toxic alkaloid (around 80%) in the sclerotia from *C. africana* in Australia (Blaney et al., 2003; McLennan et al., 2017; Molloy et al., 2003). Concentrations of dihydroergosine ranged from 1100 to 6400 mg/kg (Blaney et al., 2003). Kopinski et al. (2007) reported the presence of dihydroergosine (400 mg/kg), dihydroelymoclavine (37 mg/kg) and festuclavine (27 mg/kg) in *C. africana* from Australia. Shimshoni et al. (2017) reported much lower concentrations for sclerotia of *C. africana* found in Israel: dihydroergosine (15.3 mg/kg), dihydrolysergol (10.9 mg/kg), festuclavine (2.2 mg/kg), dihydroergotamine (0.85 mg/kg) and chanoclavine (0.83 mg/kg).

Claviceps fusiformis

Agroclavine, elymoclavine, chanoclavine, penniclavine and setoclavine were identified in pearl millet samples contaminated with ergot from *C. fusiformis* (Krishnamachari & Bhat, 1976). Lorenz et al. (2007) reported the formation of agroclavine, chanoclavine and elymoclavine in cell cultures of *C. fusiformis*.

Epichloë / *Neotyphodium*

Epichloë is a genus of fungal species that grows as endophytes in various cool season grass species. Some of these grasses are of agronomical importance such as perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*) and tall fescue (*F. arundinacea*, syn *Lolium arundinaceum*, syn *Schedonorus arundinaceus*) (Caradus et al., 2022; Strickland et al., 2011; Vikuk et al., 2019). Often a specific fungal endophyte is symbiotic to a specific grass species. Thus *L. perenne* is predominantly infected by *Epichloë festucae*, var. *lolii*, *F. pratensis* is infected by *E. uncinata* and *F. arundinacea* is infected by *E. coenophiala* (Vikuk et al., 2019). The presence of these endophytes is thought to be beneficial for the plant host as it improves drought tolerance and increases resistance to certain insect pests and diseases (Graff et al., 2020; Vikuk et al., 2019). Endophytes are propagated through the grass seeds (Dobrindt et al., 2013). Originally native to meadows and pastures in Europe, they have been introduced to many other temperate regions in the world, often along with the introduction of wool, meat and dairy production (Caradus et al., 2022). The level of endophyte infection varies widely and is influenced by environmental, climatological, geological and nutritional factors (Caradus et al., 2022; Dobrindt et al., 2013; König et al., 2018; Repussard et al., 2014a). Several commercial tall fescue varieties specifically have been developed with a high level of endophyte infection, while also varieties with endophytes with a lower or absent production of EAs or without endophytes are available (Dillard et al., 2019).

Epichloë species can produce several classes of fungal toxins, the most relevant for livestock being the ergot alkaloids and the indole-diterpene lolitrem B. The main EAs produced by *E. coenophiala* and *E. festucae* var. *lolii* *Epichloë* are ergovaline and its epimer ergovalinine (Caradus et al., 2022; Strickland et al., 2011). Other EAs that can be present are the clavines agroclavine, chanoclavine-I, elymoclavine and penniclavine (Cagaš et al., 1999; Porter, 1995; Reed et al., 2016) and the lysergic acid derivatives ergine and lysergyl-alanine (Mace et al., 2014). Occasionally present, but at much lower concentrations, are the ergopeptines ergotamine, ergocryptine, ergosine and ergocornine (Caradus et al., 2022; Strickland et al., 2011). Levels of ergovaline can vary strongly and have been shown to peak when the plants reach the fully grown stage, generally at the beginning of summer (Cagaš et al., 1999; Repussard et al., 2014a, 2014b). Ergovaline concentrations in perennial ryegrass reaching up to 2.5 mg/kg DM have been reported in Europe as well as other parts of the world (Cagaš et al., 1999; Hovermale & Craig, 2001; Reed et al., 2016; Repussard et al., 2014a).

Methods of analysis

A detailed description of the analytical procedures and methods used in EA analysis can be found in the EFSA 2012 Opinion. Since this Opinion, no major new developments with respect to the analysis of EAs in cereals and cereal-based products have surfaced. LC–MS/MS and LC–HRMS have become the most often applied analytical techniques, because they combine high selectivity with high sensitivity. Reported Limits of Quantification (LOQ) for individual EAs typically range from 0.1 to 5 µg/kg (Carbonell-Rozas et al., 2022; Diana Di Mavungu et al., 2012; Guo et al., 2016; Poapolathep et al., 2021). LC methods with fluorescence detection (FLD) are still occasionally used, with LOQs in a similar range (Schummer et al., 2018). More variety is seen in methods of sample preparation and clean-up of extracts. Acidic or alkaline extraction in the presence of an organic modifier has been used, as well as QuEChERS approaches (Chung, 2021). For sample clean-up Solid Phase Extraction (SPE) has been used, as well as dispersive PSA (primary secondary amine). Dedicated immunoaffinity columns for EAs are commercially available. Some methods only use ultrafiltration to remove matrix components, but classical liquid–liquid extraction procedures are still used as well (Chung, 2021). Most of these methods aim at the determination of the EAs present in *C. purpurea*. Ergovaline/–inine are not routinely included. For the analysis of ergovaline/–inine LC–MS/MS and LC–FLD methods are applied as well (Strickland et al., 2011; Tardieu et al., 2015). An ELISA assay for the semi-quantitative determination of ergovaline in tall fescue is commercially available (Hill & Agee, 1994).

Analytical standards of 12 regulated EAs (see 1.2.3) are available from a limited number of commercial sources, but no pure standards of β-ergocryptine and β-ergocryptinine. Ergovaline is available from a few suppliers. For EAs of the dihydropeptide, clavine and lysergic acid type, the situation is not much different. Several of these EAs are offered by only one or two suppliers, but not always with a certificate of analysis. Some isotopically labelled analogues of the EAs are commercially available.

Proficiency test (PT) schemes are offered by several providers. The matrices include rye and wheat milling products and baby food cereals. The participants are requested to analyse all regulated EAs and report either all individual EAs or the sum of the epimer pairs, as well as the total EA content based on the sum of all regulated EAs.

There is one certified reference rye material available, but with relatively high uncertainties in the certified concentrations of the EAs.

1.2.2 | Previous animal and human health risk assessments

In 2005, EFSA's CONTAM Panel published an Opinion on contaminants in the food chain related to ergot as undesirable substance in animal feed (EFSA CONTAM Panel, 2005). The data available at the time did not allow the Panel to identify marker EAs to be monitored in feed materials. In addition, evidence on toxicity in animals was limited, with some indications that adverse effects may occur, particularly in pigs. Mode of actions were unclear, although it was observed that adverse effects were associated with interactions with α-adrenergic, dopaminergic and serotonergic receptors. Tissue distribution data were incomplete, but no evidence was identified to suggest accumulation of EAs in edible tissues.

In 2012, the EFSA CONTAM Panel published a Scientific Opinion related to the presence of ergot alkaloids (EAs) in food and feed (EFSA CONTAM Panel, 2012). In this Opinion, a NOAEL of 0.15 mg EAs/kg body weight (bw) per day⁴ (corresponding to 3.57 mg EAs/kg complete feed with 88% DM) was identified for piglets. In the same EFSA Opinion, the Panel identified that new studies indicated a NOAEL of 1.4 mg EAs/kg feed for chickens for fattening. Reference Points (RP) for other animal species were not identified. The Opinion also concluded that, although scarce, the available data provided no evidence that EAs accumulate in edible tissues and therefore these are unlikely to be an important source of human exposure to EAs.

In 2017, EFSA published a scientific report on Exposure to EAs in food and feed. Occurrence data on compound feeds were scarce, therefore the CONTAM Panel considered only occurrence data on grain cereals and forages and roughage to calculate animal exposure. The cereal grain with the highest probability to be contaminated by EAs was rye. In addition, exposure from diets containing rye up to the highest recommended levels for some animal species was estimated. With regard to animal exposure, piglets were identified as the most exposed animal group for both the mean and the high concentration scenarios, followed by poultry (broilers), rabbits and beef cattle, fish and companion animals.

The publication by Schwake-Anduschus et al. (2020), attached by the Commission to the ToR, presented German data collected for sclerotia of *C. purpurea* and EAs in compound feed and feed materials in the period 2012–2014. One of the main objectives of the study was to investigate the potential relationship between the count of sclerotia in the sample and EA concentrations. The study concluded that such a relationship cannot be established, and therefore the evaluation of risk for animal health should be assessed using EA concentrations rather than sclerotia weight. The study also recommended guidance values for a number of animal species.

In 2022, JECFA published a summary on EAs in food within the evaluation of certain food additives and contaminants, WHO Technical Report Series 1036 (FAO/WHO, 2022). The document includes a short section on observations in domestic animals, reporting impacts on growth, reproductive performance and lactation. In addition, the summary reports that the very limited data available on transfer from feed to food of animal origin show no accumulation of EAs in tissues. A monograph for EAs is being elaborated by JECFA with regard to human health.

⁴See footnote in section 1.1.

1.2.3 | Legislation

Directive 2002/32/EC⁵ on undesirable substances in animal feed establishes maximum levels for these substances, which are listed in Annex I to that Directive. Section II of Annex I contains a maximum level for 'rye ergot' (*C. purpurea*) in feed materials and compound feed containing unground cereals. The maximum content is 1000 mg/kg feed with a moisture content of 12%. No maximum levels are established for EAs in animal feed. In a draft Commission Delegated Regulation amending Directive 2002/32/EC, it is foreseen to lower the maximum level for ergot sclerotia. The draft Commission Regulation is foreseen to enter into force in 2024.

In 2012, the Commission adopted a Recommendation on the monitoring of the presence of EAs in food and feed (2012/154/EU).⁶ It recommended that Member States should perform monitoring on the presence of EAs in cereals and cereal products intended for human consumption or animal feeding, in pasture/forage grasses for animal feeding and in compound feed and food. The monitoring should include, as a minimum, the following EAs: ergocristine, ergocristinine, ergotamine, ergotaminine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergocornine and ergocorninine. According to the Recommendation, Member States should determine, whenever possible, simultaneously the sclerotia content in the sample in order to be able to improve the knowledge on the relation between the content of sclerotia and the level of individual EAs. A new Recommendation will be adopted in early 2024.

With regard to EAs in food, Regulation (EU) No 2023/915⁷ introduced maximum levels of ergot sclerotia for certain unprocessed cereals and maximum levels for the aforementioned EAs in milling products of barley, wheat, spelt and oats, rye milling products, wheat gluten and processed cereal-based food for infants and young children.

2 | DATA AND METHODOLOGIES

2.1 | Occurrence data submitted to EFSA

As per the terms of reference (ToR), the dietary exposure previously derived within the EFSA 2017 report was to be taken into account for the risk characterisation. However, following a preliminary appraisal of currently available occurrence data of EAs in feed, the CONTAM Panel decided to perform a new exposure assessment, based on analytical data from samples collected in the period 2013–2022.

2.1.1 | Data collection and validation

Occurrence data for the presence of EAs 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 178/2002.⁸ The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a).

The analytical results on EAs referred to the main *C. purpurea* EAs that cover a total of 14 different EAs: ergometrine (synonym: ergonovine), ergosine, ergocornine, ergotamine, ergocristine, ergocryptine (α - and β -isomers) and the corresponding 'inine' (S)-epimers (ergometrinine, ergosinine, ergocorninine, ergotaminine, ergocristinine, ergocryptinine (α - and β -isomers)). Analytical data on the two isomers (α - and β -) of ergocryptine/–inine could be reported either as individual results or as the sum of both isomers.

Analytical data on EAs in feed were extracted from the EFSA Data Warehouse on 16 September 2023.

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.⁹ 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 left-censored data¹⁰ were treated by the substitution method using the lower bound (LB) and upper bound (UB) approach (EFSA, 2010b; WHO/IPCS, 2009). Applying the LB approach, results below the LOD/LOQ were replaced by zero; for

⁵Directive 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.

⁶Commission Recommendation 2012/154/EU of 15 March 2012 on the monitoring of the presence of ergot alkaloids in feed and food. OJ L 77, 16.3.2012, p. 20–21.

⁷Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, C/2023/35, OJ L 119, 5.5.2023, p. 103–157.

⁸Regulation (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.

⁹https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/SOP-040_S.pdf

¹⁰Left-censored data refer to the analytical results reported to be below the limit of detection (LOD) or limit of quantification (LOQ).

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.

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 cut-off values to exclude samples reported with relatively 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 approximately 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 (broilers, laying hens, turkeys for fattening and ducks for fattening); (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 animals' exposure to EAs 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 & 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; EFSA CONTAM Panel, 2012). In May 2023 the CONTAM Panel¹¹ modified/updated the default values 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) 2022/1104.¹²

2.4 | Methodology for data collection and study appraisal

A comprehensive search for literature was conducted for peer-reviewed original research relating to the effects of EAs on food-producing and non-food producing animals. Search strings were designed to identify potentially relevant studies published between 1/7/2011 (based on the year of publication of the previous Opinion (EFSA CONTAM Panel, 2012) and 1/7/2022 (the date when the actual search was performed) and dealing with analytical techniques to detect EAs in feed, toxicokinetics, toxicity in animals, mode of action and transfer to food of animal origin. Web of Science was identified as the database appropriate for retrieving literature for the present evaluation. An overview of the search terms is given in Appendix B. The total number of publications identified were 1066 for chemistry, 1549 for absorption, distribution, metabolism and excretion (ADME), 1164 for toxicity in experimental animals, 1895 for toxicity in food-producing and non-food producing animals, 143 for transfer to food of animal origin. Further details are included in Appendix B. After removal of duplicates and applying inclusion/exclusion criteria, potentially relevant references were identified. The abstracts considered as potentially relevant were screened by the experts of the WG. If considered relevant for the scope of the mandate by applying expert judgement, the papers were used in the assessment. In addition to the literature search, a 'snowballing' approach¹³ was applied by the WG members in order to obtain further papers published up to 1/7/2022. Additionally, relevant scientific evaluations by national or international bodies and reviews were considered for the current risk assessment.

In addition to the initial literature search, the WG decided to undertake an additional search for the publication period between 1/7/2022 and 1/5/2023 to ensure that new, potentially relevant literature, is not overlooked. The same search criteria (Appendix B) were applied, only with the changed publication period. In total 1126 new entries were identified of which, after abstract screening, 20 were selected as possibly relevant for the Opinion. The decision regarding relevance to the mandate, and thus inclusion in the Opinion, was made by the experts of the WG.

¹¹The approach to estimate feed consumption and the default values for feed intake and body weight used to estimate the exposure to EAs were endorsed by the EFSA CONTAM Panel in its 133rd Plenary meeting (<https://www.efsa.europa.eu/en/events/133rd-plenary-meeting-contam-panel>).

¹²Commission 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.

¹³Identifying papers that have been cited in papers found in a search.

2.5 | Methodology applied for dietary exposure assessment

As detailed in Section 2.1, following a preliminary appraisal of currently available EA occurrence data in feed, the CONTAM Panel decided to perform a new animal dietary exposure assessment, using analytical data produced from samples collected in the period 2013–2022 (section 3.2.1). The methodology followed in the elaboration is described in this section.

Model diets for each animal species and category were used to calculate the exposure to EAs. Similarly to animal feed intake data, the estimated diets have been derived from information described by the CONTAM Panel in previous Scientific Opinions (EFSA CONTAM Panel, 2011, 2012) and modified in May (see 2.2). The diets are described in Appendix C.

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 with Commission Regulation 2022/1104¹⁴ 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 D.

Occurrence data in feed materials were used to derive two scenarios of exposure, one based on the consumption of complete feeds and complementary feed for ruminants supplemented by forages, and another based on the consumption of model diets composed of feed materials such as cereals and oil seeds, again including forages for ruminants and horses. The occurrence data on feed materials, forages and compound feeds reported in Table 9 were used to calculate animal exposure. The outcomes of both scenarios, the compound feed and the model diets were compared.

In the calculations of animal exposure, two levels were considered: a mean occurrence level, in which the mean LB and UB values for each feeding stuff were used to estimate EA concentrations in the diet; and a high occurrence level, in which the highest reliable percentile LB and UB values were used (95th or lower). The calculated mean and high EA concentrations in the diet (reported in Appendix C) were combined with the estimated feed intake (also described in Appendix C) to obtain the estimated exposure per kg bw for the different animal species and categories in the two scenarios at both levels (Appendix E).

3 | ASSESSMENT

3.1 | Hazard identification and characterisation

3.1.1 | Toxicokinetics – absorption, distribution, metabolism and excretion

There is very limited information available on the ADME of EAs. This section of the Opinion includes papers which had already been considered for the 2012 Opinion.

Absorption

In ruminal and omasal tissues isolated from sheep, Hill et al. (2001) studied the transport of a mixture of EAs (lysergol, lysergic acid, ergometrine, ergocryptine and ergotamine). Transport of EAs from mucosal to serosal sides of the tissues was performed using parabiotic chambers. The studies were conducted to compare alkaloid transport in these tissues. Ruminal tissue had greater EA transport potential than omasal tissue because of larger surface area. The greatest transport capacity of the tissues tested were shown for lysergic acid, lysergol and ergometrine.

Distribution

Two hours after *i.v.* injection of 1 mg/kg bw [³H]-ergotamine in rats, higher radioactivity was measured in liver, lungs, kidneys and heart compared to blood, whereas a low concentration was observed in the brain (Kalberer, 1970). The more lipophilic EAs and their 'inine' epimers are (e.g. ergotamine, ergocristine), the more efficiently they cross the blood–brain barrier compared to more hydrophilic alkaloids such as ergometrine and its 'inine'.

Reddy et al. (2020) studied the distribution of ergotamine in male mice and identified ergotamine in kidney, liver and brainstem but not in other regions of the brain. Depending on the dose, levels observed in kidney were 4 to 11 times higher than in liver. Highest levels of ergotamine were found in the brainstem, however, individual variation in the measured concentrations was significant.

Metabolism

EAs are biotransformed in the liver, but the structures of most metabolites have not been fully elucidated yet (Lorenz, 1979; Tfelt-Hansen et al., 1995).

Incubation of ergotamine with bovine liver microsomes for 60 minutes produced two mono- as well as two dihydroxy metabolites, all thought to result from oxidation at positions 8 and 9 in the proline ring and suggested by the authors to

¹⁴Commission 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.

be mediated by CYP3A. Upon prolonged incubation the metabolites were converted into further metabolites of unknown composition (Moubarak & Rosenkrans Jr, 2000). CYP3A was also implicated in the primary oxidation of the semi-synthetic EA bromocriptine to 8-hydroxybromocriptine, as observed in isolated rat hepatocytes (Maurer et al., 1982).

In vitro incubations of ergotamine, α -ergocryptine, ergocristine and ergovaline with human or equine S9 fractions, as well as equine whole liver preparations, revealed the formation of various mono- and dihydroxy metabolites in the proline as well as the lysergic acid moieties of the EAs. Based on LC–HRMS spectral analysis it was concluded that there was a high amount of overlap of metabolites formed between the EAs produced in the different metabolic systems. Metabolites formed by *N*-oxidation and *N*-demethylation were also observed (Rudolph et al., 2019).

Reddy et al. (2020) studied the metabolism of ergotamine in male mice and based on LC–HRMS data, identified two ergotamine metabolites hydroxylated in the proline ring, with higher concentrations in liver tissue compared to kidney.

In ruminants, the rumen microflora plays an important role in the metabolic fate of EAs. Fermentation in the rumen can liberate EAs from the plant tissues in case of endophyte-infested tall fescue, increasing the amount available for absorption, but the rumen microflora was also shown to hydrolyse ergopeptines to lysergic acid (Ayers et al., 2009; De Lorme et al., 2007).

Excretion

The excretion of EAs is often biphasic depending on the degree of enterohepatic recirculation. Following hepatic metabolism, biliary excretion is the main elimination pathway of ergotamine and its metabolites, accounting for about 80%–90% of the absorbed dose in Rhesus monkeys and humans (Aellig & Nüesch, 1977; Nimmerfall & Rosenthaler, 1976), whereas urinary elimination of unchanged ergotamine was observed as a minor excretion pathway in humans (Aellig & Nüesch, 1977).

Urinary excretion of EAs appeared to be predominant in cattle grazing infected tall fescue (~96% of the estimated EA intake) and generally a low faecal excretion (~5% of EA intake) was observed. The different excretion profile is likely attributable to the specific absorption profile of EAs in ruminants, although the role of the ruminal microflora metabolism leading to cleavage of the peptide moiety cannot be disregarded (Schumann et al., 2009; Stuedemann et al., 1998; Westendorf et al., 1993).

To summarise, data on toxicokinetics are sparse. The available literature suggests that, in food producing species, EAs are absorbed from the gastrointestinal tract and subjected to oxidative biotransformation by CYP3A to form mono- and di-hydroxylated metabolites.

Transfer

Concentrations of EAs in serum, bile, liver, meat and back fat in piglets exposed to 6.96 mg/kg feed were in all cases lower than the detection limits (10 μ g/kg) for the respective EAs (Mainka, Dänicke, Böhme, Ueberschär, et al., 2005).

Young and Marquardt (1982) fed chickens various concentrations of ergotamine tartrate. Only at the highest concentration (810 mg/kg feed) residual amounts of ergotamine could be detected in liver and muscles, which did not exceed 10 μ g/kg. Chickens given diets containing 0.31, 2.09 and 6.01 mg T-EAs/kg, showed no EA concentrations in serum, bile, liver and breast meat (LOQ 5 μ g/kg) suggesting a negligible transfer of intact EAs (Dänicke, 2017). EAs were not found (LOQ of 5 μ g/kg) in egg yolk and albumen, blood, liver and breast muscle of laying hens exposed to 14.56 mg T-EAs/kg (Dänicke, 2016).

EA concentrations in liver, breast meat and serum of Pekin ducks given diets containing 0.63, 6.96, 11.39 and 16.39 mg T-EAs/kg feed were below the LOQ (5 μ g/kg) (Dänicke, 2015).

Wolff et al. (1995) exposed dairy cows to feed with a T-EA concentration corresponding to 1.835 mg/animal per day (equivalent to approximately 3 μ g/kg bw) and did not find residues in milk (LOQ not stated). They concluded that the transfer rate into milk is less than 10% of the dose applied. Durix et al. (1999), in a study with lactating goats, found 0.71 ± 0.17 μ g/L of ergovaline in the milk 8 h after intravenous injection of ergovaline at a dose of 32 μ g/kg bw.

Based on the above observations, the CONTAM Panel concluded that transfer of intact EAs to tissues of chickens, laying hens, Pekin ducks or piglets is negligible. The same holds for the transfer of intact EAs to eggs in laying hens and milk in dairy cows. No information is available for EA metabolites.

3.1.2 | Effects in food-producing and non-food producing animals

This section describes the current data on effects of EAs in food- and non-food producing animals. Since occurrence data, required for the exposure assessment and risk characterisation, are only available for EAs produced by *C. purpurea*, the studies on other EAs produced by *C. africana* and endophytes are described in Appendix F but overall summaries are included below. The Tables show the NOAEL/LOAEL for experimental studies or for case studies the feed level causing an effect considered adverse. This information was used for deriving an RP for adverse animal health effects. Serum prolactin levels are a sensitive biomarker of effect, but in the absence of adverse effects, the decrease in prolactin level by itself was not considered as a critical endpoint.

3.1.2.1 | Pigs

C. purpurea

The previous EFSA Opinion reported a series of studies on the effects of EAs on pigs with special emphasis on performance. The main conclusions are summarised below.

The first study (Mainka, Dänicke, Böhme, Ueberschär, et al., 2005) involved 36 pigs for fattening (18 gilts and 18 barrows) fed ad libitum with diets contaminated with rye ergot resulting in 0.05, 0.60 or 4.66 mg T-EAs/kg feed. At the highest dose, body weight gain was significantly lower than the control on trial day 102. At the mean body weight of 115 kg the animals were slaughtered, showing a significant increase of relative heart and spleen weights at the highest dose. Although the previous CONTAM Panel considered a 'no observed-effect level' (NOEL) between 0.60 and 4.66 mg/kg feed, the current CONTAM Panel concludes that 0.6 mg T-EAs/kg feed is the NOAEL for this study.

In the second study (Mainka, Dänicke, Böhme, Wolff, et al., 2005), eight castrated male and 8 female piglets per group were fed for 35 days with a control diet or diets contaminated with 1.39, 2.78, 5.56 or 11.12 mg 'total alkaloids'/kg feed, determined by LC-FLD. In the analytical method used only one third of the 'total alkaloid' content was identified and quantified as five EAs (ergometrine, ergotamine, ergocornine, α -ergocryptine and ergocristine; the 'inine' epimers were considered less toxic by the authors and therefore not quantified). For these five EAs the content in the diet was 0, 0.47, 0.94, 1.88 and 3.76 mg/kg. Of note, the alkaloid content was not measured in the feed, but was calculated from the content in the rye ergot sclerotia that were mixed into the diets. Feed intake and weight gain were significantly decreased in the highest dosed group. In addition, serum albumin concentrations showed a significant linear decrease, while serum aspartate aminotransferase (AST) level was significantly increased at the highest dose level.

The third study (Mainka et al., 2007b) included 80 crossbred piglets allocated to five groups and fed for 35 days with control diet or diets contaminated with EAs at a low level (3.39 and 3.75 (mean 3.57) mg T-EAs/kg feed) or high level (6.97 and 7.66 mg T-EAs/kg feed). These diets were obtained from two batches of rye ergot with contrasting patterns of EAs (especially the ergotamine content was nearly three times higher in one batch than in the other one). It was concluded that the pattern of alkaloids had no effect on growth performance or serum biochemical parameters. The cumulative body weight gains of the two highly contaminated groups were significantly decreased relative to the control group and showed a linear dose-response. The serum protein content was also significantly decreased in the high dosed groups as compared to the control. In the previous Opinion (EFSA CONTAM Panel, 2012), this last study was used to derive a NOAEL of 3.57 mg T-EAs/kg feed for pigs.

Two recent papers, not included in the previous Opinion, investigated through the analysis of case reports, the implication of EAs in porcine ear necrosis (PEN). The first one (Weissenbacher-Lang et al., 2012) aimed to identify the causative factor of this syndrome in 72 pigs, 5.5–10 weeks of age, housed on nine farms. The prevalence of ear necrosis on the farms ranged from 10% to 100% of the piglets. Biopsy samples of ear pinnae revealed contamination with *Streptococci*, *Staphylococci*, porcine reproductive and respiratory syndrome virus or *Mycoplasma suis*. Twenty-seven feed samples were analysed for a selected set of mycotoxins, including the *Fusarium* toxins deoxynivalenol and zearalenone and the EAs ergocornine, ergosine, ergotamine, ergocryptine and ergocristine. With respect to EAs, the medians for ergocristine, ergocryptine, ergosine and ergotamine levels were 0.023, < LOQ, < LOQ and 0.016 mg/kg, the highest levels were 2.02, 0.82, 0.49 and 0.048 mg/kg, respectively. Ergocornine was practically absent (< 0.015 mg/kg). A correlation between mycotoxin concentration and the progressive development of PEN was performed. For deoxynivalenol, the correlation was found only in the initial phase of PEN. There were also significant correlations between increased ergosine, ergotamine and ergocristine concentrations and the microscopic alterations in the superficial ear tissue layers. The authors concluded that PEN is of multi-factorial origin and that infectious agents are not the exclusive triggering factor although they may be involved in the development of the syndrome.

The second case report (Malik et al., 2021) investigated the prevalence of PEN in a Belgian farm with this problem in nursery pigs. In six consecutive batches of weaned piglets, the authors observed that 11%–32% of the animals were affected. Mild, moderate, severe and very severe lesions represented 84.6%, 14.0%, 1.3% and 0.1% of all lesions, respectively. The average daily gain (ADG) of animals that had PEN lesions and the one that did not have PEN lesions were not statistically different. Different mycotoxins, especially type A and type B trichothecenes, enniatins, zearalenone, tenuazonic acid, alternariol-monomethyl and EAs (average 0.143 mg/kg, range 0.068–0.26 mg T-EAs/kg) were present in the feed, but all at low concentrations. The prevalence of PEN was not modified by the inclusion of a mycotoxin detoxifier (hydrolytic enzyme) in the feed.

