SCIENTIFIC OPINION

Scientific Opinion on Ergot alkaloids in food and feed[1]

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

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ABSTRACT

The European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion on ergot alkaloids (EAs) in food and feed. EAs are produced by several members within the fungal orders of Hypocreales and Eurotiales. In Europe, *Claviceps purpurea* is the most widespread *Claviceps* species within the Hypocreales. A total of 20 558 analytical results for EAs in 1 716 food, 496 feed and 67 unprocessed grain samples were considered in this opinion. Based on the EAs identified in sclerotia of *C. purpurea*, and recent literature data, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) based its risk assessment on the main *C. purpurea* EAs, namely ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (which is a mixture of α- and β- isomers), ergocornine, and the corresponding –inine epimers. The CONTAM Panel performed estimates of both chronic and acute exposure for various age groups across European countries. A $BMDL_{10}$ of 0.33 mg/kg b.w. per day was calculated for the incidence of tail muscular atrophy in a 13-week rat feeding study of ergotamine. This effect was considered representative of the vasoconstrictive effects of EAs and provided a suitable reference point for establishment of a group acute reference dose of 1 µg/kg body weight (b.w.) and a group tolerable daily intake of 0.6 µg/kg b.w. per day. The Panel concluded that whilst the available data do not indicate a concern for any population subgroup, the dietary exposure estimates relate to a limited number of food groups and a possible unknown contribution from other foods cannot be discounted. Estimates of exposure for livestock based on example diets and levels of EAs in cereal grains reported suggest that under normal conditions the risk of toxicosis is low.

KEY WORDS

Ergot alkaloids (EAs), origin, chemistry, analysis, exposure, risk assessment, health-based guidance value

[1] On request from the European Commission, Question No EFSA-Q-2010-01000, adopted on 28 June 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on alkaloids: Till Beuerle, Diane Benford, Leon Brimer, Bruce Cottrill, Daniel Doerge, Birgit Dusemund, Peter Farmer, Peter Fürst, Hans-Ulrich Humpf and Patrick Mulder for the preparatory work on this scientific opinion and EFSA staff: Marco Binaglia, Alessandro Carletti, Gina Cioacata and Jose Angel Gomez Ruiz for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798. [158 pp.] doi:10.2903/j.efsa.2012.2798. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the risks to human and animal health related to the presence of ergot alkaloids (EAs) in food and feed. EAs are known to be produced by several members within the fungal orders of Hypocreales and Eurotiales. All species of the *Claviceps* genus within the Hypocreales may infest plant species belonging to *Poaceae* (family of the true grasses). The fungal hyphea invade the ovule of the host grass and colonize the whole ovary. Three to four weeks after infection the wintering body of the fungus becomes visible and replaces the kernels of the grain ears. These so-called sclerotia are dark, crescent shaped and protruding from the regular grains of the ear and represent the final stage of the disease. The sclerotia of *Claviceps* species are known as ergot.

Biosynthetically, EAs are classified as tryptophan-derived alkaloids and the physiological effects of this class of compounds have been known since biblical times. In the Middle Ages, the consumption of EA contaminated grains, flour or bread caused severe epidemics of the condition known as St. Anthony's fire. Today, the cause of the disease, called "ergotism" is well understood. The increased scientific understanding and improvements in agricultural practices and milling techniques (grading, sieving and sorting) has eliminated severe epidemic outbreaks of ergotism in the last decades. Besides their role in life threatening epidemic food contaminations, EAs show a broad spectrum of pharmacological effects and were used in medical applications for hundreds of years. EAs and EA-derived compounds were applied or tested for prolactin inhibition, treatment of Parkinsonism, cerebrovascular insufficiency, venous insufficiency, thrombosis, emboli, stimulation of cerebral and peripheral metabolism, and are still applied for migraine and uterine stimulation. More than 50 different EAs have been identified in the past.

In Europe, *Claviceps purpurea* is the most widespread *Claviceps* species. It is known to infect more than 400 plant species, including some economically important cereal grains such as rye, wheat, triticale, barley, millet and oats. Based on the EAs identified in sclerotia of *C. purpurea*, and recent literature data, the CONTAM Panel concluded that chemical analysis should focus on the main *C. purpurea* EAs, namely ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (which is a mixture of α- and β- isomers), ergocornine, and the corresponding -inine epimers. Although the -inine forms are described to be biologically inactive, interconversion occurs under various conditions and thus the CONTAM Panel based its risk assessment on both forms (-ine and -inine) of ergometrine, ergotamine, ergosine, ergocristine, α- and β-ergocryptine, and ergocornine.

Information for other EA-producing fungi, in particular *C. fusiformis*, relevant for pearl millet, and *C. africana*, relevant for sorghum, is limited. Endophyte infections of cool-season grasses, such as tall fescue (*Lolium arundinaceum*) with *Neotyphodium* toxins, are well known and characterised for their toxicity outside Europe, especially for ruminants and horses. However, as there are currently no indications of exposure of livestock to *Neotyphodium* toxins in Europe, the hazards of these toxins in forage crops have not been addressed in this opinion.

At present, only high performance liquid chromatography with fluorescence detection (HPLC-FLD) and high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) allow the determination of individual EAs in food and feed commodities at relevant levels. An HPLC-FLD method for the determination of the relevant *C. purpurea* EAs in grain and flour has been internationally validated. As the epimeric forms of EAs can interconvert, analytical methods should include the determination of both epimeric forms. Screening for ergot sclerotia content in grains to meet current European Union (EU) legislation can be achieved by visual inspection, near infrared hyperspectral imaging or by determination of ricinoleic acid by gas chromatography - flame ionization detector (GC-FID).

Following a European Food Safety Authority (EFSA) call for data on EAs in food and feed, 14 European countries submitted a total of 25 840 results. After a validation and cleaning step,

20 558 analytical results for EAs in 2 279 samples, of which 1 716 corresponded to food, 496 to feed and 67 to unprocessed grains of unknown end-use were considered in this opinion. These data on the occurrence of ergot alkaloids include 803 samples of food and feed obtained through an Article 36 call. All samples were collected between 2004 and 2011.

Almost 60 % of the food samples were sampled in just one Member State. Similarly, for feed around 50 % of the samples were also collected in a different single Member State. Most of the reported food samples were non- or minimally processed foods (mainly grain milling products with 1 193 samples), with only approximately 250 samples of processed food available. The occurrence data on feed were also limited, with the main focus on rye and rye by-products (253 samples) and wheat and wheat by-products (161 samples). Results were available for only a few samples of barley, despite the importance of this cereal as a feed material. Among the food and feed samples, the number of EAs for which data were submitted ranged between 1 and 12 with different limits of detection (LOD) and limits of quantification (LOQ). Around 60 % of the food samples and more than 75 % of the feed samples were left-censored, i.e. below LOD or LOQ.

The occurrence data on EAs in food and feed submitted to EFSA indicated that ergotamine, ergocristine, ergosine and ergocornine are generally more abundant than α- and β-ergocryptine and ergometrine. In order to include the maximum number of samples with a representative number of EAs in the assessments, the occurrence data were selected based on the presence of these four most abundant EAs, and a minimum number of six reported EAs per sample. The highest concentrations of EAs were reported for rye grains, rye milling products and rye by-products.

During processing, in particular baking, the total amount of EAs decreases and the ratio between the epimeric forms in general shifts towards the -inine forms. Milling processes result in redistribution of sclerotia particles in different milling fractions. For products consisting mainly of whole grains and consumed as such, distribution and diluting effects by milling and other steps of grain processing do not apply. In those cases, a single sclerotium could get into a food serving and consequently lead to comparatively high EA-exposure for consumers of those products.

Estimation of human dietary exposure to EAs was highly influenced by the facts that most of the consumption data in the EFSA's Comprehensive European Food Consumption Database refers to processed food, and that a limited amount of occurrence data on these type of foods was available. The chronic dietary exposure in the adult population varied between 0.007 and 0.08 µg/kg body weight (b.w.) per day for average consumers and 0.014 and 0.19 µg/kg b.w. per day for high consumers. The acute dietary exposure in the adult population ranged between 0.02 and 0.23 µg/kg b.w. per day for average consumers, and between 0.06 and 0.73 µg/kg b.w. per day for high consumers.

The highest chronic exposure to EAs (considering minimum lower bound (LB) and maximum upper bound (UB) across European dietary surveys) was estimated in toddlers and 'other children'. For average consumers, the estimated chronic exposure in toddlers ranged between 0.03 and 0.17 µg/kg b.w. per day, and between 0.02 and 0.17 µg/kg b.w. per day in 'other children'. For high consumers, estimated chronic exposure values in toddlers ranged between 0.07 and 0.34 µg/kg b.w. per day, and for 'other children' between 0.03 and 0.30 µg/kg b.w. per day.

Toddlers and 'other children' also showed the highest acute exposure to EAs (considering minimum LB and maximum UB across European dietary surveys). For average consumers, the estimated acute exposure in toddlers ranged between 0.08 and 0.42 µg/kg b.w. per day, and between 0.05 and 0.36 µg/kg b.w. per day in 'other children'. For high consumers, estimated acute exposure values in toddlers ranged between 0.21 and 1.03 µg/kg b.w. per day, and for 'other children' between 0.12 and 0.82 µg/kg b.w. per day.

Those countries with relatively high consumption of rye bread and rolls showed the highest dietary exposures (both chronic and acute) across the different age groups.

The assessment of the dietary exposure to EAs in specific groups of the population (vegetarians and consumers of unprocessed grains) was based on limited data. The results indicated no significant differences between vegetarians and the general population, while consumers of unprocessed grains could have slightly higher dietary exposure to EA than the general population.

Exposure to EAs by livestock and domestic animals is most likely to occur as a result of consuming rations containing cereal grains and cereal by-products, and in particular rye, sorghum and millet and by-products derived from them. Within the EU, rye, sorghum and millet are not widely used as livestock feeds, although where they are grown commercially these feeds may be more extensively used in livestock rations. Contaminated forages present a potential risk in those areas in which environmental conditions support the development of the sclerotia, but husbandry measures are available that reduce this risk.

Data on toxicokinetics are sparse and are mainly limited to those EAs that are used as pharmaceuticals in humans. The available literature suggests that EAs are absorbed from the gastrointestinal tract and subjected to oxidative biotransformation, primarily by cytochrome P450 3A4, and some EAs (e.g., ergometrine) can subsequently be conjugated with glucuronic acid.

Based on LD_{50}s, EAs exhibit moderate oral acute toxicity. Sublethal acute exposure induces signs of neurotoxicity, including restlessness, miosis or mydriasis, muscular weakness, tremor and rigidity. EAs act on a number of neurotransmitter receptors, particularly adrenergic, dopaminergic and serotonergic receptors. On repeated dosing of various EAs, these effects on receptors result in ischaemia, particularly in the extremities, such as the tails of rats, decreased body weight gain and changes in the levels of some hormones. Repeat dose studies in rats demonstrate no major quantitative difference in the toxicity of ergotamine, ergometrine and α-ergocryptine, with no-observed-adverse-effect levels (NOAELs) in the region of 0.22 - 0.60 mg/kg b.w. per day.

EAs have a number of effects on the reproductive process in rodents, including prevention of pregnancy by interfering with implantation, embryotoxicity, and inhibition of lactation. These effects have generally been observed at higher doses than the lowest-observed-adverse-effect levels (LOAELs) in the repeat dose studies.

With the exception of ergotamine, only limited genotoxicity studies have been carried out on naturally occurring EAs. The available data on ergotamine did not indicate bacterial or mammalian cell mutation. Early studies showed it had some chromosome damaging effects *in vitro* and *in vivo* although the latter were weak and inconsistent. In an investigation of carcinogenicity, rats treated with ergotoxine for periods up to two years developed a slight increase in neurofibroma of the ears, and crude ergot induced a 6-fold higher level of these tumours. The CONTAM Panel concluded that the available information on genotoxicity and carcinogenicity of EAs indicate that the observed tumours were related to a non-genotoxic mode of action.

The interaction of EAs with neurotransmitter receptors could result in acute as well as longer term effects, therefore the CONTAM Panel considered it appropriate to establish both an acute reference dose (ARfD) and a tolerable daily intake (TDI) for EAs.

The CONTAM Panel concluded that the vasoconstrictive effect represented by tail muscular atrophy in rats was the critical effect for hazard characterisation and derivation of the health-based guidance values (HBGVs). A $BMDL_{10}$[4] of 0.33 mg/kg b.w. per day was calculated for the incidence of tail muscular atrophy in a 13-week rat feeding study of ergotamine.

In establishing an ARfD, the CONTAM Panel concluded that an uncertainty factor of 3 was required to take into account deficiencies in the database, such as incomplete information on reproductive

[4] $BMDL_{10}$ (Benchmark dose lower confidence limit) is the 95 % lower confidence limit of the benchmark dose associated with a 10 % response.

toxicity. Together with the default uncertainty factor of 100 for intra and inter species differences, the CONTAM Panel applied an overall uncertainty factor of 300 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established an ARfD of 1 µg/kg b.w. (rounded to one significant figure).

In establishing a TDI, and in line with the recommendation of the EFSA Scientific Committee, the CONTAM Panel concluded that an additional uncertainty factor of 2 should be applied for extrapolation from sub-chronic to chronic studies. The CONTAM Panel applied an overall uncertainty factor of 600 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established a TDI of 0.6 µg/kg b.w. per day (rounded to one significant figure).

The available data do not allow determination of relative potencies of all EAs, but for those EAs for which comparable studies are available there appear to be no marked differences. The CONTAM Panel therefore concluded that the established HBGVs are a group ARfD of 1 µg/kg b.w. and a group TDI of 0.6 µg/kg b.w. per day for the sum of the EAs covered in this opinion, assuming equal potency.

Knowledge on dose-effect relations in humans is mainly based on the therapeutic use of ergotamine and ergometrine salts. A lowest single dose of 2 µg/kg b.w. ergometrine has been used to induce uterine contractions. This dose has been used as a starting point therapeutically and if ineffective, repeated and/or higher doses were administered. The CONTAM Panel concluded that 2 µg/kg b.w. ergometrine is likely to be close to a no-observed-effect level (NOEL) and that the margin between this dose in a sensitive subpopulation and the group ARfD of 1 µg/kg b.w. is adequate.

In the treatment of migraine, the lowest prescribed dose of ergotamine (which has not shown convincing evidence of pharmacological activity) is 13-26 µg ergotamine/kg b.w., which is 10 to 20 times higher than the group ARfD and 20 to 40 times higher than the group TDI. Furthermore, the maximum recommended oral therapeutic dose of ergotamine for adults is 8 µg/kg b.w. per day over a period of 30 days to avoid possible severe adverse effects associated with overdosage, such as peripheral vasoconstriction. This is 13 times higher than the group TDI. These comparisons with doses used in human medicine provide additional support for the value of the established group ARfD and group TDI.

The mean and high level estimated chronic dietary exposure to the sum of EAs for all age groups across European dietary surveys are all below the group TDI of 0.6 µg/kg b.w. per day established by the CONTAM Panel. These estimates reflect the predominant EAs present in foods, and even allowing for a possible additional contribution from EAs that were not measured or reported, they do not indicate a health concern. This conclusion also applies to chronic dietary exposure of the specific subgroups with the potential for higher exposure than the general population, i.e. vegetarians and consumers of raw grains. Due to the limited available data, it was not possible to estimate exposure to EAs for subgroups following other specific diets.

The mean estimates of acute dietary exposure to the sum of EAs for all age groups across European dietary surveys are below the group ARfD of 1 µg/kg b.w. established by the CONTAM Panel, both for the general population and for the specific subgroup of raw grain consumers, and do not indicate a health concern. In the case of high consumers' acute dietary exposure to the sum of EAs, maximum UB levels estimated for toddlers in the general population and 'other children' in the raw grain consumer subgroup were similar to the group ARfD. Taking into account the influence of left-censored data on the UB estimates, these exposures do not indicate a concern.

Whilst the available data do not indicate a health concern, the dietary exposure estimates relate to a limited number of food groups and a possible unknown contribution from other foods cannot be discounted.

Since the publication of the EFSA (2005) opinion on ergot alkaloids in feed, no relevant information was identified that would alter the previous risk assessment. Estimates of exposure based on example

diets and levels of EAs in cereal grains reported in Europe would suggest that under normal conditions the risk of toxicosis in livestock is low. Furthermore, the risk of ergotism in livestock as a result of consuming contaminated cereal grains, or compound feeds manufactured from them, is reduced where appropriate seed cleaning is carried out.