Waret-Szkuta et al. (2019) reported a case study related to a farrow-to-finish pig farm with 160 sows, where excessive neonatal mortality was reported in association with a loss of appetite and agalactia of sows. Observation of wheat samples revealed ergot sclerotia and EAs (6 'ine' epimers) were measured. The corresponding six 'inine' epimers were not reported. Exposure of sows to 3.49 mg EAs/kg gestation feed for 10 to 15 days before the end of gestation and to 8.06 mg EAs/kg lactation feed over 3 to 4 days at the beginning of lactation led to the abovementioned effects in pigs. Ergocryptine, described for its effect on prolactin levels, was present at 0.60 mg/kg and 0.69 mg/kg in the two diets. No clinical signs associated with vasoconstrictive effects were observed. It is not possible to determine an RP from this study, however, it indicates that 3.5 mg of the 6 'ine' EAs/kg complete feed during the gestation phase followed by 8.1 mg of the six 'ine' EAs/kg complete feed during the lactation phase induces effects in sows, leading to very high neonatal mortality (up to 76%).

Four other studies have been published since the last EFSA CONTAM Opinion (2012) and are described below. Maruo et al. (2018) investigated the effect of two doses of EAs in pigs with special emphasis on the liver and the intestine. Three groups of 24 weaned piglets (21 days of age) were exposed for 28 days to control feed or feed contaminated with wheat

ergot sclerotia resulting in 2.4 or 5.1 mg T-EAs/kg feed. Experimental diets containing EAs significantly reduced feed intake and consequently growth performance. A significant decrease in plasma concentration of creatine kinase and cholesterol was observed in animals receiving diets containing EAs. Histopathological examination of the liver showed inflammatory infiltrates, vacuolisation, apoptosis and necrosis of hepatocytes as well as the presence of enlarged hepatocytes (megacocytes) in animals receiving the diets containing EAs. In the jejunum, EAs reduced villi height and the number of mucus-producing cells. In animals fed the highest dose of EAs a significant increase of intestinal epithelium damage as well as an upregulation of mRNA coding for different tight junction proteins were also observed. This study indicates that a diet containing 2.4 mg T-EAs/kg complete feed, the lowest dose tested, induced effects in piglets.

Dänicke and Diers (2012) aimed to evaluate EA effects on performance and liver function of 31-day-old piglets with the ^{13}C -methacetin breath test,¹⁵ using two routes of tracer administration (orally and intramuscularly). Two rye ergot batches with different EA composition were mixed into piglet diets resulting in 21 and 16.8 mg T-EAs/kg feed and compared with a control diet with minimal level of EAs (0.1 mg T-EAs/kg feed). The animals received the different diets for 35 days. Feed intake and growth rate were significantly depressed after feeding the experimental diets starting at the lowest concentration. The time at maximum $^{13}\text{CO}_2$ exhalation and the half-life were not influenced by treatments. The cumulative $^{13}\text{CO}_2$ -recovery was significantly lower due to feeding the 21 mg T-EAs/kg diet compared with the 16.8 mg T-EAs/kg diet and the control diet. This study indicates that a diet containing 16.8 mg T-EAs/kg complete feed, the lowest dose tested, induces effects in piglets.

The objective of another study by Dänicke and Diers (2013a) was to assess the effect of feeding EAs to 31-day-old piglets on performance (feed intake, bodyweight gain, feed to gain ratio) and liver function by using the ^{13}C -methacetin breath test. Two rye ergot batches with different EA compositions were used. They were mixed into piglet diets resulting in experimental diets with four different EA concentrations: 5.3, 6.0, 9.2 and 20.9 mg T-EAs/kg feed. Of note, the control diet was shown to contain 0.8 mg T-EAs/kg feed. The animals received the different diets for 35 days. Body weight gain decreased in all tested groups compared to the control and the effect was already significant at the lowest dose of 5.3 mg T-EAs/kg. Feed intake also decreased with the concentration of T-EAs in the diet, but this effect was only significant in animals fed 6.0, 9.2 and 20.9 mg T-EAs/kg feed. The time of the maximum $^{13}\text{CO}_2$ exhalation occurred significantly earlier in control piglets compared to the groups fed diets containing 9.2 and 20.9 mg T-EAs/kg feed, whilst the elimination half-life remained uninfluenced whatever the diet. The cumulative $^{13}\text{CO}_2$ -recovery was significantly reduced in piglets fed the high EA concentration experimental diet (20.9 mg T-EAs/kg feed) compared to the animal receiving the control diet or the experimental diet containing 9.2 mg T-EAs/kg feed. This study indicates that a diet containing 5.3 mg total EAs/kg feed, the lowest dose tested, induces effects in piglets.

In Dänicke and Diers (2013b), the aim of the study was to assess the effects of EAs on performances as well as microsomal and mitochondrial liver function of piglets using the ^{13}C -methacetin and ^{13}C - α -ketoisocaproic acid breath test,¹⁶ respectively. Two rye ergot batches with different EA compositions were used. They were mixed into piglet diets, resulting in experimental diets with four different concentrations of T-EAs: 9.1, 10.6, 13.8 and 22.1 mg T-EAs/kg feed. The control diet was free of EAs. The animals received the different diets for 35 days. Feed intake and body weight gain were significantly decreased in all animal groups receiving experimental diets. Feeding the diet containing 22.1 mg T-EAs/kg feed decreased the microsomal and mitochondrial liver function but not significantly. The two highest doses of EAs also decreased the concentrations of bilirubin and increased that of glucose in the plasma. This study indicates that a diet containing 9.1 mg T-EAs/kg feed, the lowest tested dose, induces effects in piglets.

The 2005 Opinion described 3 studies concerning the effect of EAs from *C. purpurea* on sows. A study from 1945 indicated that 0.1% ergot sclerotia from barley reduced milk production and a level of 0.5% ergot sclerotia in the diet prior to farrowing resulted in an increased number of weak or stillborn piglets (Nordskog & Clark, 1945). Another study, published in 1972, indicated that abortions occurred when sows were fed diets containing 0.53% barley ergot sclerotia (Campbell & Burfening, 1972). The EA content was not assessed in these studies. The third study was performed with ergotized barley and although animals were more irritable, no significant effect on the maintenance of pregnancy and on lactation, nor on piglet weights and growth were found at the highest level tested, i.e. 0.2% ergot sclerotia corresponding to 4.4 mg of the sum of 7 EAs/kg feed (Digneau et al., 1986). The total EA content was 4.8 mg/kg feed (including the 'inines', reported as inactive EAs). The Panel noted that the ergot sclerotia used in the study of Digneau et al. (1986) contained high relative amounts of ergocristine compared to ergotamine, while in the study of Maruo et al. (2018) this was the opposite. Ergotamine may be more potent than ergocristine in generating effects (Craig et al., 2015; Klotz et al., 2010).

In summary, the study by Maruo et al. (2018) demonstrated adverse effects at the lowest applied dose of 2.4 mg T-EAs/kg feed. Applying an uncertainty factor of 3¹⁷ to deduce a NOAEL from a LOAEL, a NOAEL of 0.8 mg T-EAs/kg feed was derived. For pigs for fattening, a level of 0.6 mg T-EAs/kg feed was considered a NOAEL based on the study by Mainka, Dänicke, Böhme, Ueberschär, et al. (2005). The very limited data available for EAs from *C. purpurea* for sows suggest that they are not more susceptible than piglets or growing pigs.

The CONTAM Panel derived an RP for adverse animal health effects of 0.6 mg T-EAs/kg complete feed for piglets and pigs for fattening (Table 2).

¹⁵The aim of the ^{13}C -methacetin breath test, resulting in the release of $^{13}\text{CO}_2$, is to assess the P450 dependent activity of liver, providing a non-invasive liver function assessment.

¹⁶The aim for the ^{13}C - α -ketoisocaproic acid breath test is to assess the hepatic mitochondrial function, providing another non-invasive assessment of liver function.

¹⁷The use on 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, 2012) and ECHA's 'Guidance on information requirements and chemical safety assessment Chapter R.8' (ECHA, 2012).

TABLE 2 Studies on adverse effects on pigs allowing to derive a NOAEL or LOAEL.

N ^a /group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	NOAEL/LOAEL (mg T-EAs /kg feed) ^b	Reference
18 gilts 18 barrows	102 to 158 days 0.05, 0.6 and 4.66 mg T-EAs/ kg feed	Relative weight of heart and spleen, body weight gain	NOAEL 0.6	Mainka, Dänicke, Böhme, Ueberschär, et al. (2005)
40 gilts 40 barrows	35 days 0; 1.39; 2.78; 5.56 and 11.12 mg EAs/kg feed Only five EAs quantified ^c	Growth performances Biochemical serum parameters	NOAEL 5.56	Mainka, Dänicke, Böhme, Wolff, et al. (2005)
80 crossbred piglets	35 days 0; low doses (3.39 and 3.75, mean 3.57); high doses (6.97 and 7.66) mg T-EAs/kg feed	Growth performances Biochemical serum parameters	NOAEL 3.57	Mainka et al. (2007b)
36 crossbred castrated males 36 females	28 days 0, 2.4 or 5.1 mg T-EAs/kg feed. Trace of deoxynivalenol	Reduced feed intake and growth performance Plasmatic concentration of creatinase alteration of the histology of the liver and the intestine	Effect observed at 2.4	Maruo et al. (2018)
36 castrated males 36 females	35 days 0.2, 16.8 and 21 mg T-EAs/kg feed	Decreased feed intake and growth rate	Effect observed at 16.8	Dänicke and Diers (2012)
Crossbred animals 40 castrated males 40 females	35 days 0.8, 5.3, 6.0, 9.2 and 20.9 mg T-EAs/kg feed	Decreased body weight gain	Effect observed at 5.3	Dänicke and Diers (2013a)
40 castrated males 40 females	35 days 0, 9.1, 10.6, 13.8 and 22.1 mg T-EAs/kg feed	Decreased feed intake and body weight gain	Effect observed at 9.1	Dänicke and Diers (2013b)

Abbreviations: LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level.

^aIncluding the number of animals in the control group.

^bIn studies where only one dose was used and effects observed, the concentration was considered as 'concentration with effects', not necessarily a LOAEL.

^cEAs quantified: ergometrine, ergotamine, ergocornine, ergocristine, α -ergocryptine.

C. africana

In the previous EFSA Opinion the effect of EAs from *C. africana* was reported (Kopinski et al., 2007, 2008). These studies are summarised in Appendix F. Concerning EAs from *C. africana*, effects on milk production were observed in primiparous sows at 1.4 and 2.8 mg EAs/kg feed which was considered adverse. Considering 1.4 mg EAs/kg feed as a LOAEL and using an uncertainty factor of 3¹⁷, would result in a NOAEL of 0.5 mg EAs from *C. africana*/kg feed for sows.

3.1.2.2 | Poultry

Chickens for fattening

In its previous Opinion on EAs (EFSA CONTAM Panel, 2012), the CONTAM Panel referred to Mainka, Dänicke, Böhme, Wolff, et al. (2005) as the only study available for poultry at the time. The authors fed five groups of 28 birds (seven cages with four 1-day-old male chickens each [Lohmann Meat]) for 21 days with diets to which ground rye ergot sclerotia were added at inclusion rates of 0, 0.5, 1.0, 2.0 and 4.0 g/kg. The diets consisted mainly of maize, wheat and soybeans and were offered in mash form for ad libitum intake. The EA content of the ergot was determined by liquid chromatography with fluorescence detection (LC-FLD). All detectable peaks were considered to reflect the 'total alkaloid' content (2775 mg/kg). Five EAs were determined individually, ergometrine, ergotamine, ergocornine, α -ergocryptine and ergocristine, resulting in a sum content of 931 mg/kg. The 'total alkaloid' content in the experimental diets was calculated from the content in ergot sclerotia to be 0, 1.4, 2.8, 5.6 and 11.1 mg/kg. No ergot related difference was seen in feed intake, body weight gain and the AME_n/gain ratio in the individual weeks and for the entire study period.

Organ weights determined on all chickens at study end were not different for liver and Bursa fabricii but showed a dose dependent decrease for heart. However, the difference between the control group and the groups with 1.4 and 2.8 mg 'total alkaloids'/kg was not significant. Routine blood chemistry and intestinal mucosa were examined for three groups only, the control and the groups with 2.8 and 11.1 mg 'total alkaloids'/kg feed. Serum activities of GLDH and ALT were not significantly affected by the dietary treatment, whereas γ -GT as well as bilirubin showed a significant linear increase in serum. Serum albumin was significantly lower in the groups exposed to EAs than in the control group.

Feeding EAs for 21 days at the level of 2.8 mg 'total alkaloids'/kg feed caused inflammation of the mucous membrane of the proximal duodenum in 11 out of 28 animals but this was only of slight and medium severity. The higher level tested (11.1 mg 'total alkaloids'/kg) irritated the mucous membrane in only 4/25 animals but caused more severe macroscopic inflammation. The authors concluded that the mucous membrane of the duodenum of young chickens reacted quite sensitively

to the ergot in the used doses. However, these impairments had no influence on performance. The CONTAM Panel noted that the final 3-week body weight of about 730 g is rather low indicating also a low feed intake. Effects on zootechnical parameters, such as feed intake, become more visible when maximum performance is obtained.

From this study, the 2.8 mg 'total alkaloids'/kg (0.94 mg/kg for the sum of 5 EAs) could be regarded as a LOAEL. However, no data were available for the lower dose of 1.4 mg 'total alkaloids'/kg concerning the critical endpoint (i.e. inflammation of the mucous membrane of the proximal duodenum). Thus, the conclusion made in the 2012 Opinion (EFSA CONTAM Panel, 2012) that a NOAEL could be identified at 1.4 mg EAs/kg is not supported in the current Opinion.

One new broiler study (Dänicke, 2017) was identified in the literature search. The study was designed as a three-factorial experiment, considering also inclusion of two levels of soybean oil and the addition of a xylanase as feed additive. Group size was 10 cages with 8 birds each, study length 35 days. All diets were calculated to be isoenergetic and similar in crude protein content. Since there were no relevant interactions between the additions of fat and xylanase with the EA content (with the exception of feed intake for the group given ergot and enzyme), mean values for groups exposed to the same dietary EA concentration were calculated. Ergot rye containing 211.4 mg T-EAs/kg was used as source of EAs and included in the diets. The analysed levels in feed were 0.15 (for the control group), 0.31, 2.09 and 6.01 mg T-EAs/kg.

After 14 days, ADG for the control and the groups with low, medium and high T-EA levels in feed was 25, 27, 26 and 13 g, average daily feed intake (ADFI) 33, 35, 35 and 21 g, and feed to gain (F/G) ratio 1.33, 1.31, 1.34 and 1.57, respectively. Due to serious health problems in the group with the highest dietary EA level (13.8% total losses after 14 days), the study was terminated for this group. Chickens of these groups were partly unable to stand and displayed uncoordinated movements. Necropsy showed dilatation of the proventriculus, multifocal bleedings of gastric mucosa, mild duodenitis and multifocal haemorrhages and necrotic lesions in the liver. Serum enzymes AST, ALT and GLDH, significantly increased, as well as relative liver weight, whereas serum albumin levels and relative weight of Bursa Fabricius decreased. It was noted that (i) the control feed without addition of ergot contained on average 0.15 mg T-EAs/kg (0.13, 0.25, 0.14 and 0.08 mg T-EAs/kg feed) and (ii) the mortality of the control group in the first 14 study days was high (10%), comparable to the group with highest EA exposure, for which no explanation was given.

At study end, EAs significantly increased ADG and decreased dose dependently ADFI (91, 88 and 81 g for the control and the groups with 0.31 and 2.09 mg T-EAs/kg, respectively) resulting in an improved F/G ratio (by 6 and 12% for the groups with 0.31 and 2.09 mg T-EAs/kg, respectively, compared to the control). However, the effect on feed intake depression by 0.31 mg T-EAs/kg was significant only for the groups with xylanase supplemented feed.

Feed intake and reduced body weight gain appeared to be the most sensitive endpoint being reduced by dietary EAs. This was seen at 6.01 mg T-EAs/kg feed already after 14 days exposure. Reduced feed intake was also observed after 35 days for the next lower T-EA level (2.09 mg/kg). Normally, lower gain (final body weight) can be expected when feed intake is reduced. This was not the case for the 35-day results, less feed was required per unit body weight gain. Considering the 35-day data, lowering of feed intake can, therefore, not be considered as adverse. Consequently, the NOAEL of the study was 2.1 mg T-EA/kg feed.

Laying hens

A study with laying hens (Dänicke, 2016) considered the effect of heat treatment (by expansion, 95°C for 2 min preconditioning, 120°C for 5 s, 40 bar pressure) of ergot rye (25% ergot), which was used as the source of dietary EAs. Groups of 39 Lohmann-Brown laying hens (22 weeks old) were fed for 20 weeks diets containing levels for the untreated ergot rye of 0.1, 0.56, 1.97, 3.72 and 14.56 mg T-EAs/kg and for the treated ergot rye of 0.03, 0.42, 2.27, 3.81 and 13.03 mg T-EAs/kg, respectively. All diets were calculated to be approximately isoenergetic and isonitrogenous. Heat treatment of the ergot rye had an inconsistent effect on the T-EA concentration in feed.

The highest EA concentration in feed (14.56 mg T-EAs/kg) significantly reduced laying rate, daily egg mass (laying rate × egg weight), feed to egg mass ratio, relative eggshell weight, egg yolk colour and nitrogen and crude fat retention of the body; it increased serum albumin and total bilirubin, relative weight of liver and stomach/gizzard. It reduced also the weight of hatched chicks. Thermal treatment of ergot rye lowered the toxic effects in hens. The CONTAM Panel derived a NOAEL for this study of 3.7 mg T-EAs/kg feed.

Ducks

A study with Pekin ducks (Dänicke, 2015) was conducted with 1-day-old birds for 7 weeks. Group size was 54 birds (nine pens with six ducks each). The diets were formulated to be isoenergetic and isonitrogenous. An ergot rye batch (45.2% ergot, 435.8 mg T-EAs/kg) was used as the source of dietary EAs. The analytical values for the supplemented diets were 0.63, 6.96, 11.39 and 16.39 mg T-EAs/kg feed, respectively. Control feed was < LOQ (5 µg/kg). Feed intake decreased significantly during the first week of the study with increasing dietary EA levels and was already significant at the lowest dose group. Body weight gain was also reduced in all dose groups during the first 2 weeks. In week 2, ducks of the groups with the two highest EA levels in feed consumed 51% and 61% less feed than the control group, respectively. Owing to the magnitude of the feed intake depression in the first 2 weeks, these treatments were not continued further.

Cumulative data at study end showed significant differences to the control group for lower feed intake, body weight gain and F/G ratio for the 6.96 mg T-EAs/kg feed group (FI: 154 vs. 173; ADG: 65 vs. 69; F/G: 2.37 vs. 2.51). There were only a few findings in haematology, clinical chemistry and necropsy. The percentage of monocytes was significantly reduced in

both EA groups. In the 6.96 mg T-EAs group, there was a significant increase of γ -GT and of the relative liver weight, compared to control.

Based on the effects on growth observed during the first 2 weeks, a LOAEL of 0.6 mg T-EAs/kg feed was derived for ducks from this study, which by applying an uncertainty factor of 3, results in a NOAEL of 0.2 mg T-EAs/kg feed.

To summarise on EAs from *C. purpurea*, for broilers a NOAEL of 2.1 mg T-EAs/kg feed was derived from the study by Dänicke (2017), while for laying hens, a level of 3.7 mg T-EAs/kg feed was considered a NOAEL based on the study by Dänicke (2016).

In ducks reduced body weight gain and feed intake was seen at 0.6 mg T-EAs/kg feed already in the initial growing phase. This dietary concentration is considered as a LOAEL and allows a deduction of the NOAEL of 0.2 mg T-EA/kg feed by applying an uncertainty factor of 3.

The CONTAM Panel derived an RP for adverse animal health effects of 2.1 mg T-EAs/kg complete feed for broilers, 3.7 mg T-EAs/kg for laying hens and 0.2 mg T-EAs/kg for ducks (Table 3).

TABLE 3 New studies on adverse effects on poultry which have become available since the 2012 Opinion (EFSA CONTAM Panel, 2012).

N^a/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	NOAEL/ LOAEL (mg T-EAs/kg feed)	Reference
1280/16 groups with 10 replicates (80 animals) per treatment, male broilers of the strain Lohmann Meat	0.15, 0.31, 2.09 mg T-EAs/kg feed for 35 days, 6.01 mg T-EA/kg for 14 days	At day 14, reduced feed intake, body weight gain, various other serious effects; no effects at 2.09 mg T-EA/kg after 35 days	NOAEL of 2.1	Dänicke (2017)
390 / 39 Lohmann-Brown laying hens, 22 weeks old	0.10, 0.56, 1.97, 3.72 and 14.56 mg T-EAs/kg feed for 20 weeks	Reduced laying rate, daily egg mass, feed to egg mass ratio, relative eggshell weight, egg yolk colour, nitrogen and crude fat retention Increased serum albumin and total bilirubin, relative weight of liver and stomach/gizzard	NOAEL of 3.7	Dänicke (2016)
324/six groups with nine replicates each (54 animals) per treatment, 1-day-old Pekin ducks of mixed gender	0 (< LOD), 0.63, 6.95, 11.39 and 16.37 mg T-EAs/kg feed for 7 weeks	Reduced feed intake and body weight gain at 0.63 mg T-EAs/kg feed	LOAEL of 0.6	Dänicke (2015)

Abbreviations: bw: body weight; NOAEL; no observed adverse effect level; LOAEL; lowest observed adverse effect level.

^aIncluding the number of animals in the control group.

3.1.2.3 | Ruminants

Bovines

C. purpurea

In its previous Opinion (EFSA CONTAM Panel, 2012) the Panel was unable to identify an RP for adverse health effects in bovines. One of the studies, performed by Schumann, Dänicke, Meyer, et al. (2007), was conducted with Holstein Friesian bulls fed a diet containing rye ergot at dose levels of 0, 1.4 and 8.6 μ g T-EAs/kg bw per day for 230 days. Animals were kept in a stable. No significant differences in body weight gain (increase from 227 to 550 kg bw), dry matter intake, carcass composition, relative liver and kidney weight or serum liver enzyme activities (AST, GLDH, γ -GT) were observed. T-EA levels in the total mixed ration (TMR) were 0, 0.069 and 0.421 mg/kg DM with ergotamine (25%), ergocristine (15%) and ergosine (13%) contributing most to the T-EA content.

In a second study by Schumann, Dänicke, Hübner, et al. (2007), male Holstein calves were treated with cereal-based rations containing 0, 1 and 5 g rye ergot/kg feed for 84 days. Average T-EA levels in the concentrates were 0.04, 0.378 and 1.496 mg/kg with ergotamine (26%), ergocristine (14%) and ergosine (12%) contributing most to the T-EA content. Again, no differences in growth (increase from 50 to 110 kg bw), overall feed intake and feed to gain ratios were observed, although there was a reduced intake of concentrate during the first weeks in both dose groups. The intake of concentrate increased from 0.7 to 1.7 kg DM/day, as compared to 0.2 to 1.3 kg DM/day for roughage. Based on concentrate intake and body weight, the intake of EAs can be estimated to be around 5 and 20 μ g/kg bw per day for the low and high dose groups, respectively.

Other studies reviewed in the previous Opinion, in which animals were affected by ergot, did not provide estimated doses of EAs.

Grusie et al. (2018) exposed 36 pregnant Hereford cows (average weight 576 kg) for 9 weeks to rations containing 0.005, 0.048, 0.201 and 0.822 mg EAs/kg DM (from wheat contaminated with *C. purpurea*), in the last part of the gestation.

Concentrations are the sum of the six 'ines', but no information was given about the composition. Ambient temperatures were 21°C. There were no significant treatment related effects on body weight, dry matter intake, rectal temperatures, serum levels of prolactin and progesterone of the cows, and no effects on body weight of the delivered calves.

Stanford et al. (2022) investigated the effect of heat treatment and pelleting of rye ergot-contaminated (*C. purpurea*) feed in 48 Angus-cross steers (bw 290 ± 4.4 kg). The animals were dosed daily with feed containing either no, 1.6 and 2.7 mg T-EAs/kg feed without any effect on body weight, daily gain, serum prolactin levels or rectal temperature. There were some, but inconsistent, effects on blood counts.

Cowan et al. (2023) examined the effects of EAs from *C. purpurea* on sperm production and quality in 14 adult Angus bulls (bw 864 ± 111 kg). Two groups of animals were examined during a pre-treatment period of 12 weeks, a treatment period of 9 weeks (March to May, ambient temperatures between -18°C and 19°C) where they received a TMR containing either 1.11 ($n=8$) or 2.23 ($n=6$) mg EAs/kg DM, followed by a 10-weeks post-treatment period. The EAs were included via pellets contaminated with wheat ergot; with ergocristine, ergocryptine and ergotamine contributing 51, 18 and 14% to the total EA content; 'inines' were not reported. There were no treatment related effects on body weight. Serum prolactin levels decreased two-fold on both dosages and recovered during the post-treatment period. There were no clear effects on rectal temperature and scrotum circumference. No effects on spermatogenesis or sperm morphology were observed.

In addition to these controlled studies, several case reports were published.

Rösch et al. (2013) described a case study in Switzerland with 25 cows. Animals showed hyperthermia and decreased milk production following consumption of contaminated triticale (maximum 2 kg per day). Determined levels were 4.0 mg/kg for ergocornine/-inine, 3.8 mg/kg for ergosine/-inine, 2.4 mg/kg for ergotamine/-inine, 2.0 mg/kg for ergocryptine/-inine, 1.5 mg/kg for ergocristine/-inine and 0.4 mg/kg for ergometrine/-inine, in total 14.3 mg T-EAs/kg triticale. The authors estimated a daily intake of 50 µg T-EAs/kg bw.

Leuschen et al. (2014) described a case in Iowa, USA, with 80 red Angus calves (average body weight 227 kg) showing hyperthermia and poor weight gain. Eight of them died. The animals were fed with contaminated creep feed that contained 0.30 mg ergotamine/kg, 0.10 mg ergosine/kg, 0.06 mg ergocornine/kg and 0.04 mg ergovaline/kg, in total 0.5 mg/kg. The animals consumed on average 6.8 kg creep feed per day, amounting to an intake of 15 µg EAs/kg bw per day. It was noted that in the disease period the ambient temperatures during several days reached 38°C or higher.

Miskimins et al. (2015) described a case in South Dakota, USA, where 12 calves (181–272 kg bw) out of 100 showed loss of tail switches and tips, and 3 of them showed early lameness and swelling of the feet. Animals received creep feed (4.5–6.8 kg per day) contaminated with 0.025 mg ergosine/kg, 0.030 mg ergotamine/kg, 0.020 mg ergocornine/kg, 0.035 mg ergocryptine/kg and 0.095 mg ergocristine/kg, in total 0.205 mg/kg.

Craig et al. (2015) described eight case studies from Canada and the USA in bovines and the feed levels responsible for the occasionally very severe effects. All cases occurred in winter or spring, when temperatures were low, between -20°C and 1°C. The EA levels included ergosine, ergotamine, ergocornine, α-ergocryptine and ergocristine (epimers and ergometrine were not measured due to limitations in the method). LOQs were in the range of 40–50 µg/kg. Depending on the EAs detected in the feed, the sum of EAs varied by a factor 1–15. The lowest absolute level was 0.47 mg/kg feed DM resulting in tail loss. Only ergotamine was detected above the LOQ. Two other cases with EA levels of 1.5 (ergotamine+ergocornine) and 2.9 mg (ergotamine+ergocornine+ergocryptine)/kg feed DM, resulted in moderate lameness and decreased feed intake, respectively.