The CONTAM Panel recommended, *inter alia*, that efforts should continue to collect analytical data on occurrence of EAs in relevant food and feed commodities. The EAs monitored should include at least the compounds identified in this opinion as the major constituents of *C. purpurea* sclerotia. As *C. africana* and *C. fusiformis* may be relevant for ethnic foods, special diets or imported feed into the EU, the occurrence of their predominant EAs, in particular dihydroergosine and agroclavine, respectively, should be monitored. Moreover, there is a need for commercially available reference standards, in particular for isotope-labelled internal standards and for certified reference materials.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The term ergot refers to fungal structures from *Claviceps* species replacing kernels on grain ears or seeds on grass heads, being visible as large discoloured sclerotia. These sclerotia contain different classes of alkaloids, the most prominent being ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their related -inines.

THE OPINION OF THE PANEL ON CONTAMINANTS IN FOOD CHAIN ON A REQUEST FROM THE COMMISSION RELATED TO ERGOT AS UNDESIRABLE SUBSTANCE IN ANIMAL FEED.

The Panel on Contaminants in Food Chain issued on 19 April 2005 on a request from the Commission an opinion related to ergot as undesirable substance in animal feed.[5]

Data on the sensitivity of agricultural animal species towards ergot alkaloids are incomplete and do not allow the establishment of tolerance levels for individual ergot alkaloids and mixtures thereof. Available data indicate that adverse effects may occur in agricultural animals particularly in pigs after intake of feed contaminated with ergot at levels close to the current EU limit of 1 g of ergot sclerotia per kg un-ground cereals.

The limited and often incomplete data on tissue distribution and residual concentrations in edible tissues, milk and eggs do not allow an estimate of carry-over rates. Available data, however, provide no evidence that ergot alkaloids accumulate in edible tissues.

AVAILABLE INFORMATION ON ERGOT ALKALOIDS IN FOOD

In accordance with Article 36 of Regulation (EC) No 178/2002, a report "Scientific information on mycotoxins and natural plant toxicants" has been produced following a grant agreement between the European Food Safety Authority (EFSA) and the author(s) of the report (CFP/EFSA/CONTAM/2008/01). The report presents information, inter alia, regarding ergot alkaloids in food and is available on the EFSA website (http://www.efsa.europa.eu/en/scdocs/doc/24e.pdf)

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risks to human and animal health related to the presence of ergot alkaloids in food and feed.

The scientific opinion as regards the presence of ergot alkaloids in food should, *inter alia*, comprise the:

- evaluation of the toxicity of the ergot alkaloids for humans, considering all relevant toxicological endpoints and identification of the ergot alkaloids of toxicological relevance present in food;
- exposure of the EU population to ergot alkaloids, including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc).

The scientific opinion as regards the presence of ergot alkaloids in animal feed should, *inter alia*, comprise an update, if necessary, of the Opinion of the Panel on Contaminants in Food Chain on a request from the Commission related to ergot as undesirable substance in animal feed, taking into account new data (toxicological, occurrence and other relevant information) which has become available since 2005.

[5] Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to ergot as undesirable substance in animal feed, Adopted on 19 April 2005, The EFSA Journal (2005) 225, 1 – 27, http://www.efsa.europa.eu/en/scdocs/doc/225.pdf.

ASSESSMENT

1. Introduction

Several members within the fungal orders of Hypocreales and Eurotiales are known to produce ergot alkaloids (EAs). All species of the *Claviceps* genus within the Hypocreales are known to infest plant species belonging to Poaceae (family of the true grasses), including commercially important grains like rye, wheat, rice, corn, barley, millet or oat (Bové, 1970). In particular, the most widespread *Claviceps* species in Europe, *Claviceps purpurea*, is known to infect more than 400 plant species with the disease known as ergot (Haarmann et al., 2009).

The fungal hyphea invade the ovule of the host grass and colonize the whole ovary. Three to four weeks after infection the wintering body of the fungus, it becomes visible and replaces the kernels of the grain ears. These so-called sclerotia are dark, crescent shaped, protruding from the regular grains of the ear and represent the final stage of the disease. The sclerotia of *Claviceps* species are known as ergot. Referring to the sclerotia of fungi from the plant parasitic genus *Claviceps*, the word ergot is derived from the old French word for the cockerel's spur "argot".

Biosynthetically, EAs are classified as tryptophan derived alkaloids and the physiological effects of this class of compounds have been known since biblical times. In the Middle Ages, the consumption of EA contaminated grains, flour or bread caused severe epidemics of the condition known as St. Anthony's fire. Today, the cause of the disease is well known, and the condition is known as ergotism. The increased scientific understanding and improvements in agricultural practices and milling techniques (grading, sieving and sorting) has eliminated severe epidemic outbreaks of ergotism in the last decades. Besides their role in life threatening epidemic food contaminations EAs show a broad spectrum of pharmacological effects and were used in medical applications for hundreds of years. EAs and EA-derived compounds were applied or tested for prolactin inhibition, treatment of Parkinsonism, cerebrovascular insufficiency, venous insufficiency, thrombosis, emboli, stimulation of cerebral and peripheral metabolism, and are still applied for migraine and uterine stimulation (Battilani et al., 2009). In addition, lysergic acid diethylamide (LSD) is a semi synthetic derivative of the EA-family. Legally introduced as apharmaceutical in the mid-1950s and known for its potent psychoactive effects, it is an illegal drug of abuse today.

1.1. Chemistry

EAs are mainly produced by the fungal ascomycetous genus *Claviceps*. Most of the naturally occurring EAs show a tetracyclic ergoline ring system (Figure 1). Appendix A contains the chemical structures, as well as Chemical Abstracts Service (CAS) registry numbers and molecular weights of the relevant EAs discussed in this opinion. More than 50 different ergot alkaloids have been identified in the past (Flieger et al., 1997). The total EA-amounts and patterns vary between fungal strains, geographic regions and host plants (Krska et al., 2008).

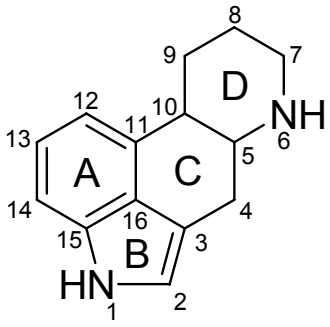


Figure 1: Ergoline ring system including numbering and assignment of rings.

EAs are classified into four major groups based on the substitutions at C-8 (Flieger et al., 1997; Mukherjee and Menge, 2000).

- Clavine alkaloids and 6,7-secoergolenes
- Simple lysergic acid derivatives
- Ergopeptine alkaloids – cyclol ergot alkaloids
- Ergopeptam alkaloids – lactam ergot alkaloids

In most of the naturally occurring EAs, no matter which of the four subgroups they belong to, the ring system is methylated at N6, substituted at C8 and possesses a double bond at position C8/C9 ($\Delta^{8,9}$-ergolenes) or C9/C10 ($\Delta^{9,10}$-ergolenes) with asymmetric centres at C5/C10 or C5/C8, respectively. The hydrogen at C5 is always in β-configuration; while in $\Delta^{8,9}$-ergolenes the hydrogen at C10 is always trans- (α) to the C5-H, the asymmetric centre at C8 in $\Delta^{9,10}$-ergolenes gives rise to two epimers, the β-$\Delta^{9,10}$-ergolenes (indicated by the suffix -ine) and the α-$\Delta^{9,10}$-isoergolenes (indicated by the suffix -inine).

1.1.1. Clavine alkaloids

Members of this class of EAs are derived from precursors of lysergic acid (e.g. agroclavine or elymoclavine). They are hydroxyl- and/or dehydro-derivatives of 6,8-dimethyl ergolenes, and include the group of 6,7-secoergolenes, which are characterised by an open D-ring.

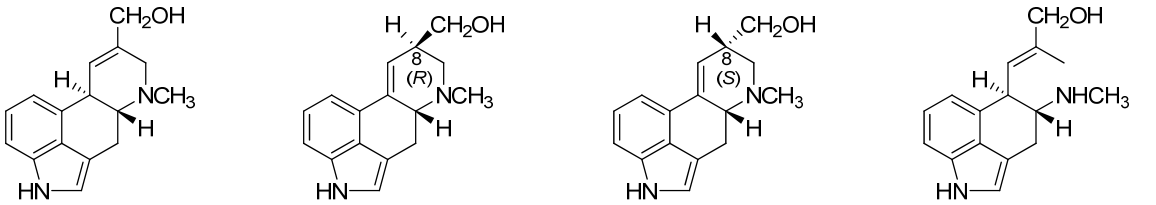


Figure 2: Structures exemplifying clavine alkaloids and 6,7 secoergolenes.

1.1.2. Simple lysergic acid derivatives

This class of compounds are amides (alkylamides or small peptides) of lysergic ($\Delta^{9,10}$) or paspalic ($\Delta^{8,9}$) acids.

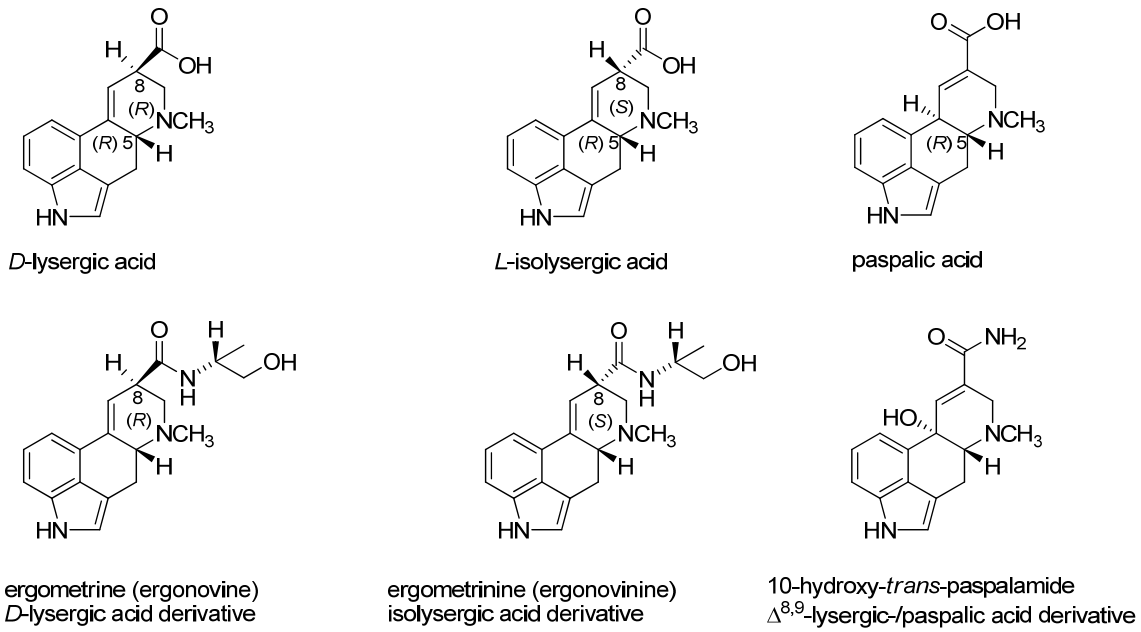


Figure 3: Structures exemplifying simple lysergic acid alkaloids.

Lysergic acid has two chiral centres (C5 and C8), both possessing *R*-configuration (see Figure 3). Derivatives of lysergic acid (8*R*-epimers; suffix –ine) represent the series of left-hand rotation isomers while the 8*S*, 5*R* diastereomers are termed right-hand rotation isolysergic acid derivatives (8*S*-epimers; suffix –inine) (Komarova and Tolkachev, 2001a).

1.1.3. Ergopeptine alkaloids – cyclol ergot alkaloids

Ergopeptines (synonym: ergopeptides) are composed of two parts, namely lysergic acid and a tripeptide moiety which is condensed to a tricyclic system.

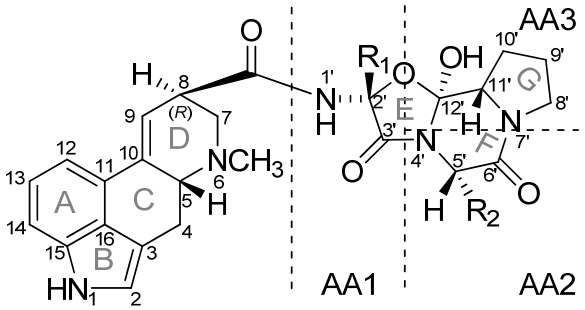


Figure 4: Examples for ergopeptine alkaloids, including the assignment of the rings and assignment of individual amino acids (AA) of the tricyclic peptide ring system. R_1 and R_2 correspond to the side chain of the AAs involved.

The cyclic part of the tripeptide results from cyclisation of AA1 which is α-hydroxylated (position: 2') during the biosynthesis and ***L*-proline at position AA3**. Variability of ergopeptines is driven by the interchangeability of possible AA for AA1 and AA2. So far, the AAs alanine, valine, phenylalanine, methionine, leucine, isoleucine, homoleucine and α-aminobutyric acid have been described. Since ergopeptines are assembled by non-ribosomal peptide synthetases, the incorporation of non-coded AA is possible. This gives rise to four subclasses of ergopeptines, namely: ergotamines (AA1: alanine), ergoxines (AA1: α-aminobutyric acid), ergotoxines (AA1: valine) and ergoannines (AA1: isoleucine). In addition, the corresponding isomers derived from isolysergic acid (characterised by the suffix –*inine*) are included in the corresponding subclasses.

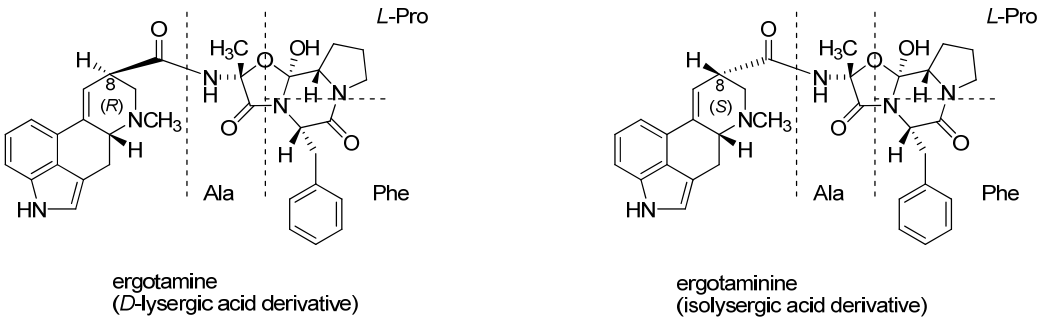


Figure 5: Structures exemplifying the (cyclol) ergotamine subclass: ergotamine and ergotaminine. AAs involved in the cyclic tripeptide part are shown.

1.1.4. Ergopeptames – noncyclol lactam ergot alkaloids

Ergopeptames resemble ergopeptine alkaloids with the exemptions that **AA3 is *D*-proline** and AA1 is not α-hydroxylated (position: 2'). Ergopeptames are correspondingly subclassified into ergotamams (AA1: alanine), ergoxams (AA1: α-aminobutyric acid), ergotaxams (AA1: valine) and ergoannams (AA1: isoleucine). The isomers built with isolysergic acid (characterized by the suffix –*inam*) are sub grouped correspondingly.

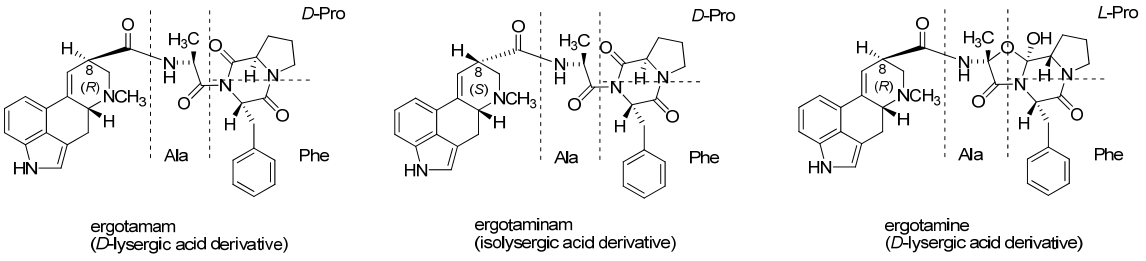


Figure 6: Structures exemplifying the (non-cyclol) ergotamam subclass: ergotamam, ergotaminam and for comparison ergotamine from the cyclol-subclass of ergotamines.

1.1.5. Physico-chemical properties

Pure EAs form colourless crystals that are soluble in organic solvents like acetonitrile, methanol or organic/buffer mixtures (Lauber et al., 2005; Krska et al., 2008, Müller et al., 2009). Several EAs (especially from the classes of simple lysergic acid derivatives and ergoclavines) are soluble to some extent in water (Schardl et al., 2006). EAs are positively charged at low pH-values and uncharged at neutral or higher pH-values.

As indoles, EAs without a $\Delta^{9,10}$-double bond show the characteristic ultraviolet (UV)-absorption at 280 nm, while $\Delta^{9,10}$-ergolenes can readily be detected by fluorescence (Flieger et al., 1997). Excitation wavelengths of 254, 313, 325 or 366 nm and an emission wavelength of 445 nm were reported (Scott, 2007).