To summarise, the experimental studies failed to show any effect using high levels varying between 0.4 and 1.7 mg/kg DM. However, several case reports suggest that lower levels may cause adverse effects (decreased feed intake, poor weight gain, hyperthermia, loss of tail switches and tips, early lameness and swelling of the feet, reduced milk production, death), possibly aggravated by weather conditions, as detailed in Tables 4 and 5. The study by Craig et al. (2015) demonstrated adverse effects at 0.47 mg EAs (sum of five EAs)/kg feed, Miskimins et al. (2015) at a concentration of 0.2 mg EAs (sum of five EAs)/kg feed and Leuschen et al. (2014) at a concentration of 0.5 mg EAs (sum of four EAs)/kg feed. The CONTAM Panel noted the controversy between the controlled studies and case studies. However, weather conditions appear to play an important role in the adverse effects of EAs, as also shown for EAs in infected grass (see Appendix F).

A LOAEL of 0.2 mg EAs/kg feed was derived from the study by Miskimins et al. (2015). The Panel noted that the ergosine and 'inines' were not included in the analysis. Assuming that the concentration of ergosine and the 'inines' was 50% of the concentration of the 'ine' epimers, this results in a LOAEL of 0.3 mg T-EAs/kg. Applying an uncertainty factor of 3¹⁷ to deduce a NOAEL from a LOAEL, a NOAEL of 0.1 mg T-EAs/kg feed would be derived.

The CONTAM Panel derived an RP for adverse animal health effects of 0.1 mg T-EAs/kg complete feed for bovines.

TABLE 4 Controlled studies on adverse effects on bovines exposed to EAs from *C. purpurea*.

N^a/group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	Concentration showing adverse effects (mg EAs/kg feed)^b	Reference
38 Holstein Friesian bulls	0, 0.069 and 0.421 mg T-EAs/kg feed DM for 230 days	Body weight, feed intake, carcass composition, kidney and liver weight, liver enzyme activity	No effects observed	Schumann, Dänicke, Meyer, et al. (2007)
35 male Holstein calves	0.04, 0.378 and 1.498 mg T-EAs/kg concentrate for 84 days	Body weight, feed intake, feed to gain ratios, health parameters	No effects observed	Schumann, Dänicke, Hübner, et al. (2007)
36 pregnant and postpartum Hereford cows	0.005, 0.048, 0.201 and 0.822 mg EAs/kg feed DM for 9 weeks Only the 'ines' quantified ^a	Body weight (cows and calves), rectal temperature, serum levels of prolactin and progesterone, ovarian parameters, pregnancy rates	No effects observed	Grusie et al. (2018)
48 Angus-cross steers	0, 1.6 and 2.7 mg T-EAs/kg feed	Body weight, daily gain, serum prolactin levels, rectal temperature	No effects observed	Stanford et al. (2022)
14 adult Angus bulls	1.11 and 2.23 mg EAs/kg feed DM for 9 weeks Only the 'ines' quantified ^a	Body weight, rectal temperature and scrotum circumference, spermatogenesis or sperm morphology not affected. Serum prolactin levels decreased	No effects observed	Cowan et al. (2023)

^aIncluding the number of animals in the control group.

^bEAs quantified: ergometrine, ergotamine, ergosine, ergocristine, ergocornine, ergocryptine.

TABLE 5 Case studies on adverse effects on bovines exposed to EAs from *C. purpurea*.

N/group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	Concentration showing adverse effects (mg EAs/kg feed)^b	Reference
25 dairy cattle	14.3 mg T-EAs/kg triticale, max 2 kg triticale/cow per day	Hyperthermia, heavy dyspnoea, increased water intake, reduced milk production	1.5 ^d	Rösch et al. (2013)
60 Red Angus beef calves	0.5 mg EAs/kg creep feed Only four EAs quantified ^a	Death, hyperthermia, poor growth, high rectal temperature, increased respiratory rates, ataxy	0.5	Leuschen et al. (2014)
100 beef calves (Hereford, Simmental, Maine Anjou)	0.20 mg EAs/kg creep feed for more than 4 weeks Only five EAs quantified ^b	Loss of tail switches and tail tips, early lameness, swollen feet	0.2	Miskimins et al. (2015)
Cattle	Case (a) 0.47 ergotamine/kg feed DM	Tail loss (−20°C, Canada, February)	0.47	Craig et al. (2015)
Cattle	Case (b) 1.5 mg ergotamine + ergocornine/kg feed DM	Moderate lameness (1°C, Oregon, December)	1.5	Craig et al. (2015)
Steers	Case (c) 2.9 mg ergotamine + ergocornine + α-ergocryptine/kg feed DM	Decreased feed intake (−2°C, Idaho, January)	2.9	Craig et al. (2015)
Dairy cattle	Case (d) 3.6 mg EAs/kg feed DM Only six EAs quantified ^c	Early term abortions, low milk yield (−5°C, Oregon, February)	3.6	Craig et al. (2015)
Cattle	Case (e) 6.0 mg EAs/kg feed DM Only six EAs quantified ^c	No feed consumption (−5°C, Idaho, January)	6.0	Craig et al. (2015)
Steers	Case (f) 11.5 mg EAs/kg feed DM Only six EAs quantified ^c	Sloughing of hooves, ears and tails (−4°C, Canada, April)	11.5	Craig et al. (2015)
Cows	Case (g) 54.9 mg EAs/kg feed DM Only six EAs quantified ^c	Early term abortions (1°C, Oregon, January)	54.9	Craig et al. (2015)

N/group, breed gender	Dosage and duration (mg/kg feed)	Endpoint(s)	Concentration showing adverse effects (mg EAs/kg feed) ^b	Reference
Cattle	Case (h) 62.2 mg EAs/kg feed DM Only six EAs quantified ^c	Hooves sloughing completely off (-1°C, Idaho, January)	62.2	Craig et al. (2015)

*In studies where only one dose was used and effects observed, the concentration was considered as 'concentration with effects', not necessarily a LOAEL.

^aEAs quantified: ergotamine, ergosine, ergocornine, ergovaline.

^bEAs quantified: ergotamine, ergosine, ergocornine, ergocryptine, ergocristine.

^cEAs quantified: ergometrine, ergotamine, ergosine, ergocristine, ergocornine, α -ergocryptine.

^dEstimated by the CONTAM Panel, assuming 600 kg body weight and 20kg feed DM intake per day.

C. africana

Two studies by Blaney et al. (2011) and McLennan et al. (2017) were identified with steers fed rations containing sorghum contaminated with EAs from *C. africana* (see Appendix F). The study by Blaney et al. (2011) demonstrated adverse effects at 1.1 mg EAs/kg feed DM. Applying an uncertainty factor of 3¹⁷ to deduce a NOAEL from a LOAEL, a NOAEL of 0.4 mg EAs/kg feed DM was derived. The two studies confirm that weather conditions play an important role in the toxicity of EAs in bovines.

The CONTAM Panel derived an RP for adverse animal health effects for EAs from *C. africana* of 0.3 mg EAs/kg complete feed (88% DM) for bovines.

Epichloë coenophiala (ergovaline/–inine)

In the previous Opinion, the CONTAM Panel described the effects of EAs present in tall fescue, produced by *E. coenophiala*. However, an RP was not derived for these compounds. Therefore, papers published before the previous Opinion as well as newer studies were reviewed and are described in Appendix G. Many studies have been published on the effects of tall fescue in cattle, followed by the identification of endophytes and the production of ergovaline/–inine by these organisms. In addition, studies were performed to compare the effects of pastures containing tall fescue infected with endophyte producing ergovaline/–inine with grass containing a novel endophyte not producing the toxin or endophyte-free grass. Most studies show reduced growth or poor hair coat scores. Concentrations of ergovaline/–inine as low as 0.3 mg/kg DM were shown to cause such effects. This type of studies does not include different levels which would allow the derivation of a NOAEL. Applying a factor 3¹⁷ to the 0.3 mg/kg DM would result in a NOAEL of 0.1 mg/kg DM. This is close to the level of 0.06 mg/kg DM grass not causing reduced ADG, as derived by Liebe and White (2018) who performed a meta-analysis of available studies. An RP for adverse animal health effects of 0.1 × 0.88 rounded to 0.1 mg/kg complete feed (88% DM) was derived for ergovaline/–inine. This RP is similar to that derived for EAs from *C. purpurea* and lower than that for EAs from *C. africana*.

Sheep

C. purpurea

The purpose of a study by Coufal-Majewski, Stanford, McAllister, Wang, Blakley, McKinnon, Swift, and Chaves (2017) was to test different concentrations of cereal EAs, including those around the maximum limit currently applied by the Canadian Food Inspection Agency (CFIA) regulation, which is 2–3 mg/kg for ruminant feed. Two experiments were conducted, measuring nutrient digestibility, growth and carcass composition of ram lambs fed diets with different EA concentrations from barley ergot screenings. In both experiments a control group was compared with three dose groups receiving diets shown to contain respectively 0.034, 0.930, 1.402 and 2.447 mg T-EAs/kg feed. Contaminated barley screenings were used to spike the diets of the dose groups.

In the first experiment 8 Canadian Arcott × Rideau Arcott ram lambs with an average bw of 30.0 ± 3.1 kg were randomly assigned to one of the four diets fed for 21 days. Nutrient digestibility was not influenced by EA concentration up to 2.447 mg T-EAs/kg.

In the second experiment 47 Canadian Arcott × Rideau Arcott ram lambs with a bw of 30.0 ± 5.8 kg were randomly assigned to one of the four diets for approximately 9 weeks (6–13 weeks) until they reached a minimum slaughter weight of 45 kg. Lambs receiving the highest EA dose had a lower ADG compared to the other groups, although there was no difference in DM intake between the four diets. The concentration of serum prolactin linearly decreased with increasing dose of EAs in the diet. Compared to the control group, the lambs of all the dose groups had 0.33°C higher body temperatures. The authors concluded that 2.447 mg T-EA/kg feed reduces the animals' growth in lambs, while at the dose of 1.402 mg EAs/kg feed the animals' growth performance is not negatively affected, however the carcass dressing percentage was reduced.

Coufal-Majewski, Stanford, McAllister, Wang, Blakley, McKinnon, and Chaves (2017) compared the effects of feeding diets contaminated with EAs from barley ergot in pelleted or mash form on the nutrient digestibility and growth performance in lambs. The mash diets contained 0.003 (control), 0.153 and 0.434 mg EAs/kg, the pelleted diets 0.002 (control), 0.185, 0.432 mg EAs/kg. Six R-isomers ('ines') were analysed (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine), but no S-isomers ('inines'). Although total EA concentrations in mash and pelleted feed were similar,

ergocornine, ergocristine and ergometrine were two to three times higher, and ergotamine and ergosine were 2 to 3 times lower in pelleted feed compared to the mash feed. The predominant alkaloids were ergocristine, ergotamine and ergocryptine. Forty-eight Canadian Arcott rams and ewes (24 each) with an average bw of 24.6 ± 1.08 kg were randomly assigned to one of the six diets for up to 12 weeks until they reached a slaughter weight of minimum 45 kg. The authors did not observe any sign of alkaloid toxicosis (e.g. lameness) or effect on the carcass characteristics. However, higher doses of EAs caused decreased serum prolactin concentrations and prolactin was generally lower for mash than for pelleted diet. Although equal T-EA concentrations were fed to the lambs, the ADG was significantly higher for the animals that received pelleted feed compared to those fed mash diets. There was no effect on the DM intake, however ADG was generally lower for the high dose groups compared to the low dose group and the control group.

To summarise, a NOAEL of 0.185 mg EAs/kg feed was derived from the study by Coufal-Majewski, Stanford, McAllister, Wang, Blakley, McKinnon, and Chaves (2017). The Panel noted that the 'inines' were not included in the analysis. Assuming that the concentration of the 'inines' was 50% of the concentration of the 'ine' epimers, this results in a NOAEL of 0.3 mg T-EAs/kg feed. The NOAEL is supported by the other study of Coufal-Majewski, Stanford, McAllister, Wang, Blakley, McKinnon, Swift, and Chaves (2017), showing an increased body temperature at the lowest tested concentration of 0.93 mg T-EAs/kg feed, which is considered a LOAEL. Applying an uncertainty factor of 3^{17} to deduce a NOAEL from a LOAEL, a NOAEL of 0.3 mg T-EAs/kg feed would be derived for sheep.

The CONTAM Panel derived an RP for adverse animal health effects of 0.3 mg T-EAs/kg complete feed for sheep.

C. africana

No studies on toxicity in sheep from ergot alkaloids produced by *C. africana* were identified in the extensive literature search.

Epichloë coenophiala (ergovaline/–inine)

In the previous Opinion, the CONTAM Panel described the effects of EAs present in tall fescue, produced by *E. coenophiala* however, an RP was not derived for these compounds. Papers published before the previous Opinion as well as newer studies were reviewed and are described in Appendix G, which should be consulted to further details on this matter. A number of studies have been published on the effects of ergovaline/–inine in tall fescue in sheep, with specific focus on effects on late gestation, resulting in impact on the placental development and lactation. In a study by Duckett et al. (2014), 0.8 mg ergovaline/–inine/kg feed DM resulted in adverse effects mainly on the health of the progeny. This concentration is considered a LOAEL from which a NOAEL of 0.3 mg ergovaline/–inine/kg feed DM can be derived by applying an uncertainty factor of 3^{17} .

The CONTAM Panel derived an RP for adverse animal health effects of 0.2 mg ergovaline/–inine/kg complete feed for sheep.

3.1.2.4 | Horses

C. purpurea

No studies suitable for deriving an RP for EAs from *C. purpurea* for horses were retrieved neither in the 2005 and 2012 Opinions nor thereafter.

C. africana

No studies on toxicity in horses from ergot alkaloids produced by *C. africana* were identified in the extensive literature search.

Epichloë coenophiala (ergovaline/–inine)

Considering horses, mares were reported to be rather sensitive to ergovaline with various adverse effects like delayed parturition, agalactia (associated with altered prolactin levels) and incidentally neurotoxic symptoms (Appendix G). A review states that horses may show effects at levels as low as 0.05–0.1 mg/kg. However, this statement appears to be based on expert knowledge and it was not possible to retrieve the underlying studies.

3.1.2.5 | Rabbits

In a case study, Korn et al. (2014) reported the association between tail necrosis in rabbits and mycotoxins in rabbit feed. The animals were fed with hay and a commercial pelleted diet ad libitum. Tail necrosis was observed in 14 out of 103 rabbits. Alopecia, erosions, crusts and necrosis were restricted to the tail area and occurred exclusively in young rabbits aged 113 ± 20 days. Histopathologically, the lesions were characterised by muscle fibre degeneration and chronic dermatitis, suggesting that ischemia had caused the necrosis. Feed analysis using enzyme immunoassays showed a mean and maximum EA content of 0.41 ± 0.25 mg/kg and 1.7 mg/kg, respectively. The mean and maximum dietary intake of EAs were 0.017 and 0.071 mg/kg bw, respectively. No EAs were detected in straw or hay samples. Using an enzyme immunoassay (EIA) for total EAs and an EIA specific for ergotamine, 44 samples from three batches of the commercial rabbit feed were screened for their EA content. Total EAs were detected in all samples, at a mean concentration of 0.41 mg T-EAs/kg (range

0.14–1.7 mg/kg). Ergotamine was reported in all samples in a mean concentration of 0.37 mg/kg (range 0.14–0.91 mg/kg). In feed samples of animal groups from husbandries without clinical symptoms, the mean concentration of T-EAs was 0.16 mg/kg (range 0.085–0.29 mg/kg; $n = 3$), the concentration of ergotamine was 0.13 mg/kg (range 0.12–0.14 mg/kg; $n = 2$). Based on these data the authors concluded that the NOAELs for rabbits are 0.29 mg T-EAs/kg feed and 0.14 mg ergotamine/kg feed. The CONTAM Panel noted that the results obtained with the EIA specific for ergotamine were in the same range as the results obtained with the EIA for total EAs. However, it is unlikely that the ergot contamination of the feed samples only consisted of ergotamine. Furthermore, the results are hampered by the simultaneous occurrence of other mycotoxins (DON, T2 toxin, zearalenone) in the experimental feed.

Considering the high uncertainty in the reported concentrations, a NOAEL could not be derived and thus the CONTAM Panel could not establish an RP for adverse animal health effects for rabbits.

3.1.2.6 | *Fish*

Herbivorous fish like carp may be exposed to EAs by feed materials, however only one study could be identified, where different ergot levels were fed but no data on the levels of EAs were provided (Svobodova et al., 1981).

3.1.2.7 | *Non-food producing animals*

Cats and dogs

No new information was identified in the current literature search nor detailed in the previous EFSA Opinions (2005 and 2012).

Farmed mink

No new information was identified in the current literature search nor detailed in the previous EFSA Opinions (2005 and 2012).

3.1.3 | Modes of action

Elucidation of the MoAs of the EA-induced effects is complicated due to the highly variable individual animal response to the exposure to EAs. This is due mainly to the complex plant–fungus–animal–microbe–environmental interaction that results in changing alkaloid concentrations, availability and distribution of various isomeric forms throughout the animal. The primary modes of action of the EAs, whether of tall fescue endophytic or ergot origin, involve vasoconstriction and/or hypoprolactinemia, due to reduced secretion of prolactin by the anterior pituitary.

Vasoconstriction

The vasoconstriction can lead to gangrenous ergotism resulting in loss of extremities such as hooves (e.g. fescue foot), ear tips and tail switches. In ruminants, the vasoconstrictive effects of EAs, whether of endophytic or ergot origin, cause both the so-called gangrenous and hyperthermic forms of EA intoxication. The gangrenous syndrome generally occurs at low environmental temperatures and is characterised by diminished blood flow to the extremities, ultimately leading to dry gangrene.

Klotz and McDowell (2017) evaluated the effects of EAs on the contractile response of medial palmer artery and vein, collected from forelimbs of horses and uterine arteries from mares. All EAs tested induced a contractile response. However, ergovaline was the most vasoactive followed by ergotamine, whereas ergometrine caused the lowest response. Neither ergotamine nor ergometrine were vasoactive in the uterine artery. Cherewyk et al. (2022) demonstrated a difference in sustained vascular contractile response between the R- and S-epimer of ergocristine, suggesting differences in receptor binding.

Hyperthermic syndrome

The hyperthermic syndrome is usually observed under heat stress conditions and develops due to impairment of an animal's abilities to thermoregulate (Evans, 2011). These effects include increased body temperature but also affected breathing and heart rates (further described in the paragraph on ruminants in section 3.1.4). In both the gangrenous and hyperthermic forms of EA intoxication, animal comfort, lameness and stress, as well as interactions involving environmental temperatures and reduced caloric intake, probably play major roles in the pathogenesis of reproductive effects (Evans, 2011). Because of their grazing habits, sheep generally seem to be less susceptible to EA intoxication than cattle. Shaving might also play an important role in susceptibility. EA-exposed sheep with full fleeces are predisposed to hyperthermia when ambient temperatures increase during summer and are expected to be prone to EA-associated declines in reproductive efficiency (Evans, 2011). This seems different from cattle where the fur changes during the summer period but not when exposed to EAs. It is unclear if prolactin plays a role since levels normally increase in springtime, but much less when exposed to EAs.

Reproductive effects

Exposure to EAs could enhance heat stress which could diminish reproductive performance in both male and female animals. Bulls grazing endophyte-infected pastures during the summer showed altered sperm motility parameters, compared with bulls grazing non-infected pastures (Looper et al., 2009). Ovarian follicular dynamics can be adversely affected by tall fescue endophytic toxins, in particular by interactions involving the hyperthermic and/or prolactin-inhibiting actions of these toxins and thermal stress (Burke et al., 2001). Effects on reproduction (including altered cyclicity, suppressed hormone secretion, reduced pregnancy rates, agalactia and reduced offspring birth weights) are observed more commonly and are more severe in horses than in ruminants (Klotz, 2015; Poole & Poole, 2019).

In rats, EAs prevented pregnancy by interfering with implantation and causing embryotoxicity. EAs also inhibit lactation. These effects have generally been observed at higher doses than the LOAELs in the repeated-dose studies.

The primary mechanism of action responsible for the pathogenesis of reproductive effects of EAs is D2-dopamine receptor agonist-induced suppression of prolactin secretion by the anterior pituitary gland: the interaction of EAs with dopamine D2-receptors in the anterior pituitary gland is key to the depression of prolactin levels (Brunton et al., 2006; EFSA CONTAM Panel, 2012; Scharldt et al., 2006). This inhibited prolactin secretion is responsible for the decreased milk production as observed in cattle, sheep and especially in horses (Klotz & Smith, 2015), as well as abnormal progesterone metabolism, delayed parturition and other reproductive abnormalities, including subfertility (Evans, 2011). Ruminants, unlike horses, produce a placental lactogen during pregnancy and are, therefore, not completely dependent on prolactin for lactogenesis, although decreased milk production has been observed in bovines (see section 3.1.4). Cross et al. (2012) investigated the efficacy of domperidone (a dopamine antagonist which increases prolactin levels in humans) in the prevention of reproductive complications of fescue toxicosis in periparturient mares. Pregnant mares were fed 0.2 mg ergovaline/kg diet daily in endophyte-infected fescue hay and seed starting 30 days before their expected foaling date. Twenty mares received domperidone gel (1.1 mg/kg bw orally, once daily) and 15 mares a placebo. Domperidone treatment prevented reproductive complications of fescue toxicosis in periparturient mares.

Adrenergic, serotonergic and dopaminergic receptors

The effects of EAs result from their activity as ligands for a wide array of adrenergic, serotonergic and dopaminergic receptors including 5-HT_{1,2,5/7} receptors, dopamine-D₂-like receptors and α _{1,2}-adrenoreceptors. EA-induced vasoconstriction is associated with D₁-dopaminergic receptor inhibition and partial agonism of α ₁-adrenergic and serotonin-receptors. Because of their structural differences with the physiological monoamine neurotransmitters, EAs are generally characterised by a low specificity and selectivity with respect to the mentioned neuroreceptors and, depending on the individual structure, they can display a complex behaviour as receptor antagonists, partial agonists or partial antagonists (Mantegani et al., 1999; Pertz & Eich, 1999). Moreover, the high heterogeneity of the serotonergic, adrenergic and dopaminergic receptors, and the distribution of different receptor types and subtypes in different tissues result in a complex combination of biological responses, with a substance-specific profile for each EA. Regarding α -adrenergic receptors, ergotamine is a partial agonist and weak antagonist in blood vessels and various smooth muscles and mainly an antagonist in the CNS. Ergometrine is a partial agonist for α -adrenergic receptors in blood vessels, but less than ergotamine and has little antagonistic action. In a study with pithed rats¹⁸ Villamil-Hernández et al. (2014) suggested that ergotamine induces inhibition of the vasopressor sympathetic outflow by activation of prejunctional 5-HT_{1A/1B/1D} receptors, α _{1,2}-adrenoreceptors and D₂-like receptors. Furthermore, they found that the vasopressor responses to ergotamine in pithed rats are mainly mediated by α _{1A}-, α _{1B}-, α _{1D}- α _{2A}- and α _{2C}-adrenoreceptors (Villamil-Hernández et al., 2014). Interacting with serotonin-receptors, ergotamine is a partial agonist in certain blood vessels and a poor agonist/antagonist in the CNS. Ergometrine, through interactions with serotonin-receptors, is a partial agonist in human umbilical and placental blood vessels, a selective and potent antagonist in various smooth muscles, and a partial agonist/antagonist in the CNS.

Reddy et al. (2020) studied the physiological effects, metabolism and distribution of ergotamine and ergovaline in male mice. Blood pressure, heart rate and motor coordination were investigated in response to i.p. treatment with ergovaline (0.015 and 0.025 mg/kg bw) and ergotamine (0.025 and 0.05 mg/kg bw). Ergotamine and ergovaline showed similar cardiovascular effects, causing elevation in blood pressure and reduced heart rate. The authors concluded that the dysregulation in respiratory, thermoregulatory, cardiac and vasomotor function induced by these EAs in experimental animals in several studies, could be partially explained by dysfunction in the autonomic nervous system, located in the brainstem.

Mixture effects

Klotz & Smith, (2015) concluded that there is not a single toxin that is solely responsible for the impact of EAs on livestock. Rather it is a collective impact of EAs derived from external spore producing fungi (*Claviceps spp*) or the endophytic fungi (*Epichloë/Neotyphodium spp*) that is responsible for the broad spectrum of associated animal responses.

¹⁸The pithed rat is a model used to study peripheral cardiovascular responses to electrical stimulation of the sympathetic nervous system, excluding the central mechanisms which could modify the response to drugs for example.

Genotoxicity/Carcinogenicity

With the exception of ergotamine, which is considered non-genotoxic, only limited genotoxicity studies have been performed with naturally occurring EAs. A carcinogenicity study with ergotamine¹⁹ in rats showed a slight increase in the incidence of neurofibromas in the ears.

No studies were identified with regard to EA genotoxicity for food producing and non-food producing animals.

3.2 | Feed occurrence data

3.2.1 | Occurrence data submitted to EFSA

On September 2023, a total of 22,866 analytical results on EAs in feed (2536 samples) were extracted from the EFSA Data Warehouse covering the last 10 sampling years, from 2013 to 2022 (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 (above 70% of the analytical data) on the LB-UB estimations. This is especially relevant for this Opinion as the total amount of EAs per sample could be obtained by summing up to 14 different individual EAs. Based on the distribution of the reported LOQs and on expert judgement, a cut-off of 10 µg/kg was applied to all analytical results. This cut-off was not applied to the analytical results reported as 'Total ine/inine ergot alkaloids' and as 'Ergot alkaloids'. The whole sample was excluded even if the reported LOQs were above the selected cut-off for just one EA. By using the LOQ cut-off, a total of 882 analytical results corresponding to 77 samples were excluded (60 with all EAs reported as left-censored data), among them 58 samples of 'Cereal grains and products derived thereof' and 13 of 'Compound feed'.

Additionally, other analytical results were also excluded; a brief description is provided below:

- 538 results on sclerotia levels.
- 16 analytical results reported as 'Ergot alkaloids'.
- 557 analytical results reported as 'Total ine/inine ergot alkaloids'.
- 924 analytical results confirmed by the data provided as derived from targeted sampling of samples with visible ergot infestation (124 samples).
- 803 analytical results from samples expressed in whole weight without further information on its moisture content and where no assumptions could be made (62 samples of different types of 'Compound feed', three samples of 'Aquatic invertebrates as feed' and two of 'Tubers, roots and products derived thereof').
- 103 analytical results corresponding to T-2/HT-2 toxin analyses.
- Twenty analytical results that belong to two samples with high EA concentrations that could not be confirmed with the data providers. They were one sample reported as 'Wheat grains' and another one as 'Lucerne, field dried' with EA concentrations (whole weight) of 20,457 µg/kg and 664 µg/kg, respectively.

After the exclusion of these samples, the final dataset consisted of 19,023 analytical results expressed either as whole weight or 88% dry matter (1580 samples). All analytical results were converted into dry matter before being used for the dietary exposure estimations; for few samples expressed in whole weight without information on the moisture content, 88% dry matter content was assumed.²⁰ The uncertainty associated to this approach is discussed in Table I.2 within Appendix I. For most of the samples, information on the recovery was not reported, and in many cases no information was provided on whether the results were corrected or not for recovery. Recovery correction was applied on those analytical results where this information was available, and otherwise, they were reported as not corrected for recovery.

The total content of EAs in each sample was estimated by summing the concentration for each of the individual alkaloids reported. For most of the samples (□ 90%), 12 analytical results were provided, while in few samples ($n = 160$) 13 analytical results were available as β -ergocryptine and α -ergocryptine were submitted as individual analytical results within the same sample. The minimum number of EAs per sample was eight, reported for 19 samples. As mentioned above, the data reported on ergot sclerotia and as 'Ergot alkaloids' and 'Total ine/inine ergot alkaloids' were excluded. Table 6 shows the different types of analytical results on EAs in the final dataset.

¹⁹Ergotamine has been defined as a mixture including ergocornine, α - and β -ergocryptine, and ergocristine (Griffith et al., 1978).