$\Delta^{9,10}$-Ergolenes are susceptible to epimerisation with respect to the asymmetry at C8. The $\Delta^{9,10}$-double bond favours the rearrangement via enolisation and this process is enhanced by aqueous solution at low or high pH-values (Krska and Crews, 2008). Two studies investigated the influence of different solvents and storage conditions on the epimerisation of the major EAs of *C. purpurea* (Smith and Shappell, 2002; Hafner et al., 2008).

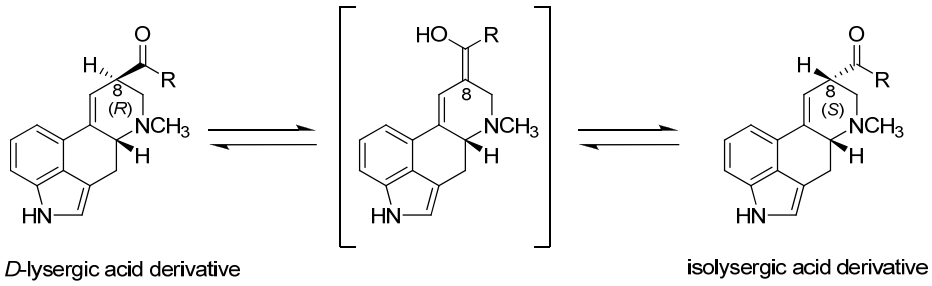


Figure 7: Epimerisation of $\Delta^{9,10}$-ergolenes via enolisation at C8 (*D*-lysergic acid (8-(*R*))-derivative into the corresponding isolysergic acid (8-(*S*))-derivative.

The ratio of the two epimers is mainly determined by the nature of the corresponding amide residue involved (Smith and Shappell, 2002). The ratio observed in a food sample or extracts might vary considerably depending on the EA structure, storage conditions, light exposure, extraction and analytical method applied. The latter was underlined by a study conducted at the BfR (BfR, 2010). It was demonstrated within one lab that sample treatment by different laboratory members had an impact on the ratio of each EA-epimeric pair, while the sum of both epimeric forms did not change. It is for this reason that a quantitative analytical approach should cover both C8-epimers of each ergopeptide.

Furthermore, EA-epimerisation and structural modifications are promoted through exposure to light. Under acid conditions the main reaction products promoted by UV-light are the so called "lumi"-derivatives, which are generated through the addition of water to the $\Delta^{9,10}$-double bond and are characterised by the loss of fluorescence (Stoll and Schlientz, 1955). In addition, "aci"-EAs are formed via acid catalyzed isomerisation of the 2'-carbon of the tricyclic peptide moiety of the ergopeptine alkaloids (McPhail et al., 1966; Smith and Shappell, 2002). "Aci" and "lumi"-derivatives are usually not covered by the analytical methods in use.

When kept in amber vials at room temperature, all major *C. purpurea* ergopeptide EAs (ergosine, ergotamine, ergocornine, α-ergocryptine, ergocristine) were found to be stable in chloroform, in terms of epimerisation and degradation (in particular "aci" and/or "lumi" products). For all other solvents or solvent mixtures tested (methanol/dichloromethane, acetonitrile/buffer, extraction mix, stabilizing solution or acetonitrile), various degrees of epimerisation at C8 and/or degradation of individual ergopeptide EAs were observed. In contrast, the simple lysergic acid derivative ergometrine showed hardly any epimerisation into ergometrinine under the tested conditions (Hafner et al., 2008)

To avoid significant epimerisation or degradation of EAs in acetonitrile (a favourable solvent for subsequent HPLC-separation) or other solvents, EAs should be stored below -20 °C and repeated freeze/thaw cycles should be avoided (Hafner et al., 2008). EA-storage in extraction buffer (acetonitrile/ammonium carbonate buffer) in autosamplers trays prior to analysis (which could last up to 18 hrs) showed acceptable results in terms of C8 epimerisation, but autosamplers with sample cooling option were recommended (Hafner et al., 2008).

1.2. Origin of ergot alkaloids in fungi and host plants

Claviceps spp.

The genus *Claviceps* belongs to the family *Clavicipitaceae* originally placed in the fungal order of Hypocreales. Doubts based on comparative studies of conidiogenous stroma development resulted in its transfer to Xylariales and later to Clavicipitales, a new order established to accommodate clavicipitaceous fungi as reviewed by Pažoutová and Parbery (1999). However, DNA analysis recently confirmed the original placement of the monophyletic family Clavicipitaceae as a member of the order Hypocreales (Pažoutová and Parbery, 1999; Sung et al., 2007).

The traditional taxonomic criteria used to delineate *Claviceps* species include the colour, size and shape of the sclerotia, although it must be emphasized that the sclerotium size is largely dependent on the plant host. Thus, *C. purpurea* sclerotia found in florets of annual meadow grass (*Poa annua*) are only about 1 - 2 mm long while those formed in florets of rye (*Secale cereale*) are up to 50 mm (Pažoutová, 2002).

The *Claviceps* fungi have also been subjected to taxonomic revisions within the genus, as based on their genetic relationships. Thus, Pažoutová (2001) constructed phylogenetic trees of 16 *Claviceps* species. The result was in strong support for two clades as follows: (1) *C. paspali, C. zizaniae, C. grohii, C. sulcata, C. fusiformis*, and *C. purpurea*; and (2) *C. citrina, C. phalaridis*, two unidentified *Claviceps* spp. (isolates PM and SG), *C. sorghicola, C. gigantea, C. sorghi, C. africana, C. viridis*, and *C. pusilla.* No relationship was found between a species placement and its morphological markers (Pažoutová, 2001).

Members of the genus *Claviceps* are parasites of sedges, rushes and grasses, specifically infecting the florets. To date about 45 teleomorph species of *Claviceps* have been described, but many species may exist only in anamorphic (sphacelial = white soft tissue producing sugary honeydew) stage and therefore go unnoticed.

In general, the distribution of *Claviceps* species among grasses is unequal, and there is for example a considerable difference in the number of species colonising the different subfamilies of the Poaceae (Arundinoideae, Bambusoideae, Chloridoideae, Panicoideae and Pooideae).

Most *Claviceps* species originate from tropical regions and colonise panicoid plant species; i.e. grasses belonging to the subfamily Panicoideae. Panicoideae includes cereals such as maize (*Zea Mays*), sorghum (e.g. Durra = *Sorghum bicolor* and other *Sorghum* spp.), sugar cane (*Saccarum officinarum*), several millets (pearl millet - *Pennisetum glaucum*, finger millet - *Eleusine coracana*, foxtail millet - *Setaria italica*, proso/broomcorn millet - *Panicum miliaceum*, little millet - *Panicum sumatrense*, barnyard millet - *Echinochloa crus-galli* and kodo millet - *Paspalum scrobiculatum*) and (white) fonio (*Digitaria exilis*). The only ergot producing parasites of grasses belonging to the subfamily Pooideae are *C. purpurea* in North temperate regions and *C. phalaridis* endemic to Australia (Pažoutová, 2002). Thus, *C. purpurea* has as its main hosts rye (*Secale cereale*) and triticale (*x Triticosecale*) (Dabkevicius and Semaskiene, 2001), but also wheat (*Triticum* spp.) and certain other Pooideae grasses (Siegel et al., 1987; Christensen et al., 2002). *C. phalaridis* occasionally infect crops of oats (*Avena sativa*) and barley (*Hordeum vulgare*) (Walker, 2004).

C. purpurea is the type species of the genus and in a food safety context worldwide, mainly infection of grains with this species is of concern, although *C. fusiformis* and *C. africana* must be mentioned as well. *C. purpurea* is an ergot fungus with a very wide host range that includes the entire subfamily Pooideae, together with many members of the Arundinoideae, and some species belonging to the chloridoid and panocoid groups (Pažoutová et al., 2000). Altogether several hundreds of grasses and cereals have been described as suitable hosts for *C. purpurea*. Ergot caused by *C. fusiformis* is found in pearl millet (*Pennisetum glaucum*) where it is a major floral disease. This fungal disease has been more severe in single-cross F_1 hybrids than in open-pollinated varieties (Thakur and Rai, 2002). Prior to the cultivation of hybrids in the late 1960's, research on pearl millet ergot was conducted primarily in India, and more due to concern about its toxicological effects on human and cattle than for its effects on grain yield

(Mantle, 1992). *C. africana*, often termed sorghum ergot, was first detected outside Africa in Australia in 1996 and has subsequently been found in all sorghum producing regions (Kopinski et al., 2007). Until now, most reports on health impairing effects have been related to production animals such as pigs (Kopinski et al., 2007) and poultry (Bailey et al., 1999). Other species that infect sorghum include *C. sorghi* and *C. sorghicola* (Bailey et al., 1999; Tooley et al., 2000; Pažoutová, 2001), but so far these species have not been associated to any toxicity for man or livestock.