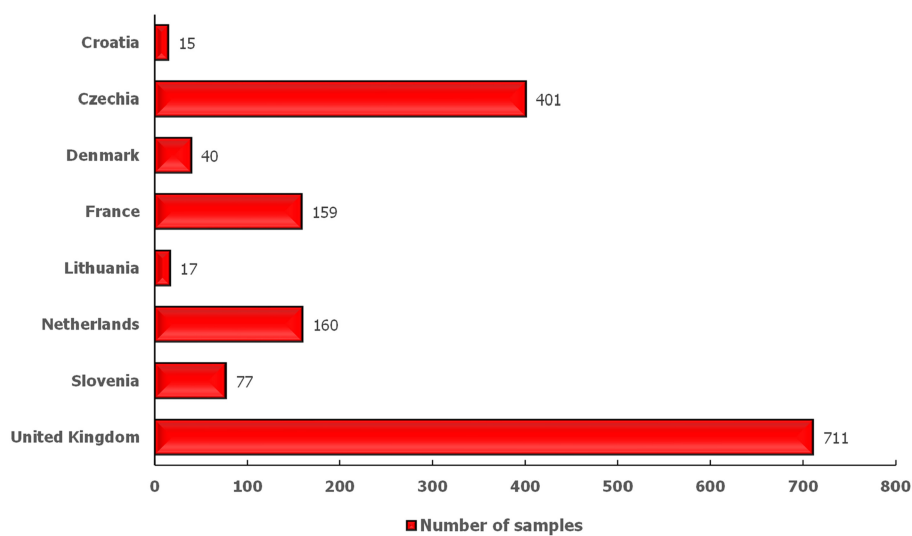
²⁰In the absence of reported moisture content, a 88% dry matter content was assumed for the following feed materials: 'Hay', 'Piglets (weaning diets) / Complete feed', 'Poultry (starter diets) / Complete feed', 'Fattening chickens (broilers) / Complete feed', 'Laying hens / Complete feed', 'Turkeys / Complete feed', 'Ducks / Complete feed', 'Rabbits / Complete feed', 'Pet food, birds / Complete feed', 'Cereal grains and products derived thereof', 'Oil seeds, oil fruits and products derived thereof', and 'Other seeds and fruits, and products derived thereof'.

TABLE 6 Distribution of the EAs reported across the different feed samples ($n = 19,023$ analytical results, 1580 samples).

		Frequency	Percent
'ine' (R)-epimers	Ergocornine	1580	8.3
	Ergocristine	1580	8.3
	Ergometrine	1551	8.2
	Ergotamine	1560	8.2
	Ergosine	1575	8.3
	α -Ergocryptine	219	1.2
	β -Ergocryptine	160	0.8
	Ergocryptine (α - and β -isomers)	1361	7.2
'inine' (S)-epimers	Ergocorninine	1580	8.3
	Ergocristinine	1580	8.3
	Ergometrinine	1557	8.2
	Ergotaminine	1560	8.2
	Ergosinine	1580	8.3
	α -Ergocryptinine	177	0.9
	Ergocryptinine (α - and β -isomers)	1403	7.4

Figure 3 shows the distribution of the samples across eight different sampling countries.²¹ Around 45% of the samples were collected in United Kingdom, followed by Czech Republic (~ 25%) and France and the Netherlands (both ~ 10%).

Figure 4 shows the different sampling years in the final dataset, from 2013 to 2022. Apart from 2013, the sampling was, overall, equally distributed across the different years, with 2017 being the year with the highest number of samples ($n = 216$).

**FIGURE 3** Number of samples by sampling country.

²¹Data from the United Kingdom refer to samples submitted before 1 January 2021.

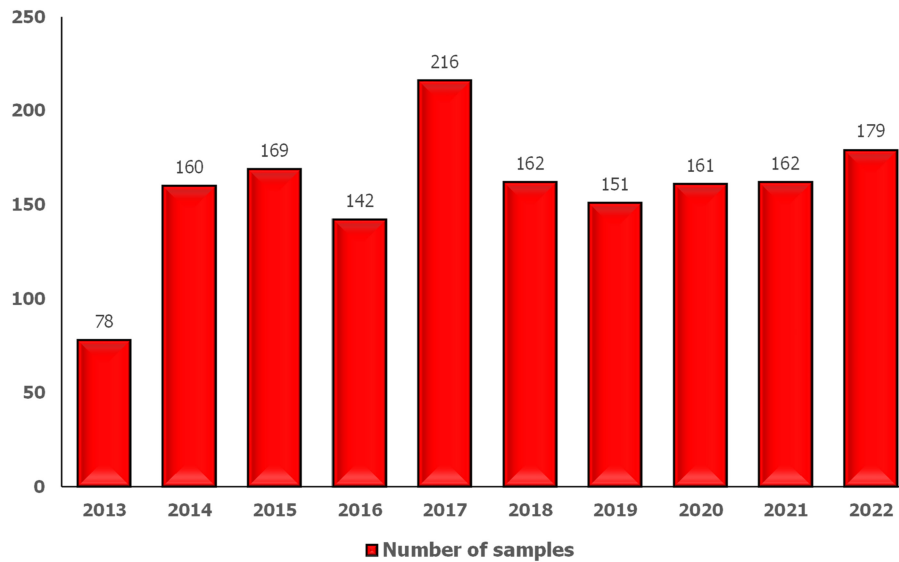


FIGURE 4 Number of samples by sampling year.

Analytical methods

Feed samples with data on EAs were reported as analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS, $n = 1227$) or by LC-MS ($n = 343$); for a small number of samples no information was reported on the analytical method ($n = 10$).

The highest sensitivity was reported for several EAs in a few samples of ‘Cereal grains and products derived thereof’ analysed by LC-MS/MS (LOQ = 0.25 µg/kg, whole weight, ww). The most often reported LOQs were 0.5 µg/kg and 5 µg/kg (ww), for ~26% and 25% of the analytical results, respectively. The median LOQ reported was 2 µg/kg.

Figure 5 shows the percentage and number of feed samples (Feed level 1) reported as left-censored (i.e. all reported EAs below LOD/LOQ) and as quantified. Around 50% of the samples ($n = 791$) were left-censored.

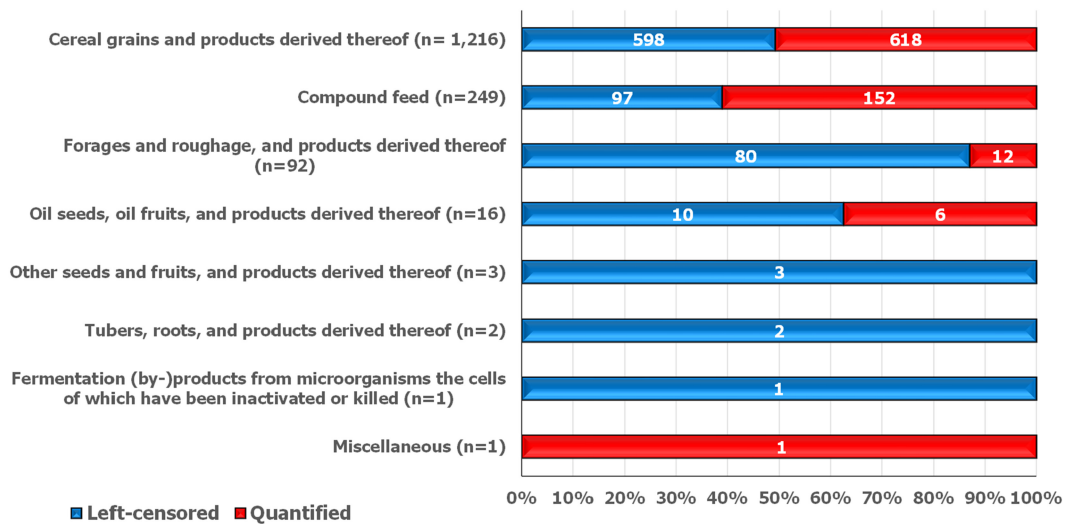


FIGURE 5 Overview of quantified and left-censored samples (all reported EAs below LOD/LOQ) at Feed level 1.

Figure 6 shows the type of analytical results for EAs across the feed samples submitted to EFSA. Only 28% of the analytical results were quantified; the percentage of quantified EAs varied from 10% for ergometrinine to 59% for β-ergocryptine. Overall, ‘ine’ epimers were reported as quantified more often than the ‘inine’ epimers.

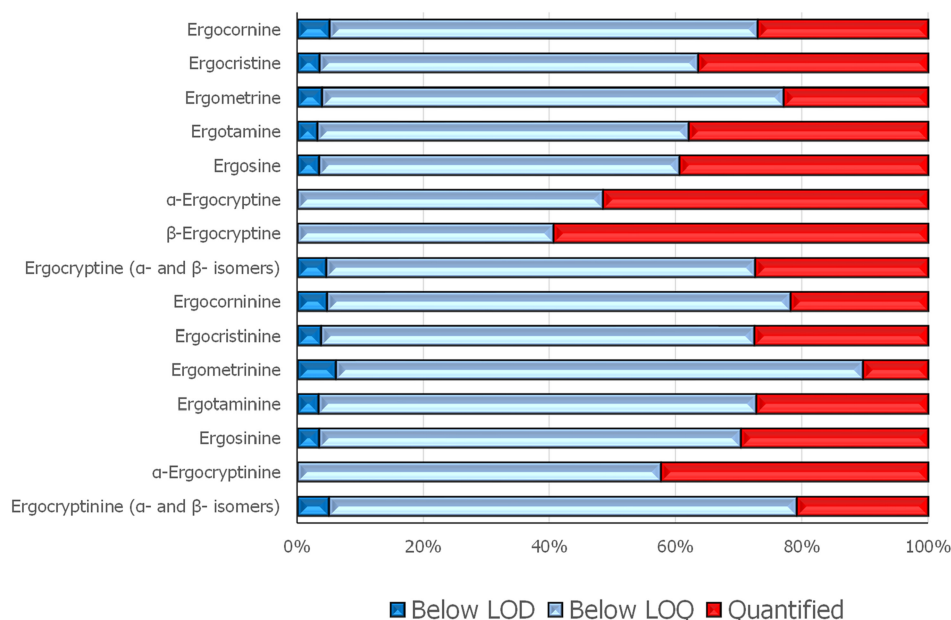


FIGURE 6 Overview of quantified and left-censored analytical results (below LOD/LOQ) across different types of EAs. Quantified samples (at least one EA quantified, $n=789$) were assessed to identify the average contribution of the different EAs to the total concentration. Overall, the 'ine' epimers represent on average 77% of the total EA concentration vs. the 23% of the 'inine' epimers. Although only a small number of feed samples referred to processed commodities (e.g. 'Compound feed', 'Malting barley and malt fines'), in these samples the proportion of 'inine' epimers was slightly higher (\square 30%) as compared to unprocessed feed (results not shown). This shift from 'ine' epimers to 'inine' epimers seems to be related to processing and it was already described in the 2017 EFSA scientific report on EAs (EFSA, 2017). The three most abundant EAs were ergotamine, ergosine and ergocristine; the three together represent on average 59% of the total EA concentration (see Table 7).

TABLE 7 Average contribution of the individual EAs to the total concentration in feed samples with at least one EA quantified ($n=789$).

		N=789 quantified samples	
		Number of quantified occasions	Average contribution (%)
'ine' epimers	Ergocornine	428	6.8
	Ergocristine	576	17.6
	Ergometrine	356	4.2
	Ergotamine	592	23.1
	Ergosine	621	17.8
	α-Ergocryptine	113	7.3
	β-Ergocryptine	95	9.3
	Ergocryptine (α- and β-isomers)	374	7.2
'inine' epimers	Ergocorninine	345	2.6
	Ergocristinine	436	5.4
	Ergometrinine	161	2.4
	Ergotaminine	426	4.6
	Ergosinine	470	4.8
	α-Ergocryptinine	75	3.3
	Ergocryptinine (α- and β-isomers)	293	2.8
	'ine' epimers	765	77.4
'inine' epimers	625	22.6	

At Feed Level 1, most of the samples with analytical data on EAs were 'Cereal grains and products derived thereof' ($n=1216$, 77%), followed by samples codified as 'Compound feed' ($n=249$) and 'Forages and roughage, and products derived thereof' ($n=92$). Table 8 shows the summary statistics of the available feed categories expressed in dry matter at Feed Level 1; detailed information of the 1580 samples available in the final dataset are shown in Annex C.

TABLE 8 EA 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	1216	49	86	1101	0	48	58	85	425	430
Oil seeds, oil fruits and products derived thereof	16	63	9	35	0	18	6	56	–	–
Tubers, roots and products derived thereof	2	100	0	66	–	–	–	–	–	–
Other seeds and fruits, and products derived thereof	3	100	0	28	–	–	–	–	–	–
Fermentation (by-)products from microorganisms the cells of which have been inactivated or killed	1	100	0	8	–	–	–	–	–	–
Miscellaneous ^a	1	0	71	73	–	–	–	–	–	–
Compound feed	249	39	71	113	11	68	68	136	249	267
Forages and roughage, and products derived thereof	92	87	19	111	0	67	0	136	84	187

Notes: 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).

^aThe 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 EA levels reported. Feed categories with less than five samples reported were excluded. Furthermore, when for a particular feed category all the samples were reported as left-censored data and there was no evidence reported in the literature that EAs 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. Further details are included in the Section 3.3.

Table 8 shows all the feed categories that were used to estimate dietary exposure in the animal species and categories considered in this scientific Opinion; mean EA 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).

For the samples codified as ‘Forages and roughage, and products derived thereof’ ($n = 92$), an ad hoc feed category was created containing the samples reported as ‘Hay’ ($n = 75$) and ‘Grass, herbs, legume plants, [green forage]’ ($n = 10$). This ad hoc feed category contained therefore a total of 85 samples, 73 of them left-censored; mean and 95th percentile EA concentrations (LB-UB, dry matter) were 21–113 µg/kg and 84–187 µg/kg, respectively.

As regards ‘Cereal grains and products derived thereof (feed)’, a total of eight feed groups were used for the dietary exposure estimations (Table 9). The highest number of samples were available for ‘Barley grain’ ($n = 224$) and the ad hoc feed group ‘Oat grain + oat feed’ ($n = 220$). In the database only 26 samples of ‘Oat grain’ were available, while there were 194 samples of ‘Oat feed’ available. As ‘Oat grain’ is one of the main ingredients of the diets used to estimate dietary exposure to EAs in horses, it was combined with ‘Oat feed’ to allow using the 95th percentile of the occurrence data to estimate dietary exposure in the high occurrence scenario. The group ‘Oat grain + oat feed’ was used in the diet for horses and for dogs. Among the different feed groups, the highest EA levels were reported for ‘Rye bran’ ($n = 12$) with mean and 75th percentile EA concentrations (LB-UB, dry matter) of 306–336 µg/kg and 482–505 µg/kg, respectively. Relatively high values were also reported for ‘Triticale grains’ ($n = 59$) with the highest 95th percentile EA concentration reported among the different feed samples (1411–1423 µg/kg dry matter, LB-UB). More details on mean LB-UB estimations as well as on the most reliable high percentiles on other samples used to estimate dietary exposure are shown in Table 9.

For ‘Compound feed’ ($n = 249$), as with other feed groups, a dedicated grouping was carried out before estimating dietary exposure. As an example, a feed category named ‘Complete feed for different pig categories’ was created containing 84 samples with complete feed targeting different type of pigs (piglets, lactating sows, etc.). For this grouped category, the one with the highest number of samples, mean and 95th percentile EA concentrations (LB-UB, dry matter) were 88–111 µg/kg and 275–275 µg/kg, respectively. Other samples of ‘Compound feed’ also used for the dietary exposure estimations are shown in Table 9.

Apart from one sample of ‘Hemp expeller’, only one feed category among ‘Oil seeds, oil fruits and products derived thereof’ was submitted to EFSA with quantified EA values. It was ‘Rape seed – expeller’ ($n = 8$, five samples quantified) for which average and median values (LB-UB, dry matter) were 16–36 and 2.8 and 24 µg/kg, respectively (Table 9).

TABLE 9 Feed samples as used for the estimation of dietary exposure to EAs (concentration expressed as 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										
Barley grain	224	63	28	61	0	28	4	68	149	174
Rye grain	66	23	129	155	60	87	146	160	598	598
Triticale grain	59	44	234	267	24	69	246	253	1411	1423
Wheat grains	204	78	38	81	0	68	0	69	176	216.0
Oat grain + oat feed	220	35	50	60	6	15	32	58	181	181
Wheat feed	213	12	174	177	95	96	234	234	601	601
Wheat bran	18	89	4	45	0	68	0	68	–	–
Rye bran	12	42	306	336	8	71	482	505	–	–
Oil seeds, oil fruits and products derived thereof										
Rape seed – expeller	8	3	16	36	3	24	–	–	–	–
Forages and roughage, and products derived thereof										
'Grass, herbs, legume plants, [green forage]' + 'Hay' ^a	85	86	21	113	0	92	0	136	84	187
Compound feed										
Complete feed for different pig categories ^b	84	15	88	111	46	69	123	136	275	275
Poultry (starter diets) + Fattening chickens/Complete feed	17	60	26	59	0	68	48	68	–	–
Laying hens/Complete feed	18	72	15	91	0	68	9	136	–	–
Rabbits/Complete feed	13	38	37	87	3	68	40	74	–	–
Dairy cows/Complementary feed	16	50	26	73	3	68	38	79	–	–
Fattening calves (weaning diets)/Complementary feed	5	0	90	105	80	98	–	–	–	–
Horses/Complementary feed	10	60	64	130	0.0	137	–	–	–	–

Note: 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).

^aAd hoc feed category consisting of the samples reported for 'Hay' and 'Grass, herbs, legume plants, [green forage]'.

^bAd hoc feed category consisting of samples of Complete feed for 'Piglets (weaning diets)', 'Growing/Fattening pigs', 'Breeding pigs', 'Sows' and 'Lactating Sows'.

3.2.2 | Previously reported occurrence data

A number of papers have been published since the last EFSA Opinion on EAs in feed was published in 2012, as summarised in Table H.1/Appendix I. Results are only included in the table if information on number of samples and the analytical method are given in the article or could be retrieved from other information in the article. The table is ordered according to the year of publication. The list of the EAs analysed for in the studies, as detailed in the table, varies in the different papers, it is therefore not possible to compare concentrations with each other within or across the different kind of feed/feed materials.

In general, LC–FLD and LC–MS/MS are the most used methods with LOQs between 2.5 and 20 µg/kg for LC–FLD and 0.05–22 µg/kg for LC–MS/MS. Enzyme immunoassay (EIA) has also been used for analysis with an LOD of 50 µg/kg for the total EA content. In some of the papers included in the table, all the analysed samples contained an EA concentration above the LOQ (Korn et al., 2014, Likar et al., 2018, Slaiding and Byrd 2013, Waret-Szkuta et al., 2019). Of note, Likar et al. (2018) reported EA concentrations in sclerotia collected from wild and cultivated infected grass species, while Waret-Szkuta et al. (2019) reported the EA concentration following a case of intoxication.

Some samples were highly contaminated, as in Schwake-Anduschus et al. (2020) and Kodisch et al. (2020). However, in these studies the sampling was targeted on feed materials which included visible sclerotia (targeted samples). Some studies were identified which reported a number of compounds/mycotoxins other than EAs from *C. purpurea* (Penagos-Tabares et al., 2022, Penagos-Tabares et al., 2022; Zachariasova et al., 2014).

3.3 | Dietary exposure assessment

The dietary exposure assessment was conducted taking into account the data on EA occurrence in compound feed and feed materials. The two scenarios, based on compound feed and model diets, described in section 2.5, were followed.

Exposure assessments based on compound feed could be made with complete feed for poultry and pig categories, and for complementary feed plus forages for ruminants and horses.

The outcome of both scenarios was then compared at both the mean and high-level exposure. Concerning compound feed, the 95th percentile could only be calculated for complete feed for pigs. For the other animal species/categories the 75th percentile was used instead.

The exposure assessment was performed making use of the flexibility in the composition of the default diets as explained in Sections 2.2 and 2.5, and using substitutions of feed materials within the groups detailed in Appendix D. In particular, 'Wheat feed' was used in lieu of 'Wheat middlings' (less than five samples reported), 'Wheat grain' instead of 'Wheat gluten' (no reported samples), as indicated in Appendix C.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 C). For all animal species for which forage is part of their diet, the occurrence data of C.2 to C.8 were grouped and added to their diets in the respective proportion.

The detailed results of the animal dietary exposure, summarised below, are tabulated in Appendix C. Within Appendix E the estimated dietary exposure levels are expressed also in $\mu\text{g}/\text{kg}$ bw per day for each animal species.

Pigs

Dietary exposure to EAs in **weaned piglets** varied between 119 (LB) and 142 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 708 and 719 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials.

EA dietary exposure in complete feed for **pigs for fattening** varied between 118 (LB) and 139 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 671 and 681 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials.

Dietary exposure to EAs in **lactating sows** varied between 127 (LB) and 145 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 709 (LB) and 717 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials.

Considering complete feeds, dietary exposure to EAs in **weaned piglets, pigs for fattening and lactating sows** varied between 88 (LB) and 111 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario and was 275 (LB and UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario for all pigs.

Poultry

Dietary exposure to EAs in **chickens for fattening** varied between 16 (LB) and 33 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 73 and 88 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Considering complete feeds, the exposure to EAs in chickens for fattening varied between 26 (LB) and 69 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 48 (LB) and 68 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

Dietary exposure to EAs in **laying hens** varied between 29 (LB) and 42 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 113 and 125 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Considering complete feeds, the exposure to EAs in laying hens varied between 15 (LB) and 91 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 9 (LB) and 136 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

EAs dietary exposure to EAs in **turkeys for fattening** varied between 21 (LB) and 46 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 105 and 126 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Dietary exposure to EAs in turkeys for fattening by complete feed could not be established, as there was not enough occurrence data available for calculation.

In **ducks for fattening** dietary exposure to EAs varied between 29 (LB) and 52 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 129 and 149 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Dietary exposure to EAs in ducks for fattening by complete feed could not be established, as there was not enough occurrence data available for calculation.

Bovines

Dietary exposure to EAs in **dairy cows** varied between 29 (LB) and 69 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 109 and 151 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Considering compound feeds, the exposure to EAs in dairy cows by complementary feed and forages varied between 25 (LB) and 85 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 52 and 111 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

Dietary exposure to EAs in **cattle for fattening** varied between 23 (LB) and 101 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 92 and 177 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet

composed of feed materials and forages. Considering compound feeds, the exposure to EAs in cattle for fattening by complementary feed and forages varied between 35 (LB) and 111 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 85 and 171 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

Dietary exposure to EAs in **veal calves** could neither be estimated for a model diet, nor for compound feeds as there was not enough occurrence data available for calculation.

Ovines

Dietary exposure to EAs in **dairy sheep** varied between 27 (LB) and 92 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 105 (LB) and 176 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Estimation of dietary exposure to EAs in dairy sheep by complementary feed and forages was not possible due to the lack of occurrence data for complementary feed.

Dietary exposure to EAs in **lambs for fattening** varied between 31 (LB) and 88 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 123 (LB) and 183 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Estimation of dietary exposure to EAs in lambs for fattening by complementary feed and forages was not possible due to the lack of occurrence data for complementary feed.

Caprines

Dietary exposure to EAs in **dairy goat** varied between 38 (LB) and 72 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 143 (LB) and 175 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Estimation of dietary exposure to EAs in dairy goat by complementary feed and forages was not possible due to the lack of occurrence data for complementary feed.

Dietary exposure to EAs in **kids for fattening** varied between 30 (LB) and 91 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 116 (LB) and 181 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Estimation of dietary exposure to EAs in kids for fattening by complementary feed and forages was not possible due to the lack of occurrence data for complementary feed.

Horses

Dietary exposure to EAs in **horses** varied between 35 (LB) and 106 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 132 and 209 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of feed materials and forages. Considering compound feeds, the exposure to EAs in horses by complementary feed and forages varied between 32 (LB) and 117 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 79 (LB) and 173 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

Rabbits

In **rabbits for fattening** dietary exposure to EAs varied between 21 (LB) and 26 (UB) $\mu\text{g}/\text{kg}$ feed DM using the mean occurrence scenario, and between 78 (LB) and 82 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Considering complete feeds, the exposure to EAs in rabbits for fattening varied between 37 (LB) and 87 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 40 (LB) and 74 (UB) $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario.

Fish

Dietary exposure to EAs in **fish** varied between 4 (LB) and 8 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 18 and 22 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Dietary exposure to EAs in **fish** by complete feed could not be established, as there was not enough occurrence data available for calculation.

Non-food producing animals

In **cats** dietary exposure to EAs varied between 21 (LB) and 26 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 78 and 82 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. Dietary exposure to EAs in **cats** by complete feed could not be established, as there was not enough occurrence data available for calculation.

Dietary exposure to EAs in **dogs** varied between 12 (LB) and 20 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 51 and 57 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model diet composed of individual feed materials. In **dogs fed a vegetarian diet** dietary exposure to EAs varied between 2 (LB) and 4 (UB) $\mu\text{g}/\text{kg}$ feed DM in the mean occurrence scenario, and between 9 and 11 $\mu\text{g}/\text{kg}$ feed DM in the high exposure scenario when using a model

diet composed of individual feed materials. Dietary exposure to EAs in **dogs** and **dogs fed a vegetarian diet** by complete feed could not be established, as there was not enough occurrence data available for calculation.

3.4 | Risk characterisation

EAs cause vasoconstriction and a decreased production of prolactin. Some of the effects are especially prominent under extreme weather conditions, particularly at high temperatures but also cold conditions. As a result, such effects seem less apparent in controlled studies and lowest effect levels are observed in so-called case studies, which by themselves introduce various uncertainties. RPs for adverse animal health effects were derived for a number of animal species.

Although decreased prolactin levels play a role in the decreased milk production and possibly other effects, prolactin was considered as a possible biomarker of EA exposure. Serum prolactin levels are a sensitive biomarker of effect but without other effects considered adverse, this was not taken into account for the assessment.

Since EAs produced by *C. africana* and the endophyte *E. coenophiala* appear to be of less relevance in the EU and no occurrence data were provided, the focus was on EAs produced by *C. purpurea*. Nevertheless, information on the adverse health effects from other EAs was reviewed and RPs for adverse animal health effects are provided if available (Table 10).

As mentioned in the text, it is likely that some EAs are more potent than others, but consolidated data are lacking, and it is therefore not possible at present to assign relative potency factors to the individual EAs and their epimers (Appendix I). For the scope of this Opinion, the individual EAs were considered equipotent in the risk assessment.

Part of the RPs were based on adverse effects observed in case studies or with only one dose level. In such cases the RP was derived by applying a factor of 3. Some RPs are based on the NOAEL from the study. In Tables 11–19, in addition to the RPs also the lowest dose showing an effect is included for comparison with the exposure.

Table 10 summarises the Reference Points for adverse animal health effects as identified by the CONTAM Panel for the various animal species.

TABLE 10 Reference Points for adverse animal health effects identified by the CONTAM Panel for the various animal species.

Species	Source	RP for adverse animal health effects (mg/kg complete feed (88% DM))	Adverse effects	Reference
Pigs				
Piglets	<i>C. purpurea</i>	0.6	Increased relative heart and spleen weight	Mainka, Dänicke, Böhme, Ueberschär, et al. (2005)
Pigs for fattening		0.6	Increased relative heart and spleen weight	Mainka, Dänicke, Böhme, Ueberschär, et al. (2005)
Sows	<i>C. africana</i>	0.5	Reduced milk production	Kopinski et al. (2007, 2008)
Poultry				
Chickens for fattening	<i>C. purpurea</i>	2.1	Reduced weight gain	Dänicke (2017)
Laying hens	<i>C. purpurea</i>	3.7	Various effects on eggs	Dänicke (2016)
Ducks	<i>C. purpurea</i>	0.2	Reduced weight gain	Dänicke (2015)
Ruminants				
Bovines	<i>C. purpurea</i>	0.1	Loss of tail switches and tips, early lameness, swelling of the feet	Miskimins et al. (2015)
	<i>C. africana</i>	0.4	Reduced growth and feed intake	Blaney et al. (2011)
Sheep	<i>Epichloë coenophiala</i>	0.1	Reduced growth	Peters et al. (1992)
	<i>C. purpurea</i>	0.3	Reduced growth	Coufal-Majewski et al. (2017a, 2017b)
	<i>Epichloë coenophiala</i>	0.2	Fetal growth during maternal exposure during gestation	Duckett et al. (2014)

Schwake-Anduschus et al. (2020) proposed preliminary guidance values for EAs from *C. purpurea* for several species. For pigs and piglets, the authors derived the same value as that derived by the CONTAM Panel. For sows a value of 0.03 mg/kg was proposed, based on a critical level of 0.33 mg/kg EAs from *C. africana* for primiparous sows (Kopinski et al., 2007) and a UF of 10, to account for the fact that EAs from *C. purpurea* were considered by the authors more toxic than those from *C. africana*. However, the lowest dose, showing an effect in one of the primiparous sows, was 1.4 rather than 0.33 mg/kg feed, for which the Panel derived a NOAEL for EAs from *C. africana* of 0.5 mg/kg feed by applying a UF of 3 (Table 10). Furthermore, Dignean et al. (1986) did not observe adverse effects in sows at a dose of 4.8 mg/kg feed

for EAs from *C. purpurea*. Therefore, the CONTAM Panel decided to compare the exposure of sows with the RP of 0.6 mg/kg feed for EAs from *C. purpurea* for pigs rather than extrapolating the RP for *C. purpurea* from the RP for *C. africana*. For chickens for fattening and laying hens, Schwake-Anduschus et al. (2020) proposed very similar values, while for ducks a three-fold lower value, based on a LOAEL of 0.6 and a UF of 10 rather than 3 to obtain a NOAEL. Similar was the case for sheep, i.e. a UF of 3 was applied rather than 10. For bovines, Schwake-Anduschus et al. (2020) proposed the same value for *C. purpurea* as the CONTAM Panel (0.1 mg/kg feed).

For risk characterisation, the CONTAM Panel took into account the dietary exposure assessment of EAs using recent analytical results on the occurrence of EAs 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 C. The estimates of exposure to EAs are presented in Section 3.3, expressed per kg DM feed and in Appendix E, expressed per kg bw.

The CONTAM Panel characterised the food producing and non-food producing animal health risk associated with dietary exposure to EAs 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 each animal species. RPs for adverse animal health effects are expressed for complete feed and on an 88% DM base to allow direct comparison with levels observed in feed. However, especially for ruminants and horses, complete feed includes forages. The comparison was performed in mg EAs/kg complete feed (88% DM), following conversion of the exposure levels into 88% DM, to be in line with the identified RPs and are summarised in Tables 11–19. The exposure was expressed as a percentage of the RP for adverse animal health effects: a percentage above 100 was considered a risk.

3.4.1 | Pigs

For **weaned piglets**, the estimated UB mean and UB high exposure to the EAs were 21% and 105% of the RP, respectively, for the model diet scenario, raising a health concern. When considering compound feeds, the estimated UB mean and UB high exposure were lower, being 16% and 40% of the RP, respectively, indicating no concern.

TABLE 11 Comparison of estimated EA Mean/High exposure levels (from model diet, compound feed) and RP for piglets (weaned).

	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Piglets (weaned)								
Model diet	0.10	0.12	0.62	0.63	17	21	104	105
Compound feed	0.08	0.10	0.24	0.24	13	16	40	40
	LOAEL/ Adverse effect concentration (mg/kg feed): 4.7				Reference Point (mg/kg feed): 0.6			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **pigs for fattening**, the estimated UB mean and UB high exposure to the EAs were below (20%) or close to the RP, respectively, for the model diet scenario, indicating no or low concern for adverse health effects. When considering compound feeds, the estimated UB mean and UB high exposure were 16% and 40% of the RP, respectively, indicating no concern.

TABLE 12 Comparison of estimated EA Mean/High exposure levels (from model diet, compound feed) and RP for pigs for fattening.

	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Pigs for fattening								
Model diet	0.10	0.12	0.59	0.60	17	20	98	100
Compound feed	0.08	0.10	0.24	0.24	13	16	40	40
	LOAEL/ Adverse effect concentration (mg/kg feed): 4.7				Reference Point (mg/kg feed): 0.6			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **lactating sows**, the estimated UB mean and UB high exposure to the EAs were 21% and 105% of the RP derived for pigs, respectively, for the model diet scenario, raising a health concern. Nevertheless, when considering compound feeds, the estimated UB mean and UB high exposure were 16% and 40% of the RP, respectively, indicating no concern.

TABLE 13 Comparison of estimated EA Mean/High exposure levels (from model diet, compound feed) and RP for lactating sows.

Lactating sows	mg EAs /kg feed ^a				Estimated exposure, % of the RP (for pigs)			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	0.11	0.13	0.62	0.63	19	21	104	105
Compound feed	0.08	0.10	0.24	0.24	13	16	40	40
LOAEL/ Adverse effect concentration (mg/kg feed): 4.7 (derived for pigs)					Reference Point (mg/kg feed): 0.6 (derived for pigs)			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

3.4.2 | Poultry

For **chickens for fattening**, the estimated UB mean and UB high exposure to the EAs were 1% and 4% of the RP, respectively, for the model diet scenario, raising no health concern. Similar results are obtained from the compound feed.

TABLE 14 Comparison of estimated EA Mean/High exposure levels (from model diet, compound feed) and RP for chickens for fattening.

Chickens for fattening	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	0.01	0.03	0.06	0.08	1	1	3	4
Compound feed	0.02	0.06	0.04	0.06	1	3	2	3
LOAEL/ Adverse effect concentration (mg/kg feed): 6.0					Reference Point (mg/kg feed): 2.1			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **laying hens**, the estimated LB/UB mean and LB/UB high exposure to the EAs were 1% and 3% of the RP, respectively, for the model diet, raising no health concern. Similar results are obtained from the compound feed.

TABLE 15 Comparison of estimated EA Mean/High exposure levels (from model diet, compound feed) and RP for laying hens.

Laying hens	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	0.03	0.04	0.10	0.11	1	1	3	3
Compound feed	0.01	0.08	0.01	0.12	0	2	0	3
LOAEL/ Adverse effect concentration (mg/kg feed): 14.6					Reference Point (mg/kg feed): 3.7			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **ducks**, the estimated UB mean and UB high exposure to the EAs were 23% and 66% of the RP, respectively, for the model diet, raising no health concern. No occurrence data was available for a comparison with compound feed for ducks.

TABLE 16 Comparison of estimated EA Mean/High exposure levels (from model diet) and RP for ducks.

Ducks	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet	0.03	0.05	0.11	0.13	13	23	57	66
LOAEL/ Adverse effect concentration (mg/kg feed): 0.6					Reference Point (mg/kg feed): 0.2			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

3.4.3 | Ruminants

For **dairy cows**, the estimated UB mean and UB high exposure to the EAs were 61% and 133% of the RP, respectively, for the model diet scenario, indicating a potential risk for adverse health effects. When considering compound feeds, the estimated UB mean and UB high exposure were 74% and 98% of the RP, respectively.

TABLE 17 Comparison of estimated EA Mean/High exposure levels (from model diet, complete feed) and RP for Dairy cows.

	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Dairy cows								
Model diet	0.03	0.06	0.10	0.13	25	61	96	133
Complete feed	0.02	0.07	0.05	0.10	22	74	45	98
	LOAEL/ Adverse effect concentration (mg/kg feed): 0.3				Reference Point (mg/kg feed): 0.1			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **cattle for fattening**, the estimated UB mean and UB high exposure to the EAs were 88% and 156% of the RP, respectively, for the model diet scenario, indicating a potential risk for adverse health effects. Similar results are obtained from the compound feed.

TABLE 18 Comparison of estimated EA Mean/High exposure levels (from model diet, complete feeds) and RP for cattle for fattening.

	mg EAs /kg feed ^a				Estimated exposure, % of the RP			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Cattle for fattening								
Model diet	0.02	0.09	0.08	0.16	20	88	81	156
Complete feed	0.03	0.10	0.07	0.15	30	98	75	150
	LOAEL/ Adverse effect concentration (mg/kg feed): 0.2				Reference Point (mg/kg feed): 0.1			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

For **dairy sheep**, the estimated UB mean and UB high exposure to the EAs were 27% and 52% of the RP, respectively, for the model diet scenario, raising no health concern. No occurrence data was available for a comparison with compound feed for dairy sheep.

For **lambs for fattening**, the estimated UB mean and UB high exposure to the EAs were 26% and 54% of the RP, respectively, for the model diet scenario, raising no health concern. No occurrence data was available for a comparison with compound feed for dairy sheep.

For dairy goats and kids for fattening, the calculated exposure was compared against the RP derived for sheep. For **dairy goats**, the estimated UB mean and UB high exposure to the EAs were 21% and 51% of the RP derived for sheep, respectively, for the model diet, raising no health concern. No occurrence data was available for a comparison with compound feed for dairy sheep. For **kids for fattening**, the estimated UB mean and UB high exposure to the EAs were 27% and 53% of the RP derived for sheep, respectively, raising no health concern. No occurrence data were available for a comparison with compound feed for dairy sheep.

TABLE 19 Comparison of estimated EA Mean/High exposure levels (from model diets) and RP for dairy sheep, lambs for fattening, dairy goats, kids for fattening.

	mg EAs/kg feed ^a				Estimated exposure, % of the RP (for sheep)			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Model diet Dairy sheep	0.02	0.08	0.09	0.15	8	27	31	52
Model diet Lambs for fattening	0.03	0.08	0.11	0.16	9	26	36	54
Model diet Dairy goats	0.03	0.06	0.13	0.15	11	21	42	51
Model diet Kids for fattening	0.03	0.08	0.10	0.16	9	27	34	53
	LOAEL/ Adverse effect concentration (mg/kg feed): 0.9 (derived for sheep)				Reference Point (mg/kg feed): 0.3 (derived for sheep)			

Abbreviations: LOAEL, lowest observed adverse effect level; LB, lower bound; RP, Reference point; UB, upper bound.

^aExpressed as Complete feed (88% DM).

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 ‘Characterizing 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 horses, rabbits, fish, dogs, cats and farmed mink, 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 EAs for animal health were listed and discussed. The complete list is presented in Appendix I, 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 Hazard identification

A number of uncertainties were identified linked to the Hazard identification with regard to the critical studies selected to derive the RP, those with the highest impact on the assessment are listed below:

- Naturally contaminated materials resulting in co-exposure to other compounds (e.g. other mycotoxins). The presence of other mycotoxins in the feed materials was often not investigated – this could lead to an over-estimation of the RP.
- Focus on zotechnical parameters, which might not identify other endpoints – this could lead to an under- or an over-estimation of the RP.
Effects of temperature and climate conditions are not systematically considered in some study designs. Case studies indicate a strong impact of extreme weather conditions (very low or very high temperatures) on the adverse effects in animals – this could lead to an under- or an over-estimation of the RP and potentially to be identified different RPs for different climate conditions.
- Relative potency of the EAs is unclear. There are indications from in vitro studies that some EAs are more potent than others, however ranking them is not possible at this stage – this could lead to an under- or an over-estimation of the RP.
- Uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect in animals. Vasoconstriction and hypoprolactemia are highly relevant factors in EA toxicity in livestock. The influence of hyperthermia is clearly indicated. There is a high heterogeneity of the involvement of serotonergic, adrenergic and dopaminergic receptors in the MoA – this could lead to an under- or an over-estimation of the RP.
- For pigs, the dose interval in the critical study was large, resulting in a conservative RP.

3.5.2 | 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:

- High amount of left-censored data. When using the LB approach, EA levels in the feed samples might have been underestimated while they have been overestimated at the UB approach.
- Low number of samples per feed category. Limited data available for EAs in forage, hay, grass and compound feeds.

3.5.3 | Uncertainty on exposure

An uncertainty was identified linked to the exposure assessment.

- The methodology follows the use of default BWs, feed intakes and example diets due to the lack of a comprehensive feed database in EU. The methodology could under- or overestimate the actual exposure, nevertheless the approach is aimed at providing a conservative estimate.

3.5.4 | 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, the CONTAM Panel considers that:

- For pigs (piglets, pigs for fattening and sows) the presence of EAs in feed is very likely (90%–95% certain) to raise a health concern, based on model diets. When using data on compound feed for pigs, the risk is very likely (90%–95% certain) to be low.
- For bovines, the presence of EAs in feed is likely (66%–90% certain) to pose a potential risk for adverse health effects.
- For chickens for fattening, laying hens, ducks, ovines and caprines the risk for adverse effects, related to EAs in feed, is very likely (90%–95% certain) to be low.

4 | CONCLUSIONS

C. purpurea is the most common source of EAs in feed materials in Europe and produces a range of ergot alkaloids: ergocristine/–inine, ergotamine/–inine, ergocryptine/–inine (α - and β -isomers), ergometrine/–inine, ergosine/–inine, ergocornine/–inine. Dihydroergosine is the main EA found in the sclerotia from *C. africana*. *Epichloë* species can produce several classes of fungal toxins, the most relevant for livestock being the EAs (ergovaline/–inine) and the indole-diterpene lolitrem B.

Toxicokinetics

- There is very limited information available on the ADME of EAs.
- The available literature suggests that, for food-producing animals, EAs are absorbed from the gastrointestinal tract and subjected to oxidative biotransformation by CYP3A to form mono- and di-hydroxylated metabolites.
- Urinary excretion is predominant.

Transfer

- Transfer of intact EAs to tissues of chickens, laying hens, Pekin ducks or piglets is negligible. The same holds for the transfer of intact EAs to eggs in laying hens and milk in dairy cows.

Toxicity

- In pigs, adverse effects were observed in terms of increased relative weight of heart and spleen, decreased body weight gain and reduced feed intake.
- In poultry, reduced feed intake and reduced body weight gain appeared to be the most sensitive endpoints as shown in chickens and ducks for fattening.
- In laying hens, significantly reduced laying rate, daily egg mass, feed to egg mass ratio, relative eggshell weight, egg yolk colour and nitrogen and crude fat retention of the body were observed.
- In bovines, the experimental studies failed to show any effect. Several case reports suggest that lower levels than applied in experimental studies may cause adverse effects (decreased feed intake, poor weight gain, hyperthermia, loss of tail switches and tips, early lameness and swelling of the feet, reduced milk production, death), aggravated by weather conditions.
- In sheep, reduced body weight gain and related changes in the carcass characteristics were identified.
- In rabbits, association between tail necrosis and EAs in rabbit feed was reported in one study. However, the results are hampered by the simultaneous occurrence of mycotoxins other than EAs in the experimental feed.
- No information is available on the adverse effects of EAs in feed for fish and non-food producing animals.

- The CONTAM Panel considered as RP for animal health adverse effects for EAs from *C. purpurea* (in mg T-EAs/kg complete feed, 88% DM): 0.6 for piglets and pigs for fattening, 2.1 for chickens for fattening, 3.7 for laying hens, 0.2 for ducks, 0.1 for bovines and 0.3 for sheep.
- For EAs from *C. africana*, the CONTAM Panel considered an RP for adverse animal health effects of 0.5 mg EAs/kg complete feed (88% DM) for sows and 0.4 for bovines. For ergovaline/–inine (*Epichloë*) an RP of 0.1 mg EAs/kg complete feed (88% DM) for bovines and 0.2 mg EAs/kg complete feed (88% DM) for sheep was derived.
- Mares appear to be sensitive to ergovaline with various adverse effects, however it was not possible to derive an RP as sufficient information was not available.

MOA

- Vasoconstriction is considered the most critical effect for experimental and food producing/non-food producing animals.
- In ruminants, the vasoconstrictive effect causes gangrenous and hyperthermic forms of EA intoxication, occurring at low and high environmental temperature conditions respectively.
- In addition, EAs interact with dopamine D2-receptors in the anterior pituitary gland causing the depression of prolactin levels, considered the primary mechanism of action responsible for the pathogenesis of reduced milk production, reproductive effects and effects on embryonic development.
- It is likely that some EAs are more potent than others, but consolidated data are lacking. It is therefore not possible to assign relative potency factors to the individual EAs and their epimers.

Occurrence

- The large majority of data comprised 14 EAs produced by *C. purpurea*. No data were submitted on EAs from *Claviceps Africana* and *E. coenophiala*.
- The three most abundant EAs were ergotamine, ergosine and ergocristine; the three together represented on average 59% of the total EA concentration in the feed samples.
- The highest mean EA levels were reported for 'Rye bran' ($n = 12$) with EA concentrations (LB-UB, dry matter) of 307–336 $\mu\text{g}/\text{kg}$. Relatively high values were reported for 'Triticale grains' ($n = 59$) with the highest 95th percentile EA concentration reported among the different feed samples (1411–1423 $\mu\text{g}/\text{kg}$, dry matter, LB-UB).

Dietary exposure

- Exposure assessment was based on model diets and, for some species, also on occurrence data in compound feeds.
- In weaned piglets, the exposure to EAs varied between 0.10 (LB) and 0.12 (UB) mg/kg feed in the mean occurrence scenario, and between 0.62 and 0.632 mg/kg feed in the high exposure scenario. In pigs for fattening, the exposure to EAs varied between 0.10 (LB) and 0.12 (UB) mg/kg feed in the mean occurrence scenario, and between 0.59 and 0.60 mg/kg feed in the high exposure scenario. In sows, the exposure to EAs varied between 0.11 (LB) and 0.13 (UB) mg/kg feed in the mean occurrence scenario, and between 0.62 and 0.63 mg/kg feed in the high exposure scenario. High exposure based on compound feed for pigs was 2.5-fold lower.
- In chickens for fattening, the exposure to EAs varied between 0.01 (LB) and 0.03 (UB) mg/kg feed in the mean occurrence scenario, and between 0.06 and 0.08 mg/kg feed in the high exposure scenario. In laying hens, the exposure to EAs varied between 0.03 (LB) and 0.04 (UB) mg/kg feed in the mean occurrence scenario, and between 0.10 and 0.11 mg/kg feed in the high exposure scenario. In ducks, the exposure to EAs varied between 0.03 (LB) and 0.05 (UB) mg/kg feed in the mean occurrence scenario, and between 0.11 and 0.13 mg/kg feed in the high exposure scenario. Similar results are obtained from the compound feed for chickens for fattening and laying hens. No occurrence data was available for a comparison with compound feed for ducks.
- In dairy cows, the exposure to EAs varied between 0.03 (LB) and 0.06 (UB) mg/kg feed in the mean occurrence scenario, and between 0.10 and 0.13 mg/kg feed in the high exposure scenario. In cattle for fattening, the exposure to EAs varied between 0.02 (LB) and 0.09 (UB) mg/kg feed in the mean occurrence scenario, and between 0.08 and 0.16 mg/kg feed in the high exposure scenario. Similar results are obtained from the compound feed.
- In dairy sheep, the exposure to EAs varied between 0.02 (LB) and 0.08 (UB) mg/kg feed in the mean occurrence scenario, and between 0.09 and 0.15 mg/kg feed in the high exposure scenario. In lambs for fattening, the exposure to EAs varied between 0.03 (LB) and 0.08 (UB) mg/kg feed in the mean occurrence scenario, and between 0.11 and 0.16 mg/kg feed in the high exposure scenario. No occurrence data were available for a comparison with compound feed for ovines.
- In dairy goats, the exposure to EAs varied between 0.03 (LB) and 0.06 (UB) mg/kg feed in the mean occurrence scenario, and between 0.13 and 0.15 mg/kg feed in the high exposure scenario. In kids for fattening, the exposure to EAs varied between 0.03 (LB) and 0.08 (UB) mg/kg feed in the mean occurrence scenario, and between 0.10 and 0.16 mg/kg feed in the high exposure scenario. No occurrence data were available for a comparison with compound feed for caprines.

Risk characterisation

- The CONTAM Panel considers that the presence of EAs in feed raises a health concern with 66%–90% certainty in bovines, and 90%–95% certainty in pigs (piglets, pigs for fattening and sows).
- The CONTAM Panel considers, with 90%–95% certainty, that for chickens for fattening, laying hens, ducks, ovines and caprines the health concern related to EAs in feed is low.

5 | RECOMMENDATIONS

- Further consolidated data to allow assigning relative potency factors to the individual EAs and their epimers is needed.
- Further information is needed on toxicokinetics of EAs in food producing animals and non-food producing animals.
- There is a need for non-animal studies to support the assessment of adverse effects of EAs in food producing and non-food producing animals.
- There should be more studies on the consequences of the decreased prolactin levels observed in various species in terms of potential adverse effects, including the degree of decrease leading to such effects.
- Occurrence data on EAs in forage (*C. purpurea*) and sorghum (*C. africana*), and on ergovaline/–inine (*Epichloë*) in forage and feed are needed.
- The EA occurrence data submitted to EFSA should contain adequate information on the feed samples analysed, including the moisture content, the target animals and the type of compound feed (complete/complementary) and sensitive methods for the analysis should be used to reduce the uncertainties linked to the LB-UB estimations (e.g. LOQ of 1 µg/kg for individual EAs).

ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
ALT	alanine transaminase
AMEn	nitrogen corrected apparent metabolisable energy low index
AST	aspartate aminotransferase
bw	body weight
BWG	body weight gain
CNS	central nervous system
CONTAM	Panel on Contaminants in the Food Chain
CT	condensed tannins
CYP3A	Cytochrome P450 3A
DM	dry matter
EA	ergot alkaloid
ECHA	European Chemical Agency
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FBS	fetal bovine serum
F/G	feed to gain
FI	feed intake
GLDH	glutamate dehydrogenase
γ-GT	gamma-glutamyltransferase
IPCS	International Programme on Chemical Safety
i.v.	intravenous
i.p.	intraperitoneal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	lethal concentration
LC–FLD	liquid chromatography with fluorescence detection
LC–HRMS	liquid chromatography with high resolution mass spectrometry
LC–MS	liquid chromatography–mass spectroscopy
LC–MS/MS	liquid chromatography with tandem mass spectroscopy
LC–UV	liquid chromatography with ultraviolet detection
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectroscopy
n/a	not available

NOAEL	no observed adverse effect level
PEN	porcine ear necrosis
PSA	primary secondary amine
PT	proficiency test
QuEChERS	quick easy cheap efficient robust safe
RP	Reference point
SPE	solid phase extraction
T-EA	sum of 14 ergot alkaloids from <i>Claviceps purpurea</i>
TK	toxicokinetics
ToR	Terms of Reference
UF	uncertainty factor
WG	working group
WHO	World Health Organization
ww	wet weight

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CONFLICT OF INTEREST

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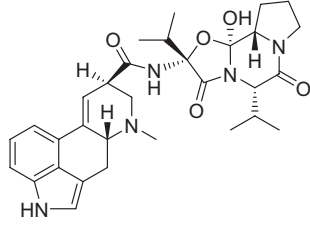
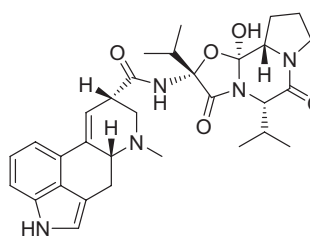
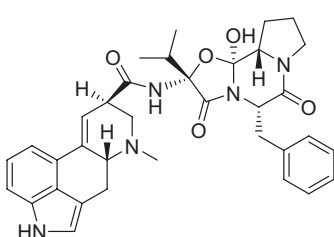
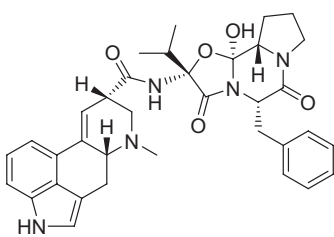
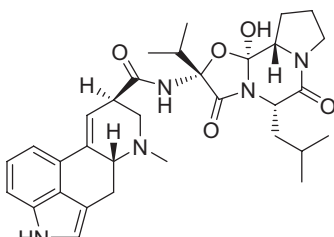
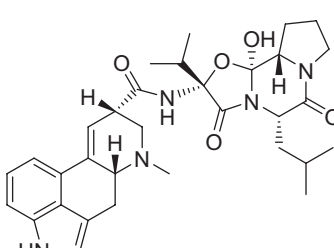
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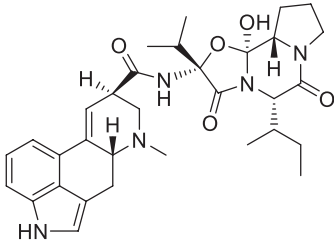
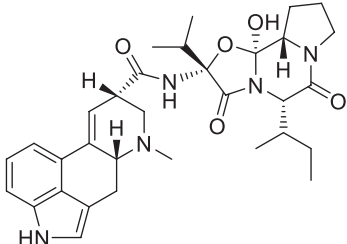
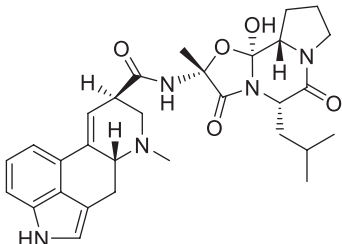
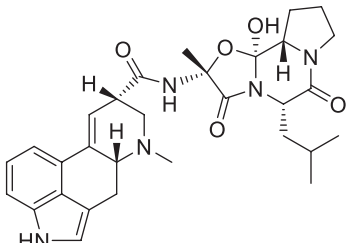
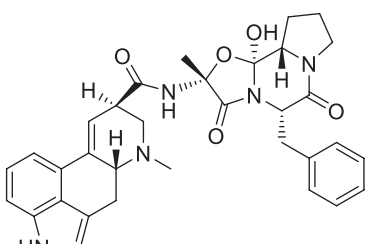
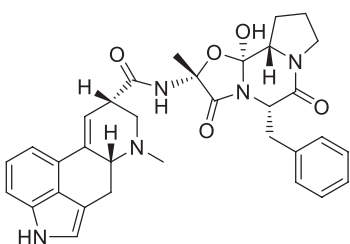
APPENDIX A

Ergot alkaloids identified in *Claviceps* and *Epichloë* species

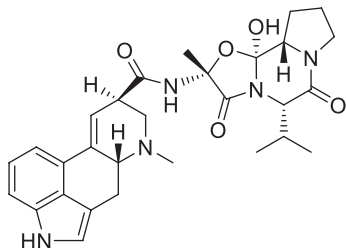
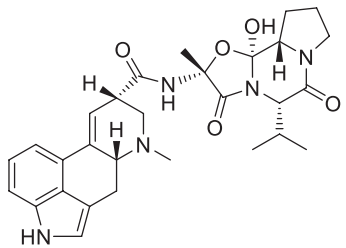
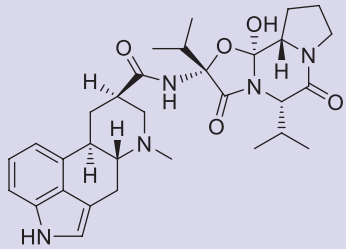
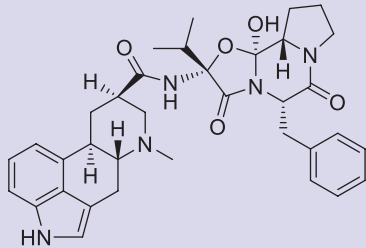
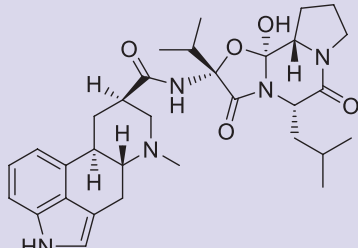
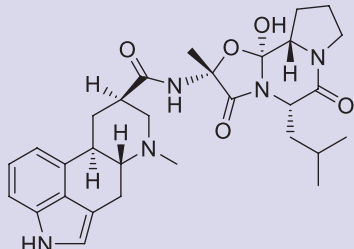
1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
Ergopeptines		
1. Ergocornine	1. C ₃₁ H ₃₉ N ₅ O ₅ 2. 564-36-3 3. 561.7 4. 3.55	
1. Ergocorninine 2. Isoergocornine	1. C ₃₁ H ₃₉ N ₅ O ₅ 2. 564-37-4 3. 561.7 4. 3.55	
1. Ergocristine	1. C ₃₅ H ₃₉ N ₅ O ₅ 2. 511-08-0 3. 609.7 4. 3.44	
1. Ergocristinine 2. Isoergocristinine	1. C ₃₅ H ₃₉ N ₅ O ₅ 2. 511-07-9 3. 609.7 4. 3.44	
1. α-Ergocryptine 2. α-Ergokryptine	1. C ₃₂ H ₄₁ N ₅ O ₅ 2. 511-09-1 3. 575.7 4. 4.08	
1. α-Ergocryptinine 2. -Ergokryptinine, isoergocryptine	1. C ₃₂ H ₄₁ N ₅ O ₅ 2. 511-10-4 3. 575.7 4. 4.08	