Another *Claviceps* species, *C. cyperi*, may cause severe ergotism in dairy cattle consuming maize silage and teff hay contaminated with *Cyperus esculentus* (yellow nut sedge) ergotised with this fungus; as described in the Highveld of South Africa (Naude et al., 2005; Van der Linde and Wehner, 2007). There has also been one reported case, presumably of *Claviceps* spp. infected wild grasses, that had severely affected the health of some free-living moose and a roe deer in Norway (Handeland and Vikøren, 2005; Uhlig et al., 2007).

In certain parts of the world *C. paspali* frequently causes intoxications (Paspali staggers) in cattle when these feed on the grass *Paspalum paspaloides* infected with this fungus. The grass belongs to the subfamily Panicoideae and has many common names, including water couch (Australia) and eternity grass (United States). The plant genus *Paspalum* differs in subtropical and tropical regions; many are tall perennial American grasses. Paspali staggers has been seen but is rare in Europe (Moyano et al., 2010). Likewise *C. cynodontis* are known to cause "bermudagrass staggers"; i.e. tremors in cattle grazing bermudagras (*Cynodon dactylon*, kweek) infected with this fungus. Many varieties and hybrids of bermudagrass have been planted throughout the United States where this disease is well known. Both *C. paspali* and *C. cynodontis* produce EAs but recent investigations have pointed to other secondary constituents as being responsible for the staggers they cause. Symptoms for these staggers and the toxins involved are further described in Section 7.3.1.

Neotyphodium spp.

Some Pooideae grasses belonging to the genus *Festuca* may be infected with a fungal endophyte also producing EAs. This is, for example, the case for the economically important forage grass tall fescue (*Festuca arundinacea*) which is often infected with the endophyte *Neotyphodium coenophialum* (= *Acremonium coenophialum*) (Roberts, 2000; Wang et al., 1992). Other members of the Clavicipitaceae, including both other *Neotyphodium* species and species of the genus *Epichloe*, also infect forage and turf grasses, including certain economically important cultivars of perennial rye grass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*) (Morgan-Jones et al., 1990), producing toxic alkaloids associated with "fescue toxicity" (CAST, 2003).

In general, many ryegrass cultivars grown for turf and forage in the United States, Australia and New Zealand are infected with *Neotyphodium lolii* (Roberts et al. 2005). In North America over 90 % of the tall fescue pastures are reported to contain plants infected with *Neotyphodium coenophialum* (Panuccione and Annis, 2001). Tall fescue is of great importance as a forage grass in several states in the United States, and significant economic losses are seen due to reduced weight gain as well as to other effects of tall fescue toxicosis (Roberts, 2000).

One approach to overcoming these problems has been to breed and grow different cultivars of endophytic infected perennial ryegrasses containing e.g. strains of *Neotyphodium lolii* that produce alkaloids that are not toxic to mammals.

In European pastures, the predominant tall fescue cultivars used are endophyte-free (Gibson and Newman, 2002). As a result, intoxications of livestock associated with *Neotyphodium* toxins are rare in grazing livestock, and therefore exposure to these toxins in forage crops will not be addressed in this opinion.

Other species

Some species of the morning glory family (*Ipomoea spp.*) have been associated with the production of EAs (Friedman et al., 1989). For long it was assumed that the EAs were biosynthesised by the plants themselves, but later on evidence has been presented that for at least one species (*I. asarifolia*) it is likely that fungal endophytes are responsible for the production of the EAs (Kucht et al., 2004). Very recently, the fungal species has been described as *Periglandula ipomoeae*, belonging to the new genus of *Periglandula* (Steiner et al., 2011). Morning glory is a noxious weed and its seeds can contaminate soybean (*Glycine max*) harvests (Friedman et al., 1989). Seeds of wild-type morning glory species were found to contain relatively small amounts of EAs, not likely to present a health risk (Wilkinson et al., 1987).

1.2.1. Ergot alkaloids in sclerotia of *Claviceps* species

As described, *Claviceps* species, infecting hosts relevant for the food chain, are primarily *C. purpurea* (ubiquitous, infects grasses and cereals, such as rye, wheat, triticale), *C. africana* (infection of sorghum) and *C. fusiformis* (infection limited to pearl millet). The sclerotia produced by these species vary considerably in their EA-composition due to differences in the alkaloid biosynthesis pathways (e.g. Lorenz et al., 2007).

Claviceps purpurea

Sclerotia from *C. purpurea*, which are the major cause of food contamination with EAs in Europe, contain lysergic acid derivatives including the complex ergopeptines. The main alkaloids in ergots from *C. purpurea* are ergocristine, ergotamine, ergocornine, α- and β-ergocryptine, ergometrine, ergosine, ergocristinine, ergotaminine, ergocorninine, α- and β-ergocryptinine, ergometrinine, and ergosinine; the alkaloid composition being highly variable (Young and Chen, 1982; Mainka et al., 2007a; Appelt and Ellner, 2009; Franzmann et al., 2010a).

The total alkaloid content of European *C. purpurea* sclerotia was determined by Silber and Bischoff in 1954 (cited in Lorenz, 1979). Using a colorimetric method 260 sclerotia were analysed and an average alkaloid content of 0.26 % was found (range 0.1-0.5 %). Individual EAs were not investigated in this study.

In a recent study by Franzmann et al. (2010a) 63 single sclerotia samples from rye (collected from harvest years 2005-2009) were analysed by liquid chromatography with fluorescence detection (HPLC-FLD). Most samples originated from various locations in Germany, but also some sclerotia from Poland (n = 6) and Lithuania (n = 4) were included. The alkaloid contents varied considerably, from 115 to 2 362 µg/g (0.01-0.24 %) with a mean value of 757 µg/g (0.076 %). The amounts of ergotamine (22 %), ergocristine (20 %) and their isomers ergotaminine (9.5 %) and ergocristinine (6.5 %) together represented 57% of the total alkaloid content with little variation (SD = 8.9 %, n = 63). The sum of the -ine epimers accounted for 71.5 % of the content, the -inine epimers represented 28.5 % (Franzmann et al., 2010a).

Appelt and Ellner (2009) analysed the occurrence of EAs in batches of ergot from German rye and triticale collected during harvest years 2007/2008 by HPLC-FLD. The results are presented in appendix B. The total EA content varied between 280 and 1826 µg/g (0.03–0.18 %) for rye ergot (n = 13) collected in 2007 and between 217 and 1574 µg/g (0.02-0.16 %) for rye (n = 6) collected in 2008. Triticale (n = 4) was only sampled in 2007 and varied between 572 - 2 214 µg/g (0.06 – 0.22 %). The average total EA content of all samples was 900 µg/g (0.090 %). The main alkaloids present were ergocristine (21 %), ergotamine (18 %), ergosine (10 %) and ergocornine (8 %). The six -ine isomers combined accounted for 69 % of the total content, the 6 -inine isomers for 31 %. Single sclerotia (n = 33) from two rye samples from 2007 were also analysed: the total EA content ranged from 2 to 4 178 µg/g (0.0002 - 0.42 %). For single sclerotia (n = 7) from triticale the range was from 5 to 3 759 µg/g (0.0005 - 0.38 %).

Mainka et al. (2007a) conducted a study on the EA content of rye ergot growing on a set of artificially infected hybrid rye varieties as well as two conventional rye varieties at three German locations in 2002-2004 using HPLC-FLD. The EA content of the sclerotia collected from the various varieties was in most instances not significantly different (probability (p) from 0.01 to 0.50, depending on location and year of harvest). The year of harvest itself was however highly significant ($p < 0.001$) with respect to EA total content. Regarding six varieties for which data from the same location were available covering the three harvest years, an average total amount was found of 512, 132 and 1 259 µg/g, for respectively 2002, 2003 and 2004. Concentrations ranged from 7 to 683 µg/g (0.007 to 0.07 %) for the harvest of 2003 and from 301 to 2 457 µg/g (0.03 to 0.25 %) for the harvest of 2004. An average EA content of 841 µg/g (0.084 %) was calculated from the presented data. The EA profiles were highly variable with respect to location and year. The major EAs present were ergocristine (30 %), ergosine (22 %) and ergotamine (11.5 %). The other EAs individually contributed amounts less than 8 %. The six -ine epimers together accounted for 79 %, the six -inine epimers for 21 %.

Mulder et al. (2012) analysed 48 batches of sclerotia collected in 2008-2010 from rye (n = 32), triticale (n = 14), wheat (n = 1) and barley (n = 1) samples primarily produced in the Netherlands. The sample size ranged from 1 to 17 pooled sclerotia per inspected lot and analysis was performed by high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS). A total of 24 different EAs were monitored, 16 of which were detected at least once (the 12 major EAs from *C. purpurea* together with β-ergocryptin(in)e, agroclavine and chanoclavine-1). Concentrations ranged from < limit of detection (LOD) (1 µg/g) to 3258 µg/g (<0.0001 to 0.33 %) for rye ergot and from < LOD to 6 003 µg/g (< 0.0001 to 0.60 %) for triticale ergot. The average concentration for the rye sclerotia was 521 µg/g (0.052 %) and for the triticale sclerotia it was 959 µg/g (0.096 %). The mean concentration for all 48 samples was 659 µg/g (0.066 %). The mean concentration for the 12 main *C. purpurea* EAs in these samples was 642 µg/g (0.064 %). One sclerotia sample from triticale contained a substantial amount of chanoclavine-1 (750 µg/g), an EA that in the other samples was present at low or non detectable concentrations. Furthermore, it was noted that a substantial number of sclerotia samples (10 from rye and 4 from triticale) did not contain measurable amounts of EAs. Major EAs found in the sclerotia were the α- and β-ergocryptine isomers (together 19 %) and ergosine (18 %), followed by ergometrine, ergocornine and ergotamine (each around 12 %), while the amount of ergocristine was relatively small (5.4 %). The -ine epimers accounted for 79 % of the total content and the -inine epimers for 21 %.

Young (1981a, b) and Young and Chen (1982) conducted a series of studies on the alkaloid content and composition of ergot in Canadian rye, wheat, triticale and barley, collected in 1978 and 1979. Total alkaloid content was determined colorimetrically, while the individual EAs were separated and analysed by HPLC coupled to UV or FLD. Sclerotia taken from different heads, fields, crops, and harvest years were analysed and the variation in ergot content and EA composition was investigated in detail. The total alkaloid content in rye, wheat, triticale and barley ergot sclerotia was found to be highly variable between sclerotia from the same head, field and region. Total alkaloid content in rye ergot ranged from 0.011 to 0.452 % with an average of 0.249 % (n = 169). For wheat ergot a slightly lower content was found: between 0.013 and 0.31 %, with an average of 0.16 % (n = 73). Alkaloid content from triticale ergot ranged from 0.042 to 0.75 % (n = 39) with a mean of 0.26 % and from barley ranged from 0.082 to 1.04 % (n = 47) with a mean of 0.26 %. The overall average for Canadian ergot was 0.24 %. Ergotamine was the predominant EA in rye ergot from Eastern Canada, while ergocristine was the major EA found in rye, wheat, barley and triticale ergot from Western Canada. Insufficient data were available for rye from wheat, barley and triticale from Eastern Canada to draw conclusions on their composition. The following overall average alkaloid composition for Canadian ergot was determined (based on 47 fields of rye, 75 of wheat, 29 of triticale and 3 of barley): ergocristine (31 %), ergocristinine (13 %), ergotamine (17 %), ergotaminine (7.6 %), α-ergocryptine (5.3 %), α-ergocryptinine (2.6 %), ergometrine (5.0 %), ergometrinine (2.2 %), ergosine (4.2 %), ergosinine (2.0 %), ergocornine (4.0 %), ergocorninine (2.1 %), 3.4 % unidentified. Ergocristine, ergotamine and their epimers accounted for 69 % of the total EA composition. The sum of the -ine epimers accounted for 67 % of the total composition, the -inine epimers close to 30 % (Young and Chen, 1982).

Fajardo et al. (1995) reported a total content of 1 100 µg/g (0.11 %) EAs for a batch of Canadian rye from spring wheat using HPLC-FLD. Distribution of the six EAs determined was as follows: ergocristine (31 %), ergotamine (24 %), ergocornine (16 %), α-ergocryptine (14 %), ergosine (10 %) and ergometrine (5 %). The -inine epimers were not included in this study.

Blaney et al. (2009) studied the content of Australian rye, barley, oats and wheat ergot sclerotia using a combination of high performance liquid chromatography – ultraviolet (HPLC-UV) and fluorescence detection. The samples were collected in 2008 during outbreaks of ergot along the coast of Western Australia. The average EA content of the samples was 2 690 µg/g (0.27 %) and varied between 1 006 and 3 766 µg/g (0.10 - 0.38 %). Ergotamine was by far the most prominent EA with an average contribution of 57 %, followed by α-ergocryptine (15 %), ergotaminine (11 %), α-ergocryptinine (6.4 %), ergocornine (4.3 %) and ergosine (2.9 %). The other EAs, including ergocristine, were only present in very small amounts.

Tandem mass spectrometry was used to investigate the ergot peptide alkaloids occurring in the sclerotia of *C. purpurea* parasitic on tall fescue and to compare those with the alkaloids produced by this fungus on barley and wheat (Porter et al., 1987). The fescue sclerotia contained ergotamine (35 %), ergocristine (31 %), and ergosine(s) (27 %) as the major peptide alkaloids. Furthermore, minor percentages of ergocryptine(s) (3.0 %), ergocornine (2.2 %), ergostine (0.85 %), ergovaline (0.30 %), ergoptine(s) (0.18 %), and ergonine (0.11 %) were detected. Although the same EAs were found in the sclerotia of *Claviceps*-infected wheat (except ergonine) and barley, there were differences in alkaloid quantities.

Sclerotia of *Claviceps* spp. collected from 10 different species of wild grasses in Norway contained ergocryptine and its epimer ergocryptinine as the most prominent EA (ranging from 14 to 9 300 µg/g) (Uhlig et al., 2007). Ergotamine (together with ergotaminine) was found in a concentration between 7.7 and 990 µg/g. Concentrations of other EAs were less than 20 µg/g. HPLC-MS/MS analysis of sclerotia extracts indicated the presence of many EAs of unknown structure.

Claviceps africana

Dihydroergosine is the principal toxic alkaloid found in the sclerotia from *C. africana* (Molloy et al., 2003). Bandyopadhyay et al. (1998) reported that the content of *C. africana* sclerotia varied between 0.02 and 0.98 % (wt/wt), dihydroergosine accounting for 88 % (no further details given).

Claviceps fusiformis

Individual alkaloids isolated from pearl millet samples contaminated with ergot from *C. fusiformis* were identified as agroclavine, elymoclavine, chanoclavine, penniclavine, and setoclavine (Krishnamachari and Bhat, 1976). A strain of *Claviceps fusiformis* also was shown to produce clavine alkaloids (principally agroclavine) when grown submerged in a sucrose-ammonium sulphate-inorganic salts medium (Banks et al., 1974).

Claviceps paspali

Sclerotia of *C. paspali* from Australia were early shown to contain up to 0.005 % of total alkaloids composed of ergine and ergonovine along with chanoclavine and some unidentified EAs (Groger et al., 1961). Later studies reported in addition the presence of low concentrations of elymoclavine (Kobel et al., 1964) and agroclavine (Brar et al., 1968). Thus, the total content of EAs would be too low to account for the induction of "paspali staggers", which also later has been shown to be caused by indole-diterpenoid tremorgens; so-called paspalitrems (Uhlig et al., 2009; see also Section 7.3.1.).

Claviceps cyperi

The main ergopeptine alkaloid in sclerotia of *Claviceps cyperi* from ergotised nut sedge was identified by HPLC-MS/MS and tandem mass spectrometry as α-ergocryptine. All sclerotia samples also yielded

ergosine whereas ergocornine and ergocristine were detected in low concentrations of freshly collected samples only (Van der Linde, 2005).

1.2.2. Ergot alkaloids from endophytes in tall fescue and other grasses and plants

The primary ergopeptine in tall fescue and perennial rye grass endophytes (*Neotyophodium* spp.) is ergovaline (Lehner et al., 2005; Porter, 1995). Many other ergot peptines (ergotamine, ergocornine, ergocryptine, ergocristine, ergosine and corresponding -inines) and lysergic acid derivatives (ergometrine, lysergol, lysergic acid, ergine) have been reported (Porter, 1995; Shelby et al, 1997; Lehner et al., 2005). Less common EAs such as ergonine, ergoptine, ergostine and various "aci"- and didehydro- forms have been detected as well using HPLC-MS approaches (Shelby et al, 1997; Lehner et al., 2005; Strickland et al., 2011).