(Continues)

(Continued)

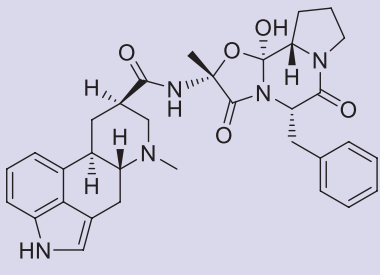
1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
1. β -Ergocryptine 2. β -Ergokryptine	1. $C_{32}H_{41}N_5O_5$ 2. 20315-46-2 3. 575.7 4. 4.08	
1. β -Ergocryptinine 2. β -Ergokryptinine	1. $C_{32}H_{41}N_5O_5$ 2. 19467-61-9 3. 575.7 4. 4.08	
1. Ergosine 2. Ergoclavine	1. $C_{30}H_{37}N_5O_5$ 2. 561-94-4 3. 547.6 4. 3.20	
1. Ergosinine 2. Ergoclavinine, isoergosine	1. $C_{30}H_{37}N_5O_5$ 2. 596-88-3 3. 547.6 4. 3.20	
1. Ergotamine	1. $C_{33}H_{35}N_5O_5$ 2. 113-15-5 3. 581.7 4. 2.5	
1. Ergotaminine 2. Isoergotamine	1. $C_{33}H_{35}N_5O_5$ 2. 639-81-6 3. 581.7 4. 2.5	

(Continued)

1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
1. Ergovaline	1. C ₂₉ H ₃₅ N ₅ O ₅ 2. 2873-38-3 3. 533.6 4. 2.67	
1. Ergovalinine	1. C ₂₉ H ₃₅ N ₅ O ₅ 2. 3263-56-7 3. 533.6 4. 2.67	
Dihydroergopeptines		
1. Dihydroergocornine 2. 9,10-Dihydroergocornine	1. C ₃₁ H ₄₁ N ₅ O ₅ 2. 25447-65-8 3. 563.7 4. 3.50	
1. Dihydroergocristine 2. 9,10-Dihydroergocristine	1. C ₃₅ H ₄₁ N ₅ O ₅ 2. 17479-19-5 3. 611.7 4. 4.40	
1. Dihydroergocryptine 2. Dihydroergokryptine, 9,10-dihydroergocryptine	1. C ₃₂ H ₄₃ N ₅ O ₅ 2. 25447-66-9 3. 577.7 4. 4.03	
1. Dihydroergosine 2. 9,10-Dihydroergosine	1. C ₃₀ H ₃₉ N ₅ O ₅ 2. 7288-61-1 3. 549.7 4. 3.15	

(Continues)

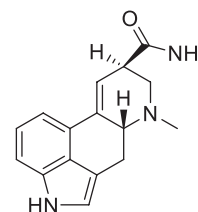
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1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
1. Dihydroergotamine 2. 9,10-Dihydroergotamine	1. C ₃₃ H ₃₅ N ₅ O ₅ 2. 511-12-6 3. 583.7 4. 3.53	

Lysergic acid derivatives

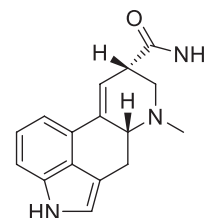
1. Ergine
2. Lysergic acid amide, lysergamide

1. C₁₆H₁₇N₃O
2. 478-94-4
3. 267.3
4. 1.18



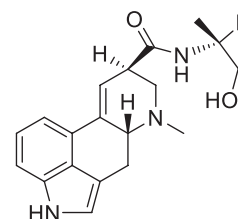
1. Erginine
2. Isolysergic acid amide

1. C₁₆H₁₇N₃O
2. 2889-26-1
3. 267.3
4. 1.18



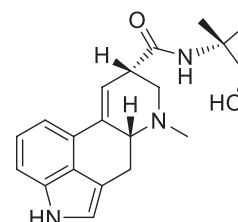
1. Ergometrine
2. Ergonovine, ergobasine

1. C₁₉H₂₃N₃O₂
2. 60-79-7
3. 325.4
4. 2.05



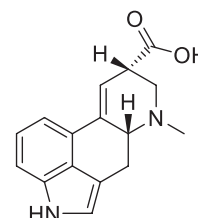
1. Ergometrinine
2. Ergonovinine, ergobasinine, isoergometrine

1. C₁₉H₂₃N₃O₂
2. 479-00-5
3. 325.4
4. 2.05



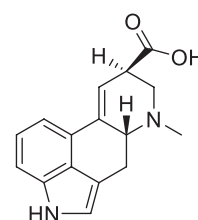
1. Isolysergic acid
2. 9,10-Didehydro-6-methylergoline-8(α)-carboxylic acid, d-isolysergic acid

1. C₁₆H₁₆N₂O₂
2. 478-95-5
3. 268.3
4. 2.13

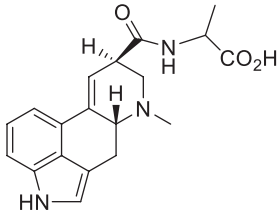
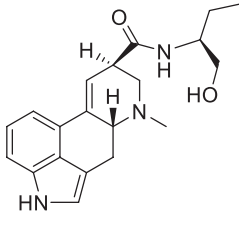
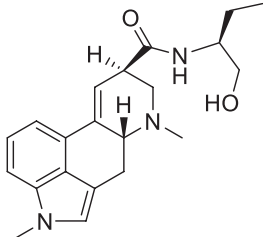
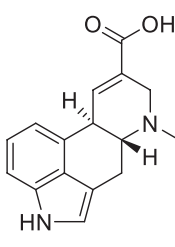
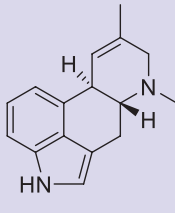
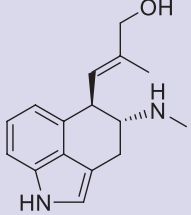


1. Lysergic acid
2. 9,10-Didehydro-6-methylergoline-8(β)-carboxylic acid, D-lysergic acid

1. C₁₆H₁₆N₂O₂
2. 82-58-6
3. 268.3
4. 2.13



(Continued)

1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
1. Lysergyl-alanine	1. C ₁₉ H ₂₁ N ₃ O ₃ 2. 339.4	
1. Methylergometrine (semi-synthetic EA) 2. Methylergonovine	1. C ₂₀ H ₂₅ N ₃ O ₂ 2. 113-42-8 3. 339.4 4. 1.67	
1. Methysergide (semi-synthetic EA)	1. C ₂₁ H ₂₇ N ₃ O ₂ 2. 361-37-5 3. 353.4 4. 2.07	
1. Paspalic acid 2. 8,9-Didehydro-6-methylergoline-8-carboxylic acid	1. C ₁₆ H ₁₆ N ₂ O ₂ 2. 5516-88-1 3. 268.3 4. 2.56	
Ergoclavines		
1. Agroclavine 3. 8,9-Didehydro-6,8-dimethylergoline	1. C ₁₆ H ₁₈ N ₂ 2. 548-42-5 3. 238.3 4. 3.38	
1. Chanoclavine 2. 8,9-Didehydro-6-methyl-6,7-secoergoline-8-methanol, chanoclavine-I	1. C ₁₆ H ₂₀ N ₂ O 2. 2390-99-0 3. 256.3 4. 1.96	

(Continues)

(Continued)

1. Trivial name 2. Synonyms	1. Elem. Comp. 2. CAS number 3. MW 4. LogP (25°C) (est.)	Chemical structure
1. Dihydroelymoclavine 2. 9,10-Dihydrolysergol, 6-methylergoline-8(β)-methanol	1. C ₁₆ H ₂₀ N ₂ O 2. 18051-16-6 3. 256.3	
1. Elymoclavine 2. 8,9-Didehydro-6-methylergoline-8-methanol	1. C ₁₆ H ₁₈ N ₂ O 2. 548-43-6 3. 254.3 4. 2.59	
1. Festuclavine 2. 6,8(β)-Dimethylergoline	1. C ₁₆ H ₂₀ N ₂ 2. 569-26-6 3. 240.3 4. 3.53	
1. Lysergol 2. 9,10-Didehydro-6-methylergoline-8(β)-methanol	1. C ₁₆ H ₁₈ N ₂ O 2. 602-85-7 3. 254.3 4. 2.17	
1. Penniclavine 2. 9,10-Didehydro-8-hydroxy-6-methylergoline-8(β)-methanol	1. C ₁₆ H ₁₈ N ₂ O ₂ 2. 519-13-1 3. 270.3 4. 1.13	
1. Pyroclavine 2. 6,8(α)-Dimethylergoline	1. C ₁₆ H ₂₀ N ₂ 2. 478-89-7 3. 240.3 4. 3.53	
1. Setoclavine 2. 9,10-Didehydro-6,8(α)-dimethylergolin-8-ol	1. C ₁₆ H ₁₈ N ₂ O 2. 519-12-0 3. 254.3 4. 2.20	

APPENDIX B

Identification of evidence relevant for the risk assessment

Search strings for ERGOT ALKALOIDS in feed

Web of Science

Limits WOS: Databases = WOS, BCI, CABI, CCC, DRCI, FSTA, MEDLINE, SCIELO, ZOOPEC

Timespan = 01/07/2011–01/05/2023

Search language = no limitation

Set	Query	Results	Comments
	#1 AND #2	7211	WOS CHEMISTRY
	#1 AND #2 AND (TS=feed)	1066	WOS CHEMISTRY in feed
	#1 AND #4 AND (#5 OR #8)	1549	WOS ADME in food-producing and non-food producing animals
	#1 AND #6 AND #8	1164	WOS TOXICITY in experimental animals
	#1 AND #5 AND #6	1895	WOS TOXICITY in food-producing and non-food producing animals
	#1 AND #7	143	WOS TRANSFER feed to food
	#1 AND #9	301	WOS OCCURRENCE feed
#1	("Ergot alkaloid*" OR ergot* OR "Claviceps purpurea" OR "C. purpurea" OR ergocornine OR ergocorninine OR ergocristine OR ergocristinine OR alpha-ergocryptine OR α-ergocryptine OR beta-ergocryptine OR β-ergocryptine OR ergocryptinine OR alpha-ergocryptinine OR beta-ergocryptinine OR α-ergocryptinine OR β-ergocryptinine OR ergometrine OR ergometrinine OR ergosine OR ergosinine OR ergotamine OR ergotaminine OR sclerotia OR 60-79-7 OR 479-00-5 OR 561-94-4 OR 596-88-3 OR 113-15-5 OR 639-81-6 OR 564-36-3 OR 564-37-4 OR 511-09-1 OR 511-10-4 OR 20315-46-2 OR 19467-61-9 OR 511-08-0 OR 511-07-9 OR 2854-38-8 OR 3268-95-9)	8393	Main search WOS Command word: TS
#2	(chem* OR analy* OR identi* OR charact* OR detect* OR determin* OR method* OR form* OR degrad* OR hydroly* OR reaction* OR "GC-MS*" OR "HPLC" OR "LC-MS" OR "ICP-MS")		Chemistry Command words: TS
#3	(intake OR feed* OR fodder OR diet* OR meal OR cereal* OR corn OR maize OR "Zea mays" OR wheat OR "Triticum aestivum" OR rye OR "Secale cereale" OR barley OR "Hordeum vulgare" OR oat OR Avenasativa OR grain* OR seed* OR forage OR silage OR grass OR Poaceae OR hay OR rape OR Raptio OR Brassicanapus OR soybean OR "Glycine max" OR DDGS OR WDG OR seaweed OR algae)		Feed Command words: TS
4	(absor* OR tissue* OR metaboli* OR excret* OR kinetic* OR toxicokinetic* OR pharmacokinetic* OR degrad* OR biotrans* OR eliminat* OR biomark*)		Toxicokinetic in vivo Command words: TS

(Continues)

(Continued)

Set	Query	Results	Comments
#5	("farm animals" OR "food producing animal*" OR aquaculture OR horse* OR stallion* OR mare* OR foal* OR equine OR ruminant* OR livestock OR herd OR cow* OR cattle OR bull* OR calf OR calves OR heifer* OR bovine OR sheep* OR ewe* OR ram* OR lamb OR goat* OR caprine OR ovine OR pig* OR swine* OR sow* OR gilt* OR boar* OR porcine OR mink* OR poultry OR chicken* OR hen* OR cock* OR rooster* OR broiler* OR duck* OR goose OR geese OR geesling* OR turkey* OR quail* OR guinea OR rabbit* OR fish* OR salmon OR trout* OR piscine OR zebrafish OR pet* OR cat* OR kitten* OR dog* OR bitch* OR pup* OR "sea bream" OR seabream OR "sea bass" OR seabass OR turbot OR carp OR sturgeon OR eel* OR tilapia OR cod OR halibut OR cobia OR "milk fish" OR tuna*)		Food-producing and non-food producing animals Command words: TS
#6	(tox* OR poison* OR cancer* OR carcino* OR tumor* OR tumour* OR organ* OR tissue* OR immun* OR neuro* OR developmental OR teratogen* OR repro* OR liver OR kidney* OR brain* OR lung* OR cardiovascular OR health OR clinical OR growth OR weight OR "feed intake" OR vascularisation OR vasoconstriction)		Toxicity Command words: TS
#7	((administration OR absorption OR distribution OR "tissue distribution" OR bioavailab* OR metaboli* OR biotransform* OR activat* OR half-li* OR excret* OR clearance OR eliminat* OR bioconcentrat* OR *kinetic* OR PBPK OR PBK OR transfer OR carry-over OR carryover OR "carry over" OR residue*) AND (ruminant* OR cattle OR cow* OR bovine* OR sheep* OR goat* OR buffal* OR swine OR pig* OR poultry OR chicken* OR turkey* OR duck* OR quail* OR goose* OR trout* OR salmon OR "sea bream" OR seabream OR "sea bass" OR seabass OR turbot OR carp OR sturgeon OR eel OR eels OR tilapia OR cod OR halibut OR cobia OR "milk fish" OR tuna* OR horse* OR ostrich*) NOT (rat* OR mice* OR mouse OR monkey* OR "guinea pig" OR "mini pig" OR hamster* OR dog* OR cat* OR mink* OR zebrafish OR "zebra fish" OR "Danio rerio" OR medaka OR "Oryzias latipes" OR mummichog* OR killifish OR "Fundulus heteroclitus" OR fundulus OR minnows OR "Pimephales promelas" OR "model fish"))		Transfer from feed to food Command words: TS
#8	Cavia OR Cricetinae OR Cricetus OR "guinea pig" OR "guinea pigs" OR Hamster* OR Mice OR Mouse OR Muridae OR Murinae OR Murine OR Mus OR Rat OR Rats OR Rattus OR Rodent* OR Mink OR Minks OR Mustela* OR Monkey OR Monkeys OR Nomascus OR primate*		Experimental animals Command words: TS
#9	Feed* OR occur*		Occurrence Command words: TS

APPENDIX C

Intakes and composition of diets used to estimate animal exposure to EAs

The feed intake and the diet composition used to estimate the exposure to EAs of the animal species considered in this report were derived from information extensively described by the CONTAM Panel in previous Opinions on the risks for animal and public health (EFSA CONTAM Panel, 2011; EFSA CONTAM Panel, 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 & 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 EAs, based on the LB and UB mean and high (95th or lower) concentrations in the feedingstuff reported in Table 9.

C.1. | Feed intake

C.1.1. | Cattle, sheep, goats and horses

TABLE C.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 ^a (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 ^b	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)

^a88% dry matter.

^bMilk replacer (94.5% dry matter).

C.1.2. | Pigs, poultry, fish and rabbit

TABLE C.2 Default values for live weight and feed intake of pigs, poultry, fish and rabbits.

	Live weight [kg]	Feed intake [kg/day]		Reference
		Dry matter (DM)	Complete feed ^a (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)
Ducks for 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)

^a88% dry matter.

C.1.3. | Dogs and cats

TABLE C.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 ^a (CF)	
Dogs	15	0.25	0.284	EFSA (2017)
Cats	3	0.06	0.068	EFSA (2017)

^a88% dry matter.

C.2. | Diet composition and EA concentration estimates

C.2.1. | Cattle, sheep, goats and horses

TABLE C.4 Compositions of feed for bovines using feed materials, and calculated mean and high lower-bound and upper-bound levels of EAs 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 gluten feed	10	10
Oil seeds, oil fruits and products derived thereof	26	22	Soybean meal	5	
			Rapeseed expeller	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		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
EAs^b					
Mean lower bound (µg/kg DM)				28.5	23.0
Mean upper bound (µg/kg DM)				69.3	100.5
High lower bound (µg/kg DM)				108.8	91.8
High upper bound (µg/kg DM)				151.1	177.2

^aThe ratio of concentrate to forages on a dry matter basis defines the daily ration.

^bEAs 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 9).

TABLE C.5 Compositions of feed for caprines using feed materials, and calculated mean and high lower-bound and upper-bound levels of EAs 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 expeller	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
EAs^b					
Mean lower bound (µg/kg DM)				37.7	30.2
Mean upper bound (µg/kg DM)				72.0	91.1
High lower bound (µg/kg DM)				142.6	116.0
High upper bound (µg/kg DM)				175.1	180.8

^aThe ratio of concentrate to forages on a dry matter basis defines the daily ration.

^bEAs 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 9).

TABLE C.6 Compositions of feed for ovines using feed materials, and calculated mean and high lower-bound and upper-bound levels of EAs 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
			Oat grain + oat feed		20
Oil seeds, oil fruits and products derived thereof	20	20	Soybean meal	4	4
			Rapeseed expeller	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
EAs^b					
Mean lower bound (µg/kg DM)				26.8	31.4
Mean upper bound (µg/kg DM)				92.0	87.7
High lower bound (µg/kg DM)				104.7	123.5
High upper bound (µg/kg DM)				176.1	182.7

^aThe ratio of concentrate to forages on a dry matter basis defines the daily ration.

^bEAs 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 9).

TABLE C.7 Compositions of feed for horses using feed materials, and calculated mean and high lower-bound and upper-bound levels of EAs in these diets.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition [%]
Cereal grains and products derived thereof	82	Oat grain + oat feed	52
		Oat feed	0
		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
EAs^b			
Mean lower bound (µg/kg DM)			35.1
Mean upper bound (µg/kg DM)			105.9
High lower bound (µg/kg DM)			131.7
High upper bound (µg/kg DM)			208.8

^aThe ratio of concentrate to forages on a dry matter basis defines the daily ration.

^bEAs 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 9).

TABLE C.8 Diet compositions for piglets, pigs for fattening and lactating sows and calculated mean and high lower-bound and upper-bound levels of EAs 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	Triticale	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 expeller	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
EAs^a							
Mean lower bound (µg/kg DM)					118.6	118.3	127.3
Mean upper bound (µg/kg DM)					141.6	139.0	145.2
High lower bound (µg/kg DM)					707.7	670.5	709.5
High upper bound (µg/kg DM)					719.0	680.7	716.9

Abbreviation: DM, dry matter.

^aEAs 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 9).

C.2.2. | Pigs, poultry, fish and rabbit

TABLE C.9 Diet compositions for chickens, turkeys and ducks for fattening and calculated mean and high lower-bound and upper-bound levels of EAs 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
EAs^a							
Mean lower bound (µg/kg)					16.1	21.1	28.9
Mean upper bound (µg/kg)					32.5	45.7	52.4
High lower bound (µg/kg)					73	104.9	128.9
High upper bound (µg/kg)					88.1	125.7	149.1

^aEAs 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 9).

TABLE C.10 Diet compositions for laying hens and calculated mean and high lower-bound and upper-bound levels of EAs 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	30
		Wheat feed	10
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
EAs^a			
Mean lower bound (µg/kg DM)			28.7

Abbreviation: DM, dry matter.

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

^aEAs 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 9).

TABLE C.11 Diet compositions for salmon^a and calculated mean and high lower-bound and upper-bound levels of EAs 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 grain	10
Minerals and products derived thereof	3	Mineral salts	3
		Feed additives	Premix
EAs^b			
Mean lower bound (µg/kg DM)			3.8
Mean upper bound (µg/kg DM)			8.1
High lower bound (µg/kg DM)			17.6
High upper bound (µg/kg DM)			21.6

Abbreviation: DM, dry matter.

^aEllingsen, H., Olaussen, J. O., & I. B. (2009). Environmental analysis of the Norwegian fishery and aquaculture industry—A preliminary study focusing on farmed salmon. *Marine Policy*, 33, 479–488.

^bEAs 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 9).

TABLE C.12 Diet compositions for rabbits for fattening and calculated mean and high lower-bound and upper-bound levels of EAs in these diets.

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition [%]
Cereal grains and products derived thereof	25	Wheat	10
		Maize	5
		Wheat feed	10
Forage dehydrated	20	Lucerne 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
EAs^a			
Mean lower bound (µg/kg DM)			21.2
Mean upper bound (µg/kg DM)			25.8
High lower bound (µg/kg DM)			77.7
High upper bound (µg/kg DM)			81.7

Abbreviation: DM, dry matter.

^aEAs 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 9).

C.2.3. | Dogs and cats

TABLE C.13 Diet compositions for dogs and calculated mean and high lower-bound and upper-bound levels of EAs 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	
			Wheat grain	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
			Potato 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
EAs^b					
Mean lower bound (µg/kg DM)				11.6	1.9
Mean upper bound (µg/kg DM)				20.3	4.0
High lower bound (µg/kg DM)				50.6	8.8
High upper bound (µg/kg DM)				57.1	10.8

Abbreviation: DM, dry matter.

^aIncludes poultry meal, lambs meal and fish meal.

^bEAs 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 9).

TABLE C.14 Diet compositions for cats and calculated mean and high lower-bound and upper-bound levels of EAs 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 ^a	35
		Hydrolysed animal products ^a	1
		Fat	4
Cereal grains and products derived thereof	30	Rice	10
		Wheat feed	10
		Wheat grain	10
Oil seeds, oil fruits and products derived thereof	5	Soybean meal	5
Tubers, roots and products derived thereof	12	Sugar beet pulp	2
		Potato protein	10
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
EAs^b			
Mean lower bound (µg/kg DM)			21.2
Mean upper bound (µg/kg DM)			25.8

(Continues)

TABLE C.14 (Continued)

Groups according to REG (EU) 2022/1104	% of diet	Feed material	Composition [%]
High lower bound (µg/kg DM)			77.7
High upper bound (µg/kg DM)			81.7

Abbreviation: DM, dry matter.

^aIncludes poultry meal, lamb meal and fish meal.

^bEAs 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 9).

C.3. | EAs concentration estimates from compound feed (complementary or complete feeds)

TABLE C.15 EA concentration in complete feed^c (mean and high lower-bound and upper-bound levels) for all animal species.

Animal species	EA concentration (µg/kg DM)			
	Mean LB	Mean UB	High ^b LB	High UB
Pigs				
Pigs all categories	88.2	110.8	275.4	275.4
Cattle				
Veal calves	n/a ^a	n/a	n/a	n/a
Poultry				
Chickens for fattening	26.0	68.7	48.4	68.2
Laying hens	14.5	91.2	8.6	136.4
Turkeys for fattening	n/a	n/a	n/a	n/a
Ducks for fattening	n/a	n/a	n/a	n/a
Fish				
Salmonids	n/a	n/a	n/a	n/a
Rabbits				
Rabbits for fattening	37.2	86.9	39.8	73.8
Dogs				
	n/a	n/a	n/a	n/a
Cats				
	n/a	n/a	n/a	n/a

Abbreviation: DM, dry matter.

^aNo data available.

^bThe highest reliable percentile based on the number of samples available.

^cConcentrations in complete feeds are also reported in Table 9.

TABLE C.16 EA concentration estimated from complementary feed and forages^b (mean and high lower-bound and upper-bound levels) for all ruminants and horses.

Animal species	EA concentration (µg/kg DM)			
	Mean LB	Mean UB	High ^c LB	High UB
Cattle				
Dairy cows	24.6	84.6	51.7	111.1
Cattle for fattening	34.5	111.4	85.2	170.6
Small ruminants				
Dairy sheep/goat	n/a ^a	n/a	n/a	n/a
Lambs for fattening	n/a	n/a	n/a	n/a
Horses				
	31.6	117.3	79.1	172.8

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

^aNo data available.

^bForages included in the diets in the ratios indicated in the section A.2.

^cThe highest reliable percentile based on the number of samples available.

APPENDIX D

Groups of feed materials²²

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.

²²As per Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials.

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, dextrans, sorbitol, fatty acids esterified with glycerol, soap stocks, glycerine, propylene glycol, chondroitin sulphate.

APPENDIX E

Exposure assessment of ergot alkaloids for animals per kg body weight

Estimated intake of EAs using mean and high, LB and UB EA concentrations in feeding stuffs and expressed per kg bw are shown in Tables E.1–E.7.

TABLE E.1 Estimated exposure to EAs 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.

Exposure											
μg/day		μg/kg bw per day		μg/day		μg/kg bw per day		μg/day		μg/kg bw per day	
Mean		High		Mean		High		Mean		High	
Dairy cows						Cattle for fattening					
Model diet plus forages											
LB	570	0.9		2176	3.3	184	0.5		734	1.8	
UB	1385	2.1		3021	4.6	804	2.0		1418	3.5	

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

^aAs detailed in Appendix C.2.

TABLE E.2 Estimated exposure to EAs 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.

Exposure												
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 goats				Lambs for fattening				Horses				
Model diet plus forages												
LB	45	0.8	171	2.9	35	1.7	136	6.8	281	0.7	1053	2.6
UB	86	1.4	210	3.5	97	4.8	201	10.0	847	2.1	1671	4.2

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

TABLE E.3 Estimated exposure to EAs 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.

Exposure												
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 piglets				Pigs for fattening				Lactating sows				
Model diet												
LB	104	5.2	623	31.1	260	4.3	1475	24.6	672	3.8	3746	21.4
UB	125	6.2	633	31.6	306	5.1	1498	25.0	766	4.4	3785	21.6

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

TABLE E.4 Estimated exposure to EAs using Mean LB/UB and High LB/UB by a 2-kg body chickens for fattening and a 2-kg body weight laying hen, using model diets.

	Exposure							
	$\mu\text{g/day}$		$\mu\text{g/kg bw day}$		$\mu\text{g/day}$		$\mu\text{g/kg bw day}$	
	Mean	High	Mean	High	Mean	High	Mean	High
	Chickens for fattening				Laying hens			
Model diet								
LB	2.5	1.3	11.5	5.8	3.0	1.5	12.0	6.0
UB	5.1	2.6	13.9	7.0	4.4	2.2	13.2	6.6

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

TABLE E.5 Estimated exposure to EAs 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.

	Exposure							
	$\mu\text{g/day}$		$\mu\text{g/kg bw day}$		$\mu\text{g/day}$		$\mu\text{g/kg bw day}$	
	Mean	High	Mean	High	Mean	High	Mean	High
	Turkey				Ducks for fattening			
Model diet								
LB	3.7	1.2	18.5	6.2	3.8	1.3	17.0	5.7
UB	8.0	2.7	22.1	7.4	6.9	2.3	19.7	6.6

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

TABLE E.6 Estimated exposure to EAs 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.

	Exposure							
	$\mu\text{g/day}$		$\mu\text{g/kg bw day}$		$\mu\text{g/day}$		$\mu\text{g/kg bw day}$	
	Mean	High	Mean	High	Mean	High	Mean	High
	Rabbits for fattening				Salmons			
Model diet								
LB	2.1	1.1	7.8	3.9	0.01	0.1	0.04	0.3
UB	2.6	1.3	8.2	4.1	0.02	0.1	0.05	0.4

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

TABLE E.7 Estimated exposure to EAs 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.

	Exposure											
	$\mu\text{g/day}$		$\mu\text{g/kg bw day}$		$\mu\text{g/day}$		$\mu\text{g/kg bw day}$		$\mu\text{g/day}$		$\mu\text{g/kg bw day}$	
	Mean	High	Mean	High	Mean	High	Mean	High	Mean	High	Mean	High
	Dogs				Dogs (vegetarian diet)				Cats			
Model diet												
LB	2.9	0.2	12.6	0.8	0.5	0.0	2.2	0.1	1.3	0.4	4.7	1.6
UB	5.1	0.3	14.3	1.0	1.0	0.1	2.7	0.2	1.5	0.5	4.9	1.6

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

APPENDIX F

Claviceps africana: Effects in food producing animals

Pigs

In the previous EFSA Opinion the effect of EAs from *C. africana* was reported (Kopinski et al., 2007, 2008). These studies are summarised below.

Ergot from sorghum, contaminated mainly with the alkaloid dihydroergosine, was investigated for its effect on sows (Kopinski et al., 2007, 2008). A control group was given a feed containing EAs at concentrations below 0.01 mg/kg. When compared to control feed, inclusion of 1.5% ergot (corresponding to 7 mg EAs/kg feed, including 6 mg dihydroergosine/kg) in the sow diet for 6–10 days preceding farrowing, led to suppression of milk production and to the death of 87% of their piglets despite supplementary feeding of natural and artificial colostrum, milk replacer and attempts to foster them onto normally lactating sows. Ergot inclusions of 0.6%–1.2% (corresponding to 2.8–5.6 mg EAs/kg feed) had less of an effect on milk release and piglet mortality. Out of the 23 animals exposed to the lowest doses of EAs (1.4–2.8 mg EAs/kg feed), lactation was affected for two first-litter (primiparous) sows (one for each dose, significance not reported). In another experiment with diets containing 3% w/w sorghum ergot (14 mg dihydroergosine/kg feed) fed to sows from 14 days post-farrowing until weaning 14 days later, a lower weight gain of litters was observed. In this experiment, ergot feeding for 7 days significantly reduced the concentration of prolactin in the sow plasma (4.8 µg/L compared to 15.1 µg/L).

A case study on *C. africana* intoxication also indicated agalactia in sows receiving 1.6 mg EAs/kg diet (dihydroergosine representing 90% of the EA content), leading to loss of litters until ergot was removed; in grower and finisher pigs persistent feed refusal, as well as severe diarrhoea after 3–4 weeks of ergot feeding was observed (Blaney et al., 2000).

Concerning EAs from *C. africana*, adverse effects on milk production were observed on two primiparous sows (out of 5) at 1.4 and 2.8 mg EAs/kg feed. Considering 1.4 mg EAs/kg feed as a LOAEL and using an uncertainty factor of 3, would result in a NOAEL of 0.5 mg EAs from *C. africana*/kg feed for sows.

Bovines

Blaney et al. (2011) treated 32 Hereford steers with rations prepared with sorghum, containing initially 0, 4.4, 8.8 and 17.6 mg EAs/kg DM (84% dihydroergosine, 10% dihydroelymoclavine and 6% festuclavine). However, due to severe hyperthermia and almost complete feed refusal, the study was continued with 0, 1.1, 2.2 and 4.4 mg EAs/kg DM for about 3 months. The study was performed in summer and autumn in Brisbane, Australia. In the first 2 months, rectal temperatures were strongly increased in all three ergot groups. During the last phase they were similar to controls, possibly related to the lower environmental temperatures. There was a strong reduction in dry matter intake and body weight gain in all three ergot groups, being 91, 88 and 84% of the controls at the end of the treatment. Serum plasma prolactin levels were strongly decreased in all treatment groups. They returned to normal after the treatment was ended.

In a follow-up study, McLennan et al. (2017) fed Hereford steers with rations containing ergot-contaminated sorghum (*C. africana*) during a period with lower temperatures in south-east Queensland, Australia. In a first study, 35 Hereford steers were fed for 125 days during winter and spring with rations containing EA concentrations of 0, 2.8, 5.6, 8.2 and 11.2 mg/kg DM (84% dihydroergosine, 10% dihydroelymoclavine and 6% festuclavine). Steers (average weight 271 kg) were fed ad libitum for 125 days. There was an effect on the hair coat in all treatment groups, being longer and rougher. In addition, more animals showed signs of heat stress, like 'open-mouth breathing, panting, excessive salivation and high respiration rates', especially during the second half (springtime) of the study with incidences being 0/6, 4/7, 6/6, 7/7 and 4/7 animals, respectively. Rectal temperatures were increased in all treatment groups on warmer and more humid days. Dry matter intake was reduced by 15% on average in the treatment groups without clear dose–response. Growth was reduced by 34% in all treatment groups, again without differences among the groups. Plasma prolactin concentrations were strongly decreased at day 21 in all treatment groups and for the three highest doses at days 63 and 125. There were no signs of gangrene. In a second study, carried out in May till September with lower temperatures, 36 steers (average weight 317 kg) were initially fed with rations containing 0, 0.28, 0.55 and 1.1 mg EAs/kg DM. After 28 days, due to a lack of effects, levels of the low and middle dose were gradually raised to 2.1 and 4.3 mg EAs/kg DM. The effects related to hair coat and heat stress were not observed in this study, likely related to the lower temperatures. Dry matter intake was only decreased at the highest dose, similar as the body weight gain, although the average body weight was not significantly lower for the highest dose group. Serum prolactin levels were not different after 21 days but after 56 and 85 days there was a dose-related decrease, more than 10-fold in the highest dose group of 4.3 mg EAs/kg DM. The study suggests a clear relationship between the animals' tolerance to ergot and environmental temperature/humidity conditions.

To summarise on EAs from *C. africana* for bovines, the study by Blaney et al. (2011) demonstrated adverse effects at 1.1 mg EAs/kg feed DM. Applying an uncertainty factor of 3 to deduce a NOAEL from a LOAEL, a NOAEL of 0.4 mg EAs/kg feed DM was derived. The abovementioned studies confirm that weather conditions play an important role in the toxicity of EAs in bovines.

APPENDIX G

Epichloë coenophiala (ergovaline/–inine): Effects in ruminants and horses

Bovines

In the previous Opinion, the CONTAM Panel described the effects of EAs present in tall fescue, produced by *E. coenophiala*. However, an RP was not derived for these compounds. Therefore, papers published before the previous Opinion as well as newer studies were reviewed and are described below. Many studies have been published on the effects of tall fescue in cattle, followed by the identification of endophytes and the production of ergovaline/–inine by these organisms. Due to the presence of endophytes, grass is more resistant to drought and insects. This started work on developing tall fescue varieties with endophytes not producing the alkaloids but being also more resistant. In addition, studies were initiated on possible ways to improve the health of the animals, like red clover and soybean. An important criterion was that ergovaline/–inine levels had been measured, allowing the derivation of an RP for adverse animal health effects. Most studies compare the effects in animals kept on pastures which makes it more difficult to determine the actual levels of ergovaline/–inine that caused the effects. In addition, the weather conditions play an important role, higher temperatures during the summer but also extremely cold weather. This is due to the vasoconstrictive properties of the alkaloids. Furthermore, they cause a strong decrease in the serum levels of prolactin with potential effects on milk yield but the impact of this on non-lactating cattle is less clear.

Peters et al. (1992) was one of the first to measure the levels of ergovaline and estimate the exposure of cows. Sixteen pairs of cows (Angus and Simmental × Angus; initial body weight around 500 kg) and their male or female calves (initial body weight around 90 kg) were kept on pastures with either Kentucky-31 (KY-31) tall fescue, endophyte-free tall fescue or orchard grass during May until September during two different years. The temperature in the first year was relatively high, in the second year relatively low. In both years, the body weight of the cows decreased much more in the cows on the KY-31 than on the other two types of grass. As a result, also weight-to-height change was increased in both years in these cows, and hair, respiration and condition scores decreased. Milk production was decreased by 25%, 6 vs. 8 kg/day. The body weight gain of the calves was lower. Organic matter intake was reduced but only in August of the first year and seems not to explain the effects. Ergovaline levels in KY-31 measured in June and August were 0.33 and 0.15 mg/kg in the first year, 0.51 and 0.24 mg/kg in the second year (average 0.3 mg/kg DM). In the endophyte-free tall fescue, levels were < 0.05 mg/kg in June and August of the first year, but 0.32 and < 0.05 mg/kg in the second year; the high level could not clearly be explained.

Stamm et al. (1994) fed straw containing 0, 0.16, 0.32 and 0.48 mg/kg DM ergovaline to steers (Hereford × Angus; 220 kg body weight; 21 animals/treatment) for 84 days. Animals were kept at low temperatures. No clear effects on intake, body weight and gain to feed ratios were observed. In a separate study with 16 ruminally cannulated steers (370 kg body weight; 4 animals per treatment), treated with the same levels of ergovaline for 7 days, also no effect on feed intake was observed, as well as no effects on heart and respiration rates and body temperature. Ruminal pH and acetate to propionate ratio were decreased at the highest dose. Prolactin levels were reduced two- to three-fold at the two highest doses but the decrease was not significant.

Parish et al. (2003) investigated the effect of two types of grass infected with non-alkaloid producing endophytes (AR542 and AR502) with those of endophyte-free grass (E-) and of wild-type toxic endophyte-infected (E+) Jesup grass (all four based on KY-31 tall fescue). The study was carried out in three subsequent years and results were averaged for the spring or autumn of the 3 years. Angus crossbred cattle (mean bw 227 kg) was assigned to pastures with one of the four types of grass, heifers in the first year and spring of the second year, steers in autumn 2000 and spring and autumn 2001. At a second location only steers were used. Mean ergot alkaloid concentrations over the 3 years in E+ grass were 0.84 (autumn) and 1.13 mg/kg DM (spring) on the first location, and 1.21 (autumn) and 0.82 mg/kg DM (spring) for the other location. In the other grass types, they were much lower, varying between 0.003 and 0.038 mg/kg. ADG in animals kept on E+ was strongly reduced, being 0.41 kg/d during the autumn on the first location vs. 0.82–0.84 kg/day for the other three treatments, 0.49 vs. 0.71–0.73 kg/day in the spring on that location, and on the second location 0.56 vs. 0.81–1.00 kg/day in the autumn and 0.31 vs. 0.78–0.97 kg/day in the spring. Rectal temperatures were increased in the spring period on the first location and spring and autumn period in the second location. There were also signs of heat stress in animals on E+ pastures during hot days (panting and seeking shade). Serum prolactin levels were much lower in animals on E+ grass, being at the first location 2 vs. 13–19 ng/mL (autumn), 6 vs. 133–148 (spring), and on the second location 1 vs. 11–21 (autumn) and 3 vs. 87–120 ng/mL (spring). In a separate experiment the authors also observed differences in grazing behaviour and water consumption, including a reduced dry matter intake.

Watson et al. (2004) compared the effects of grass infected with a non-alkaloid producing endophyte with that containing an endophyte producing ergovaline on cow-calf pairs. Cows (purebred Angus; 3–10 years) were assigned to the pastures from March to September for 3 years and used for breeding from April to mid-June (only some animals for more than 1 year). Animals were not exposed during the 90 days prior to parturition. The EA content (measured by ELISA) was on average 0.45 mg/kg DM in the wild-type grass and below or close to the LOQ in the other one. Available forage and forage growth rates were not different. There was no effect on calving rate or interval. However, birth weight was lower in the exposed animals (32.7 vs. 38.6 kg). Average daily gain of the cows was lower (0.12 vs. 0.29 kg/day) but also for the steer calves (0.97 vs. 1.15 kg/day) and heifer calves (0.9 vs. 1.03 kg/day). Also, the body condition score (BCS) did not increase in

these calves, contrary to non-exposed animals. As a result, weaning weights at 205 days of age were significantly lower (respectively 227 vs. 256 and 217 vs. 237 kg). Prolactin levels were reduced in cows (40 vs. 142 ng/mL) but also the calves (16 vs. 50, 31 vs. 119 and 55 vs. 165 ng/mL in years 1, 2 and 3, respectively).

Nihsen et al. (2004) compared the effects of tall fescue varieties, one infected with endophyte producing (KY+) and two with endophyte not producing ergovaline on growing steers. In addition, a grass not containing endophyte was included. Animals (230–240 kg bw) were kept on pastures with the different varieties during spring until October in two different years and locations. Measured levels of ergovaline/–inine in the KY9+ grass varied between 0.4 and 2.3 mg/kg DM but were not detectable (<0.05 mg/kg DM) in the other grasses. There were no interactions for year × location, forage × location or forage × year and therefore all results were combined. Steers kept on KY+ showed reduced average daily gain (0.34 kg/d vs. 0.60, 0.54 and 0.61 kg/d for, respectively, the two other endophyte and non-endophyte containing grasses). In addition, these animals showed the typical reduced hair coat scores (HCS), increased respiration rate and increased rectal temperatures. Prolactin levels were strongly reduced and also cholesterol, triglyceride, LDH, ALP levels in blood serum were significantly lower, whereas creatinine levels were increased. The KY+ grass also contained high levels of *N*-formyl and *N*-acetyl loline concentrations but that was also the case for the grasses infected with non-ergot producing endophytes, suggesting that these compounds are not responsible for the observed effects.

Brown et al. (2009) investigated potential effects on metabolic parameters in Angus beef steers grazing a low or high contaminated pasture (Mid-June till September). Levels of ergovaline/–inine were respectively 0.02 and 0.52 mg/kg DM. In addition, the grasses contained 0.002 vs. 0.065 mg/kg lysergic acid and 0.005 vs. 0.16 mg/kg isolysergic acid. ADG was decreased, being 0.40 vs. 0.58 kg, hair coats were affected and serum prolactin levels reduced by a factor 10 in the exposed animals. The lower ADG was reflected by the lower body weights, being 313 vs. 338 kg after 89–105 day grazing. In addition, serum levels of ALP, ALT, AST, LDH, cholesterol and creatine kinase were decreased, those of ammonia increased. Serum calcium levels were decreased, those for potassium increased, potentially reflecting observed differences in the forage levels of these minerals. Absolute and relative liver weights were decreased by 10 and 6.6%, possibly explaining the low serum enzyme levels. In the liver protein levels of AST and PEPCK-C (phosphoenolpyruvate carboxykinase) were increased in the treated animals, the latter enzyme being involved in the gluconeogenic pathway.

Drewnoski et al. (2009) investigated the possibility to feed cattle on infected tall fescue grass during the winter period (starting in December followed by a period of 56–86 days; 5 study years). Average temperatures were 2.8–6.2°C. Alkaloid levels were measured in year 4 and 5, being 2.35 mg/kg in infected grass (E+), 0.29 mg/kg in grass without endophyte (E–) and 0.19 mg/kg in grass containing an endophyte not producing EAs (EN). In the first year Angus steers were studied, in subsequent year heifers. There was no effect of the treatment on ADG (0.51, 0.59 and 0.56 kg/day for E+, E– and EN) or body condition score (BCS). No effect on forage disappearance was observed, being around 4.7–5.0 kg DM/animal per day. Serum prolactin levels in heifers on E+ pastures were markedly reduced compared to the two other treatments, with clear differences between years. Prolactin levels increased by the end of the treatment period (March), but the increase was slower on E+.

Aiken et al. (2009) fed 18 one-year-old crossbred (Angus × Brangus; mean bw 345 kg) heifers for 9 days with alfalfa and one of three concentrate diets, one containing tall fescue seed infected with a non-alkaloid producing endophyte (E–), one containing an alkaloid producing endophyte (E+) and a mixture of the two (E+ E–). Ergovaline levels were 0, 0.39 and 0.79 mg/kg DM, in addition to 0, 0.13 and 0.38 mg/kg DM ergovalinine (sum 0, 0.52 and 1.17 mg/kg DM). Dry matter intake was 7.9 ± 0.8 and 8.1 ± 0.4, and 7.1 ± 0.4 kg/day for the E–, E+ E– and E+ diets, the latter being significantly lower. Serum prolactin levels averaged 191 ± 19, 142 ± 16 and 102 ± 17 ng/mL. Levels decreased faster on the highest exposure but were similar after 51 h of treatment. There was some recovery on the intermediate level. There was an increased vasoconstriction at both levels as measured by caudal artery area. There was also a decreased heart rate at both levels but only significant at some time points for the highest dose. Blood flow rates through the caudal artery were also decreased but borderline significant at only some time points ($p < 0.06$). Ambient temperature during the study decreased from around 18°C at the start of the adjustment period but then decreased to around –2°C at the start of the treatment and slowly increased to 5–10°C during that period. This may have affected the outcome of the study.

Looper et al. (2009) investigated effects on sperm production by one-year-old Brahman-influenced bulls (bw 478 ± 34 kg) kept on either a toxic endophyte-infected (EI) or novel endophyte-infected (NE) pasture for 121 days (4 bulls per pasture, 2 pastures per type of grass). Ambient temperatures in July and August were 34°C and 40°C, respectively. Average ergovaline levels during April to August ranged between 0.39 and 0.74 mg/kg DM (average 0.60 mg/kg DM) in the EI pasture and was non-detectable in the NE pasture. Body weight (gain) and body temperature were not affected, and no differences were observed in scrotal circumference. Sperm concentrations and percentage of live sperm were not different, but the percentage of motile, rapid and progressive sperm was lower in bulls grazing EI pasture ($p < 0.06$). Prolactin levels were similar in April but three- to five-fold lower in May through August.

Johnson et al. (2012) compared the performance of growing steers on two types of late maturing tall fescue grasses infected with non-toxic endophyte with toxin-containing KY-31 and an endophyte-free grass. Pastures were grazed in two subsequent years, during May to July and April to June. The concentration of ergovaline/–inine was only determined at one time point in each year, beginning of June, being respectively, 0.26 and 0.54 mg/kg DM in KY-31. For the three non-toxic fescues they were less than 0.01 and 0.03 mg/kg DM in the first and second year. ADG was reduced by the treatment, being 0.63 for KY-31, as compared to 0.84, 0.81 and 0.81 kg/day for the non-toxin-containing grasses. In addition, rectal and skin temperature were significantly increased. Serum prolactin levels were 2- to 3-fold lower.

Shoup et al. (2016) investigated the effect of grazing on tall fescue with and without EA-producing endophytes during late gestation and on the progeny. Eighty 6-year-old pregnant Angus×Simmental cows were assigned to different lots. The average EA content of the grass was reported to be 1.789 mg/L, but it is unclear how to interpret this level. There was no effect on body weight, body condition score (BCS) or milk production, but respiration rate and hair coat score (HCS; implying more hair) were increased, and serum prolactin levels strongly decreased, due to the exposure. Calf birth weight and pregnancy rate were not affected.

Baldwin et al. (2016) treated three groups of eight pregnant Holstein cows with a diet containing 10% contaminated tall fescue seed (group 2) or 10% non-contaminated tall fescue seed, in which one group served as control (group 1) and one (group 3) received subcutaneous injections with bromocriptine (synthetic dopamine agonist), 3 times per week (100 µg/kg bw per injection). Based on dry matter and EA intake, the contaminated ration was calculated to contain ergovaline/–inine at a concentration of 0.53 to 0.64 mg/kg DM, amounting to 7.7 to 9.9 µg/kg bw per day. Treatment started on day 90 prepartum and ended 10 days postpartum. The group receiving contaminated seed showed a significant decrease in dry matter intake during the whole period. For the group injected with bromocriptine, this decrease was smaller and not significant during postpartum. Based on these results, the authors concluded that the reduction in feed intake is not due to a change in the palatability of the contaminated seed. There was also a 62 and 67% reduction in milk production of groups 2 and 3, respectively, at least partly caused by the reduced feed intake. Serum prolactin levels before calving were 85 and 90% reduced in the two treatment groups. The surge around parturition was reduced by 63 and 95% for groups 2 and 3, respectively. There was no effect on the development of the mammary gland based on a set of markers and histological examination, and also no effect on gestation length, milk composition and body temperature. There was a slight increase in milk production when the treatment ended.

Liebe and White (2018) performed a meta-analysis on reductions in the growth rate of cattle when grazing tall fescue pastures, based on 20 studies with a total of 138 treatments, including steers, heifers and cows (including many of the studies described above). Many of the studies compared the effects of animals kept on grass infected with toxin-producing endophyte with novel grass varieties not containing the toxins. ADG values from the different studies were compared with the alkaloid levels. Their model predicts a decrease in average daily gain of 39 and 33 g per day per increase of 0.1 mg/kg of, respectively, total EAs (most likely ergovaline plus ergovalinine but not clearly stated) or ergovaline, with a threshold of 0.06 mg/kg for ergovaline only. There was also an association with endophyte content with a decrease of 47 g per day for each increase of 10% endophyte content and a threshold of 11%, but this association was less good. Increasing temperatures decreased the growth rate when levels exceeded the threshold. The Panel noted that only nine of the studies reported on ergovaline levels in the grass.

Davis et al. (2023) showed that red clover leaf could ameliorate the effects of ergovaline/–inine in mature Holstein steers (bw 486 ± 6 kg). When focussing on the group without red clover, the three animals received a dose of 10 µg/kg bw per day for days 0 to 7 followed by 15 µg/kg bw per day for days 8 to 14. This resulted in a decreased caudal luminal area due to vasoconstriction of 55, 27, 75 and 80% on days 1, 7, 11 and 14 of the treatment. In addition, serum prolactin levels decreased with 36 and 80% on days 7 and 14. Feed intake was not affected. Based on the reported average feed intake of 2.6% of the body weight, the overall feed intake was 12.6 kg DM per day and the extrapolated ergovaline/–inine concentrations for the two doses 0.39 and 0.58 mg/kg DM.

Alfaro et al. (2023) investigated the effects of ergovaline in ergot-sensitive and -insensitive pregnant cows (Angus×Simmental) and the effect of niacin. Animals received a dose of 10–20 µg ergovaline/kg bw per day for 30 days and showed a strong reduction in prolactin levels. However, no unexposed animals were included to evaluate the effect of ergovaline on growth and milk production.

Summary

A large number of studies was performed to investigate the effects of pastures containing tall fescue infected with endophyte producing ergovaline/–inine, often in comparison with grass containing a novel endophyte not producing the toxin or endophyte-free grass. Most studies show effects like reduced growth or poor hair coat scores. The lowest ergovaline/–inine concentration showing an effect was 0.3 mg/kg DM. This type of studies does not include different levels which would allow the derivation of a NOAEL. Applying a factor 3 to the 0.3 mg/kg DM to derive a NOAEL would result in an RP for adverse effects of 0.1 mg/kg DM. This is close to the level of 0.06 mg/kg DM derived by Liebe and White (2018) based on a meta-analysis of most of the studies described above. This RP is rather similar to that derived for ergot alkaloids from *C. purpurea* and lower than that for ergots from *C. africana* (Table G.1).

TABLE G.1 Studies on adverse effects of ergovaline/–inine on bovines.

N⁵/group, breed gender	Exposure conditions	Endpoint(s)	Concentration with adverse effects (mg/kg feed)	Reference
48 pairs of cows (16 per treatment) (Angus and Simmental × Angus)	Pastures (3 types of grass)	Decreased body weight, scores for hair coat, respiration and condition, milk production in cows Decreased body weight calves	0.15–0.51 (average 0.3)	Peters et al. (1992)
Steers (Hereford × Angus), 21 per treatment (part 1) rumen cannulated steers (part 2)	Controlled study with straw, 84 days. 0, 0.16, 0.32, 0.48 mg/kg DM Part 2, 7 days	No effect on intake, body weight and gain to feed ratio No effect on heart and respiration rate, body temperature	–	Stamm et al. (1994)
Angus crossbred cattle (steers and heifers)	pastures	Reduced ADG, increased body temperature	0.82 to 1.21 (average 1.0)	Parish et al. (2003)
Angus purebred cows	pastures	No effect on calving rate, but reduced birth weight, ADG of cows and calves Low body condition scores calves	0.45	Watson et al. (2004)
Crossbred steers (72, 18 per type of grass, breed not provided)	pastures with different types of grass	Reduced ADG, reduced hair scores, increased respiration rate and rectal temperature	0.4 to 2.3	Nihsen et al. (2004)
Angus beef steers	pastures	Reduced ADG, hair coats affected	0.02 to 0.52	Brown et al. (2009)
Angus steers and heifers	pastures, winter period	No effect on ADG or body condition score		Drewnoski et al. (2009)
Angus × Brangus heifers	Concentrates plus alfalfa; 0, 0.52, 1.17 mg/kg DM	Reduced feed intake at highest dose, increased vasoconstriction at both doses, decreased heart rate at highest dose	0.52	Aiken et al. (2009)
Eight, one-year-old Brahman-influenced bulls, two group	Ergovaline: 0; range 0.39 and 0.74 mg/kg DM (average 0.60 mg/kg DM). For 121 days.	No effects on BWG. Reduced % in motile, rapid and progressive sperm. Lower prolactin levels in the hotter months (temperatures between 34 and 40°C)	0.39 to 0.74	Looper et al. (2009)
Crossbred (predominantly) Angus steers	Pastures, 0.26 to 0.54 mg/kg DM	Reduced ADG, increased body temperature	0.26 to 0.52	Johnson et al. (2012)
Eighty 6-year-old pregnant Angus × Simmental cows (8 groups with 10 cows each; 4 groups per E+ or NOV treatment)	1.789 mg EAs /L from day 110 prepartum to day 23 postpartum (E+ diet)	body weight, body condition score, milk production not affected, but respiration rate and hair coat increased, serum prolactin levels decreased	1.79 mg/L but unclear how to interpret this	Shoup et al. (2016)
24 pregnant Holstein cows (three groups with eight cows each)	0.53–0.64 mg ergovaline/–inine/kg DM from day 90 prepartum to day 10 postpartum	Reduced feed intake, milk production, serum prolactin levels, no effect on gestation length and body temperature	0.53 to 0.64	Baldwin et al. (2016)
12 Holstein steers (four mineral treatments with three steers each, no control group)	0.39 (day 0–7) and 0.58 (day 8–14) mg ergovaline/–inine/kg DM in all four groups (14 days in total at 22°C)	Serum prolactin levels and vasoconstriction reduced; feed intake not affected	0.39 to 0.54	Davis et al. (2023)
28 Angus × Simmental pregnant cows (four groups with seven cows each, no control group)	10–20 µg ergovaline/kg bw / day	Serum prolactin levels reduced		Alfaro et al. (2023)

Sheep

Duckett et al. (2014) studied fetal growth of lambs during maternal exposure to ergot alkaloids during gestation. Pregnant ewes ($n=16$, bw at mating 70 kg) were randomly assigned to one of two total mixed rations (TMR) containing endophyte-infected (*N. coenophialum*) tall fescue seed (E+) or (2) endophyte-free tall fescue seed (E-). The TMR of the E+ group contained 0.8 mg ergovaline/-inine per kg DM. The TMRs E+ and E- were provided to ewes from day 35 of gestation until parturition. Blood serum prolactin concentration was lower in E+ compared to E- at days 50 and 130. E+ ewes had approximately 4 days shorter gestation length than E- controls. Birthweight of E+ lambs was reduced by 37% compared to E-. Organ and muscle weight of male lambs ($n=8$ /group), euthanised about 12 h after birth, were also smaller for E+ than E-. Exposure to ergot alkaloids in utero reduced fetal growth and muscle development and altered skeletal muscle formation by reducing the ratio of secondary to primary myofibers.

The research group of Duckett has subsequently published 6 papers, 5 of them characterised by a common basic design, but with different endpoints examined. Pregnant ewes were exposed to 1.77 mg ergovaline/-inine per head and day by a TMR containing endophyte-infected tall fescue (E+); the control group (E-) received the same TMR with endophyte-free tall fescue (E-). The TMR consisted mainly of corn grains, cottonseed and soybean hulls and molasses.

Britt et al. (2019) studied in a 2×2 factorial design how exposure to endophyte-infected or endophyte-free tall fescue seed fed during two stages of gestation (MID, days 35–85; LATE, days 86–133) alters placental development. The groups were in MID and LATE gestation E- and E-, E- and E+, E+ and E-, and E+ and E+, respectively. Thirty-six, fescue-naïve Suffolk ewes (83.3 kg bw) were randomly assigned to the groups and fed individually with equal amounts of TMR (and seed) within each group daily. The E+ TMR contained in MID gestation 0.93 mg and in LATE gestation 0.73 mg ergovaline/-inine/kg DM (corresponding to a daily feed DM intake of approximately 1.9 kg and 2.4 kg in the MID and LATE period, respectively). Ewes fed E+ TMR had reduced serum prolactin levels during the periods when fed E+. Average daily gain was reduced by 64% for the E-/E+ group during LATE gestation and tended to be reduced by 33% overall. Ewes fed E+ during LATE gestation exhibited a 14% and 23% reduction in uterine and placentome weights, respectively. Total fetal weight per ewe was reduced for ewes fed E+ during LATE gestation compared with E-. The results suggested that exposure to ergot alkaloids during LATE (days 86–133) gestation has the greatest impact on placental development by reducing uterine and placentome weights.

The study of Greene et al. (2019) is based on the same animals as described above, fetal growth, muscle fibre development and miRNA transcriptome were examined. Foetuses from ewes exposed to E+ seed during LATE gestation had reduced bw by 10% compared with E- foetuses. Foetuses from ewes fed E+ seed during MID and LATE gestation tended to have smaller liver and kidney weights compared with E-. Fetal brain weight did not differ by fescue treatment during both gestation periods. The percentage of brain to empty body weight was greater in foetuses from ewes fed E+ fescue seed during LATE gestation. This work supports the hypothesis that E+ tall fescue seed fed during late gestation reduces fetal weight and causes asymmetrical growth, which may be indicative of intrauterine growth restriction.

The study of Britt et al. (2021) is also based on the same animals as described above. Because uteroplacental malperfusion is linked to altered placental development, the objective of the study was to examine the impact of gestational EA exposure from E+ seed on microscopic placental structure and vascular development. On day 133 of gestation, a terminal surgery was performed and two placentomes of the type B morphology were collected for microscopic analyses for each animal. Amorphous connective tissue regions were larger and more numerous in the placentome of EA-exposed ewes. Staining showed no difference in the number of vessels present, but luminal area of maternal vasculature was 117% greater in EA-exposed ewes. Results showed that exposure to EAs during gestation slowed maturation of the fetal villi as indicated by greater amorphous connective tissue regions and altered size and shape of blood vessels to counteract reductions in blood flow caused by vasoconstriction.

The objectives of another experiment (Britt et al., 2020) were to (i) determine if the dopamine receptor D2 (DRD2) genotype is associated with response to E+ and (ii) examine how feeding tall fescue seeds containing ergovaline/-inine in the second and third trimester of gestation modifies ewe performance, lactation and growth of offspring. Pregnant Suffolk ewes (final $n=52$) were genotyped for the dopamine receptor D2 (DRD2) and stratified by genotype (AA, AG, GG) and stage of gestation (MID, LATE), and fed TMR E+ or TMR E-. Group size for E- was 24, for E+ 28 ewes. Feeding TMR E+ lowered serum prolactin (but did not differ by maternal DRD2 genotype) and carotid luminal area. Lamb birth weight was lower in ewes fed TMR E+ in LATE gestation period. Pre-weaning growth rate, milk production and total weaning weight was reduced in ewes fed TMR E+ during MID and LATE gestation. Ingestion of ergovaline/-inine in the LATE period reduced lamb birth weight; however, lamb growth rate, milk production and total weaning weight were reduced in all ewes fed TMR E+ during both periods of gestation.

The study of Greene et al. (2020), based on the same animals as described above, aimed to evaluate post-weaning growth, puberty attainment and carcass quality in lambs that were exposed to E+ or E- tall fescue seed during different stages of gestation. Weaned lambs ($n=82$), born from ewes fed E+ or E- TMRs during MID and LATE stage of gestation were used. Lambs were weaned at 75 days of age and separated by sex to assess puberty in ewe lambs ($n=39$) until approximately day 215 post-weaning and to evaluate growth, carcass and meat quality in wethers until day 185 post-weaning ($n=43$). Age at puberty tended to be higher for ewe lambs born to dams fed E+ during LATE gestation vs. those fed E-. Post-weaning ADG tended to be higher for lambs born to dams fed E+ fescue seed during MID gestation compared to E-. Exposure to ergot alkaloids during fetal growth caused weight decrease of the longissimus muscle and its alteration in colour, alteration of the lipid deposition and fatty acid composition, and increase in shear force values of semimembranosus muscle in wether lambs.

A study from Chai et al. (2020) involved Katahdin ewes to investigate the rumen microbiota from gestation and lactation in ewes grazing tall fescue pastures. No control group without exposure to ergovaline was included. Since the concentration of total ergot alkaloids in the pasture increased throughout the study, accurate levels of ergovaline in tall fescue were not available. This study was not considered further for the scope of this Opinion.

The prevention of toxic effects of endophyte-infected tall fescue silage by condensed tannins (CT) in non-lactating, non-pregnant ewes was the focus of the study of Grote et al. (2023). Ewes ($n = 20$; 57 kg bw) were allocated to four groups given diets with either a novel endophyte (NE) or toxic endophyte-infected tall fescue silage (E+) with no CT (ECT0), 10 g CT (ECT10) or 30 g CT/kg silage DM (ECT30) for ad libitum access. The ergovaline content of the E+ diets was 0.193 mg/kg. Ergovaline concentrations in forages were sufficient to reduce serum prolactin levels in all treatments with E+ tall fescue. There was no influence of the treatment on body weight. The only significant difference in DM intake was between the NE and the ECT30 group, which was the lowest. Digestibility of the organic DM was lower for all E+ groups, the intake of digestible DM and of digestible organic matter per kg bw was significantly lower for all E+ groups compared to NE with a tendency for lowest values in the ECT30 group. Comparable data were obtained for N-absorption and retention. It can be concluded that endophyte-infected tall fescue silage with an ergovaline content sufficient to depress prolactin serum levels does not depress body weight, DM intake and nutrient digestibility.

In the study of Duckett et al. (2014) 0.8 mg ergovaline/–inine/kg feed DM resulted in adverse effects mainly on the health of the progeny. This concentration is considered a LOAEL from which a NOAEL of 0.3 mg ergovaline/–inine/kg feed DM can be derived by applying an uncertainty factor of 3 and converting to mg/kg complete feed with 88%DM.

The CONTAM Panel derived an RP for adverse effects of 0.3 mg ergovaline/–inine/kg feed for sheep (Table G.2).

TABLE G.2 New studies on adverse effects of ergovaline/–inine on sheep which have become available since the 2012 Opinion (EFSA CONTAM Panel, 2012).

N ^a /group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effects concentration (mg/kg feed or mg/kg feed DM) ^b	Reference
8 pregnant ewes	0.8 mg ergovaline/kg diet DM	Fetal growth during maternal exposure during gestation	Effects at 0.8 mg/kg feed DM	Duckett et al. (2014)
36 pregnant Suffolk ewes (9 groups with 4 ewes per group)	0 (control) or 0.93 and 0.73 mg ergovaline/–inine/kg DM in MID (days 35–85) or LATE (days 86–133) gestation, respectively	Serum prolactin, ADG, placental development, fetal weight	Effects at 0.73 mg/kg feed DM	Britt et al. (2019)
8 pregnant Suffolk ewes	0.35 mg ergovaline/kg feed	fetal growth, muscle fibre formation and miRNA expression	No effects at 0.45 mg/kg feed	Greene et al. (2019)
24/28 Suffolk ewes	0.35 mg ergovaline/–inine/kg feed from day 35 of gestation until parturition	Plasma prolactin, Performance of progeny	No effects at 0.35 mg/kg feed	Britt et al. (2020)
39 ewe lambs, 43 wether lambs	0.35 mg ergovaline/–inine/kg feed 188–199 days of age for ewe lambs, 161–171 days on feed for wethers	Subsequent puberty attainment in ewe lambs or carcass quality in wethers	No effects at 0.35 mg/kg feed	Greene et al. (2020)
3 Suffolk ewes	0.35 mg ergovaline/–inine/kg feed from day 35 of gestation until day 133 of gestation	Microscopic placental structure and vascular development	No effects at 0.35 mg/kg feed	Britt et al. (2021)
16 pregnant ewes	0 or 0.8 mg ergovaline/–inine/kg DM from gestation day 86 to 110 or 133	Placental structure, fetal development, serum prolactin	Reduced serum prolactin concentrations on day 110 and 133 at 0.8 mg/kg feed DM	Britt et al. (2021)
20 ewes	E+ diets 0.193 mg ergovaline/kg	Serum prolactin levels. BW, DM intake and nutrient digestibility	No effects at 0.193 mg/kg feed	Grote et al. (2023)

^aIncluding the number of animals in the control group.

^bIn studies where only one dose was used and effects observed, the concentration was considered as 'concentration with effects', not necessarily a LOAEL.

Horses

In 2005 ergovaline exposure was reported by the EFSA CONTAM Panel as being associated with various effects like delayed parturition, agalactia of mares (associated with reduced prolactin levels) and incidentally neurotoxic symptoms (Cross et al., 1995). Furthermore, mares appear to be sensitive to ergovaline at levels as low as 0.05–0.1 mg/kg. Craig et al. (2014) have stated that: 'for horses, ergovaline levels in grass should be below 0.3–0.35 mg/kg feed DM. During cold

periods these levels may actually have to be lower'. The authors also stated that 'brood mares should have no detectable ergovaline in their feed'. The LOD for ergovaline of the LC-FLD method used was 0.031 mg/kg, the LOQ 0.1 mg/kg. No study was provided related to this conclusion, however in the 2012 Opinion, there was a reference to a website of the Ministry of Agriculture, Food and Rural Affairs in Ontario, Canada, but not containing a reference to underlying studies. The effects in pregnant mares are associated with interference with the normal rise of progestagens (mainly 5-alpha-pregnanes) and prolactin in the later stages of pregnancy, and foals born without the normal increases in maternal progestagens suffer from hypoadrenocortical function and are small, weak or stillborn (Brendemuehl et al., [1995](#)). There may also be a decreased transfer of immunoglobulins via the milk.

APPENDIX H

New studies on occurrence data in feed within the EU published since the 2012 Opinion

TABLE H.1 New studies on occurrence data in feed within the EU published since the 2012 Opinion (EFSA CONTAM Panel, 2012).

Feed	Compounds analysed for	Positive/analysed samples	Information on the concentration	Analytical method	Additional information	Reference
Composite feed (11), corn and grass silage (9)	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	13/19	Range: 1–1145 µg/kg	LC–MS/MS; LOQ range: 0.15–0.96 µg/kg	Sampled in Belgium	Diana Di Mavungu et al. (2012)
Pig feed	Ergosine, ergotamine, ergocornine, ergocryptine, ergocristine	27/not reported	Range of average concentrations: 352–694 µg/kg	LC–FLD; LOQ not reported	Sampled in Austria	Weissenbacher-Lang et al. (2012)
Wheat, barley and rye	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	52/52	Range: 10–2053 µg/kg	LC–MS/MS; LOQ: 10 µg/kg	Sampled in UK on rejected grain deliveries for presence of sclerotia	Slaiding et al. (2013)
Commercial feed for rabbits	Total ergot alkaloids	44/44	140–1700 µg/kg	EIA; LOD: 50 µg/kg	Sampled in Germany	Korn et al. (2014)
Rye (157), wheat (137), triticale (27)	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	130/321	1–12,340 µg/kg	LC–MS/MS; LOQ: 1 µg/kg	Sampled in Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Poland, Sweden, Switzerland, the Netherlands	Malysheva et al. (2014)
Hay, feeding cereals (wheat, barley, maize, oat, maize silage), clover-grass and alfalfa forage, non-fermented/fermented feedingstuffs and complex compound feeds intended for feeding of various groups of animals	Agroclavine, ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine	17 ^a /343	LOQ-228 µg/kg	LC–MS/MS; LOQ range: 2.5–20 µg/kg	Sampled in Czech Republic and UK	Zachariasova et al. (2014)

(Continues)

TABLE H.1 (Continued)

Feed	Compounds analysed for	Positive/ analysed samples	Information on the concentration	Analytical method	Additional information	Reference
Sclerotia of wild and cultivated infected grass	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	39/39	59–4200 mg/kg sclerotia	LC–MS/MS; LOQ range: 0.1–1 mg/kg	Sampled in Slovenia	Likar et al. (2018)
Feed for sows and wheat (intoxication case)	Ergosine, ergocornine, ergocryptine, ergocristine, ergotamine, ergometrine	3/3	Range: 3490–16,060 µg/kg	LC–MS/MS; LOQ not reported	Sampled in France	Waret-Szkuta et al. (2019)
Wheat (206), rye (35), triticale (101), oat (16), spelt (23) and barley (136)	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	83/507	14–4217 µg/kg	LC–MS/MS; LOQ: 1 µg/kg	Sampled in Slovenia	Babic et al. (2020)
Winter rye	Ergosine/–inine, ergocornine/–inine, α-ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	347/372	800–37,640 µg/kg	LC–FLD; LOQ: 20 µg/kg	Sampled in Germany, Austria and Poland	Kodisch et al. (2020)
Rye, triticale, wheat, mixed cereal grains and compound feeds	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	522/600	20–61,951 µg/kg	LC–FLD and LC–MS/MS; LOQ range: 1–20 µg/kg	Sampled in Germany	Schwake-Anduschus et al. (2020)
Feed for pigs	Ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	29/228	6.3–158.7 µg/kg	LC–MS/MS; LOQ range: 2.1–21.7 µg/kg	Sampled in Spain	Arroyo-Manzanares et al. (2021)
Complete diet of lactating cows (partial mixed ration (87%) and exclusively forage-based mixed rations (11%), as well as total mixed rations (2%))	Chanoclavine, festuclavine, ergosine/–inine, ergocornine/–inine, ergocryptine/–inine, ergocristine/–inine, ergotamine/–inine, ergometrine	64/198	Range: 0.95–219 µg/kg DM	LC–MS/MS LOQ not reported	Sampled in Austria	Penagos-Tabares, Khiaosard, et al. (2022)
Wet spent brewery grains for dairy cow feeding	Ergosine/–inine, ergocornine, ergocryptine, ergocristine/–inine, ergotamine/–inine, ergometrine/–inine	19/21	14–210 µg/kg	LC–MS/MS; LOQ range: 0.2–3.8 µg/kg	Sampled in Austria	Penagos-Tabares, Sulyok, et al. (2022)

^aEstimated.

APPENDIX I

Information on relative potency

In the current Opinion the CONTAM Panel assumed equal potencies of the different EAs in the absence of data to derive Relative Potency Factors (RPFs). However, several authors used *in vitro* models to study the effects of one or more EAs on the contractile response of isolated muscle or blood vessels (Klotz et al., 2006, 2007, Klotz et al., 2008, 2009, 2010; Klotz & Smith, 2015; Klotz & McDowel, 2017; Foote et al., 2011; Egert et al., 2014; Pesqueira et al., 2014; Cherwyk et al., 2020, 2022). Craig et al. (2015) summarised the data from the studies by Klotz et al. (2006, 2007 & 2010) on a number of different EAs tested on bovine lateral saphenous vein tissue. Based on the dose–response curves, EC50 values were derived, i.e. the concentration showing a half-maximal response. Using ergocristine as the reference compound (tested in most studies), relative potencies can be calculated by dividing the EC50s by that of ergocristine. Factors below 1 suggest a lower potency than ergocristine. As shown in Figure 1, most EAs tested showed potencies within a factor 2, except ergocornine and lysergic acid (Figure I.1).

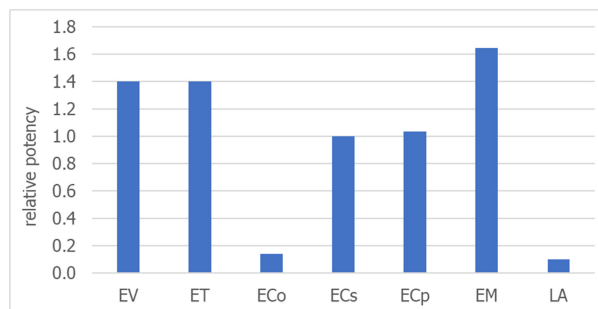


FIGURE I.1 Relative potencies for the effect of EAs in the bovine lateral saphenous vein bioassay (Craig et al., 2015). EV, ergovaline; ET, ergotamine; ECo, ergocornine; ECs, ergocristine; ECp, ergocryptine; EM, ergometrine; LA, lysergic acid.

Foote et al. (2011) used a bioassay based on bovine right ruminal arteries or veins. Figure 2 shows the relative potencies for the investigated EAs, suggesting that ergovaline is much more potent than the other EAs in the ruminal vein. In the ruminal artery the differences between the EAs tested were relatively small (Figure I.2).

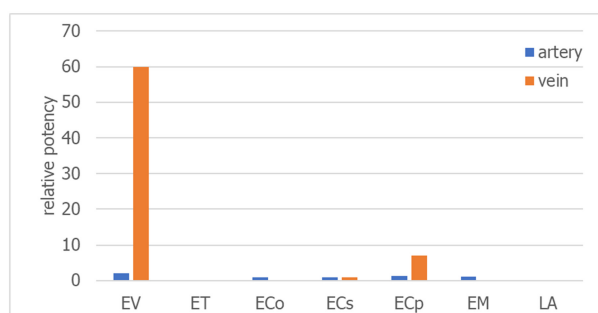


FIGURE I.2 Relative potencies for the effect of EAs in the bovine ruminal artery and vein bioassays (Foote et al., 2011). EV, ergovaline; ET, ergotamine (not tested); ECo, ergocornine (not active in vein assay); ECs, ergocristine; ECp, ergocryptine; EM, ergometrine (not active in vein assay); LA, lysergic acid (not active in both assays).

Egert et al. (2014) used a bioassay based on bovine mesenteric arteries or veins. Figure I.3 shows the relative potencies for the investigated EAs, suggesting that ergovaline is much more potent than the other EAs in the mesenteric vein.

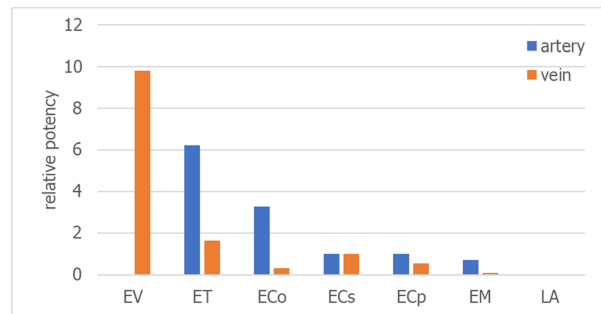


FIGURE I.3 Relative potencies for the effect of EAs in the bovine mesenteric artery and vein bioassays (Egert et al., 2014). EV, ergovaline (not tested in the artery assay); ET, ergotamine; ECo, ergocornine; ECs, ergocristine; ECp, ergocryptine; EM, ergometrine; LA, lysergic acid (not included in this study).

Klotz and McDowell (2017) investigated the effects of a number of EAs on medial palmar artery and vein tissues from horses. Figure I.4 shows the relative potencies based on this study. Ergotamine showed a higher potency compared to the other five EAs in the artery assay. Differences between the EAs were relatively small in the vein assay.

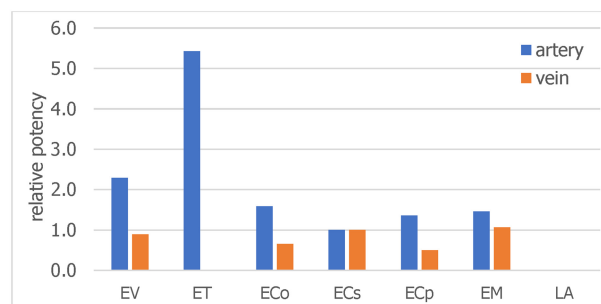


FIGURE I.4 Relative potencies for the effect of EAs in the equine medial palmar artery and vein bioassays (Klotz & McDowell, 2017). EV, ergovaline; ET, ergotamine; ECo, ergocornine; ECs, ergocristine; ECp, ergocryptine; EM, ergometrine; LA, lysergic acid. LA was not included in this study and ET was only tested on arteries.

Cherewyck et al. (2020) used a bovine metatarsal artery bioassay to investigate the effects of a number of -inines, i.e. ergotaminine, ergocorninine, ergocristinine and ergocryptinine. Ergotaminine was found to be slightly more potent than the other 'inines'. In a follow-up study Cherewyck et al. (2022) used the same assay to compare the effects of ergocristine and its -inine. Ergocristine was found to be approximately four times more potent than its epimer.

Although these data show differences in the potencies of EAs, the CONTAM Panel concluded that more data are required to derive RPFs for EAs. This should also take into account potential differences in the toxicokinetics of EAs.

APPENDIX J

Uncertainty tables

TABLE J.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 ^a
Chemical composition and analytical methods	Chemical composition	Is there uncertainty associated with the dose in the critical studies used in the risk assessment?	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	Not always all relevant EAs were analysed, particular in case studies	1
			Presence of other ergot alkaloids	Feeds can be contaminated by other EAs than the 14 that we consider, and EAs from other sources. Although this would impact both RPs and occurrence in the same direction	1
			Naturally contaminated materials resulting in co-exposure to other compounds (e.g. other mycotoxins)	Presence of other mycotoxins in the feed materials often not investigated	2
	Analytical methods		Lack of certified reference materials, proficiency tests and method validation	n/a	
		Other:	Some studies used only qualitative/semi-quantitative methods for ergovaline/-inine or total EA content. These studies were not used to derived RPs	0	

(Continues)

TABLE J.1 (Continued)

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 ^a
Hazard identification and characterisation	ADME	Is there uncertainty in any aspect of ADME in the various animal species?	Insufficient information on absorption of the different congeners	Information on absorption is limited	0
			Accumulation potential (e.g. duration of studies, sample size, sex, number of studies, direct measurements, biomarkers, metabolites)	Inconsistent information on ability of EAs to cross the BBB. Individual variation in concentrations is significant	0
			Confounders (e.g. effects of other chemicals that may affect the ADME of the tested compounds)	Not relevant	0
			Elimination	Urinary excretion appeared predominant in cattle. Ruminant microflora metabolism plays a role in ruminant excretion profile	0
			Little information on transfer rate to animal products	Transfer to animal products (milk, meat) seems low, but information is limited. This is unlikely to influence the RA for animal health	0
	Toxicity studies: critical endpoints and critical study design	Are there sources of uncertainties in the design of the studies?	Insufficient information on the extent of metabolism of the parent compounds (degradation/hydrolysis/reduction/other reactions)	Information on metabolism of parent compounds is limited	0
			Focus on zootechnical parameters, which might not identify other endpoints	Some effects might be overlooked	2
			Effects of temperature and climate conditions are not systematically considered in the study design	Case studies indicate a strong impact of extreme weather conditions (very low or very high temperatures) on the adverse effects in animals	3
	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 with other substances	In certain tox studies, the exposure to EAs happens together with other mycotoxins. A special case is the co-occurrence of EAs with the tremorgenic mycotoxin Lolitrem B in perennial ryegrass. These studies have not been used to derive RP for <i>C. purpurea</i> for animal health	1
			Relative potency of the EAs is unclear	There are indications from in vitro studies that some EAs are more potent than others, however ranking them is not possible at this stage	3
Interaction/combined effects between congeners			For the scope of the assessment, effects of the different EAs have been considered as additive	1	
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	Vasoconstriction and hypoprolactemia are highly relevant factors in EA toxicity in livestock. The influence of hyperthermia is clearly indicated. There is a high heterogeneity of the involvement of serotonergic, adrenergic and dopaminergic receptors in the MoA	2	

TABLE J.1 (Continued)

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 ^a
	Selection of reference point	What are the uncertainties in the use of NOAEL/LOAEL due to lack of appropriate BMDL?	Uncertainty factors used, dosing intervals etc	When no NOAEL could be determined then a LOAEL was established and an uncertainty factor of 3 was applied. The use on an UF of 3 is in line with EFSA's and ECHA's guidances	1
				For pigs, the dose interval was large, resulting in a conservative RP	2
		Further sources of uncertainty?	Effects of temperature and climate conditions on the RP	Case studies indicate a strong impact of extreme weather conditions (very low or very high temperatures) on the susceptibility of animals. Different RPs could potentially be identified for different climate conditions	3
	Dose-response analysis of critical endpoints	Is there uncertainty regarding the dose-response analysis e.g. trend occurrence, large data variation, possible covariants?	Lack of raw data points	n/a	n/a
				No dose-response relationship/not well defined	n/a

^a0 – U with negligible priority.

1 – U with low priority.

2 – U with medium priority.

3 – U with high priority.

TABLE J.2 Occurrence and exposure.

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty ¹	
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).	LC–MS/MS and LC–FLD are methods with sufficient selectivity	1	
			Analytical capability of the method - sensitivity (e.g. LOQ, LOD)	Not always all relevant EAs included in analytical method Older data may have higher LOQs	2	
			Consideration of recovery (e.g. correction carried out or not)	It is not always reported whether results were corrected for recovery	1	
			Lack of certified reference materials and proficiency tests	Proficiency tests for EAs from <i>Claviceps purpurea</i> are nowadays in place	1	
	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, of the compound, unit of measurement, parameter, fat vs. whole weight, etc.) – unidentified errors (not apparent from the data provided), missing information in reporting the occurrence data (e.g. analytical method, moisture content)	For some feed samples, information is missing e.g. correct classification of feed category. Some very high concentrations were observed. Some of these aspects could not be clarified by data providers	1	
			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 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	The data providers were contacted with the aim of clarifying the unclear aspects. Data were only used when these aspects could be clarified	0
	Representativeness and completeness of the data	Is there uncertainty in the occurrence data due to limited data availability?	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
			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	Little/no data available for EAs in forage, hay, grass Limited data available for EAs in compound feeds	2	
			Low number of reporting countries	Eight countries submitted data to EFSA. Around 45% of the samples were collected in United Kingdom, followed by Czech Republic (~25%) and France and the Netherlands (both ~10%)	2	
	Multiple chemicals and metabolites	Is there uncertainty in the occurrence data due to that not all relevant substances are reported?	Not optimal distribution of year of samplings (e.g. too many old data)	Apart from 2013, the sampling was, overall, equally distributed across the different years, with 2017 being the year with the highest number of samples ($n=216$)	1	
			Limited data on the co-occurrence for the chemicals belonging to the group of interest	Some minor EAs (other than the 12 EAs) are not included in the analysis. Contribution to the total EA content in general is small	1	
	Left censorship	Is there uncertainty in the occurrence data due to left censorship and the substitution method?	Missing information on metabolites or degradation products	Metabolites and degradation products are not considered in the analysis but expected to be present only in low relative concentrations	1	
High percentage of left-censored data			High amount of LC data skewed the data distribution causing the highest representative percentile to be lower than the mean for certain feed categories. In these cases, only the mean was used to calculate exposure	2		

TABLE J.2 (Continued)

Main group	Sub-group	Overarching questions	Description of uncertainty	Sources of uncertainty in the opinion	Priority ranking of the Uncertainty ¹
Animal diets	Representativeness of the data	Is there uncertainty in the animal diets (e.g. feed materials)?	Unidentified errors in the animal diets	The diets as they are designed, are aimed at following the current common practices. The use of compound feeds (when possible, on the ground of data availability) allows to provide two scenarios of exposure	1
			Body weight and feed intake of the animals	Body weight and feed intake were recently aligned to FEEDAP Panel and 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?	The methodology follows the use of default BWs and feed intake, and example diets due to the lack of a comprehensive feed database in EU	The methodology could underestimate overestimate the actual exposure nevertheless the approach is aimed at providing a conservative estimate	2

ANNEXES

Annex A – Protocol for the development of the opinion

The protocol undertaken for the scientific development of this Opinion can be found in the online version of this output ('Supporting information' section) at: <https://doi.org/10.2903/j.efsa.2024.8496>

Annex B – Raw occurrence data

The occurrence data in feed extracted from EFSA Data Warehouse for the period from 2012 to 2021 are on EFSA's Knowledge Junction Community on Zenodo at: (link: <https://doi.org/10.5281/zenodo.10474207>.)

Annex C – Occurrence samples in dry matter

EA concentrations at Feed expressed in based on dry matter are available on EFSA's Knowledge Junction Community on Zenodo at: (link: <https://doi.org/10.5281/zenodo.10474207>.)