A total EA content from 0.01 to 0.035 % in the seeds of some species of the morning glory family (*Ipomoea* spp.) has been reported by Friedman et al. (1989). Of the *Ipomoea* spp. investigated thus far, seeds of the horticultural variety Heavenly Blue were found to contain the highest amounts of EAs: up to 520 µg/g (0.052 %) as estimated by a colorimetric method (Wilkinson et al., 1987). Ergometrin(in)e, ergin(in)e, ergsin(in)e, agroclavine, chanoclavine, setoclavine, penniclavine, and elymoclavine were identified in *Ipomoea* spp., using thin layer chromatography (TLC) (Wilkinson et al., 1987).

1.3. Previous assessments

WHO-IPCS (1990) evaluated selected mycotoxins including naturally occurring EAs for the risk as contaminants in food for human consumption. The WHO Task Group on Environmental Health Criteria for Selected Mycotoxins concluded that human exposure to low levels of EAs appears to be widespread and that *Claviceps purpurea* alkaloids produced more severe effects than the alkaloids of *Claviceps fusiformis*. It was not possible to conclude whether such differences are related to different content of alkaloids of the fungal species, to the toxicological properties of the alkaloids, or to the levels of intake by different types of populations.

The Committee for Veterinary Medicinal Products of the European Agency for the Evaluation of Medicinal Products (EMEA, 1999) evaluated the veterinary use of ergometrine maleate for the control of postpartum uterine haemorrhages. Since ergometrine maleate is only occasionally administered to parturient females only, immediate slaughtering of treated animals is unlikely and the product is eliminated within a few hours after the administration, no need to establish Maximum Residue Limits (MRL) was identified by the Committee.

BfR assessed the risk of the consumption of rye flours contaminated with EAs (range of total alkaloids: 2 308 to 7 255 µg/kg) based on the estimated intakes of ergotamine and ergometrine, which are pharmacologically and toxicologically characterised due to their pharmaceutical uses. Estimated intake of ergotamine and/or ergometrine with food in the ranges of daily therapeutic doses or above were associated with the possible occurrence of severe side effects and were considered injurious to health. Considering that ergotamine accounted for a maximum of 46 % of the total alkaloid content, BfR estimated that consumption of 250 g of the most contaminated rye flour (7 255 µg total alkaloids/kg) would lead to an intake of maximum 834 µg ergotamine per day per person. This exceeded the maximum therapeutic daily dose tolerated for a month-long therapy, which is equivalent to about 670 µg ergotamine tartrate per day. On the supposition that the ergometrine contributes 5 % of the total alkaloid content, consumption of 250 g of the most contaminated rye flour (7 255 µg total alkaloids/kg) would result in an intake of maximally 91 µg ergometrine/day per person, which is below the lowest therapeutic dose equivalent to 400 µg egometrine hydrogen maleate per day (BfR, 2004; Dusemund et al., 2006).

In 2005 the EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) assessed EAs as undesirable substances in animal feed in the food chain (EFSA, 2005). The CONTAM Panel concluded that adverse effects (including vasoconstriction leading to vaso-occlusion and gangrene in cattle, neurotoxicity and delayed parturition in horses and reduced milk production in pigs) may occur in

agricultural animals after intake of feed contaminated with ergot at levels close to the current European Union (EU) limit[6] (1 000 mg of ergot sclerotia per kg unground cereals), but no tolerance levels for individual ergots could be established in view of the insufficient data on sensitivity of agricultural animal species towards EAs. Insufficient data on tissue distribution and residual concentrations in edible tissues, milk and eggs were available to reliably estimate carry-over rates. The few available data did not provide any evidence that EAs accumulate in edible tissues and thus that they are unlikely to be an important source of human exposure.

The French Food Safety Agency (AFSSA) assessed qualitatively the risk arising from the presence of EAs in the food chain for human and animal consumption (AFSSA, 2009). AFSSA concluded that nowadays the EAs produced by *C. purpurea* are not likely to represent a significant risk for humans. However, they still represent a risk for animals exposed to contaminated feed and forage.

1.4. Ergot alkaloids selected for assessment in this opinion

This opinion deals with the following EAs: ergometrine, ergotamine, ergosine, ergocristine, α- and β-ergocryptine, ergocornine, and their corresponding epimers (-inine forms). Although the -inine forms are described to be biologically inactive on the neuroreceptor sites, an interconversion under alkaline or acidic conditions can take place and thus both forms have to be considered in the risk assessment. The chemical structures of the EAs considered in this opinion are reported in Figure 8.

[6] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p.10-21. See Section 2.

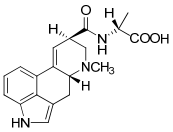
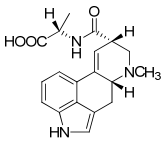
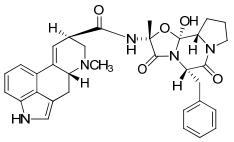
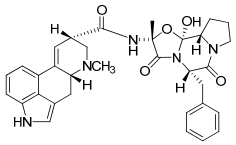
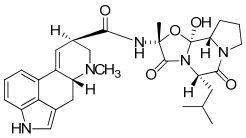
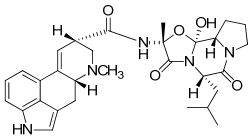
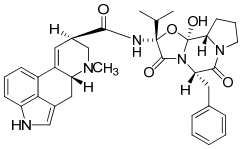
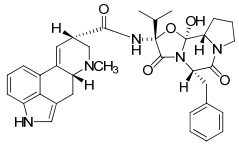
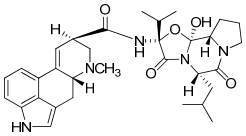
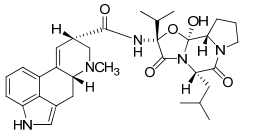
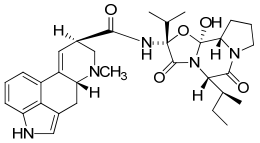
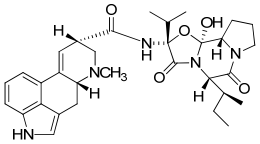
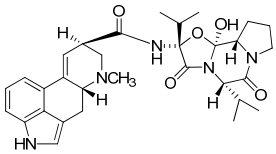
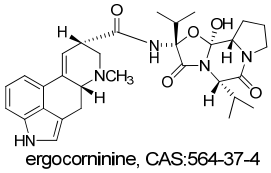
Lysergic acid derivatives	Isolysergic acid derivatives
 ergometrine (ergonovine, ergobasine) CAS: 60-79-7	 ergometrinine (ergonovinine), CAS: 479-00-5
 ergotamine, CAS: 113-15-5	 ergotaminine, CAS: 639-81-6
 ergosine, CAS: 561-94-4	 ergosinine, CAS: 596-88-3
 ergocristine, CAS: 511-08-0	 ergocristinine, CAS: 511-07-9
 α-ergocryptine (ergocryptine) CAS: 511-09-1	 α-ergocryptinine (ergocryptinine) CAS: 511-10-4
 β-ergocryptine, CAS: 20315-46-2	 β-ergocryptinine, CAS: 19467-61-9
 ergocornine, CAS: 564-36-3	 ergocorninine, CAS:564-37-4

Figure 8: Overview on the structures of the EAs selected for assessment in this opinion.

2. Legislation

In order to protect public health, Article 2 of the Council Regulation (EEC) No 315/93[7] stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Thus, a number of maximum tolerances for contaminants as well as natural plant toxicants are currently laid down in Commission Regulation (EC) No 1881/2006.[8] While maximum levels (MLs) for various mycotoxins were set for a number of food commodities, EAs as such in food are not regulated so far under this EU Regulation.

Recently, the Commission adopted a Recommendation on the monitoring of the presence of EAs in feed and feed (2012/154/EU).[9] It recommended that Member States should perform with the active involvement of the feed and food business operators monitoring on the presence of EAs in cereals and cereal products intended for human consumption or intended for animal feeding, in pasture/forage grasses for animal feeding and in compound feed and food. At least the following EAs should be monitored: ergocristine/ergocristinine, ergotamine/ergotaminine, ergocryptine/ergocryptinine, ergometrine/ergometrinine, ergosine/ergosinine and ergocornine/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.

Commission Regulation (EU) No 1272/2009 laying down common detailed rules for the implementation of Council Regulation (EC) No 1234/2007 as regards buying in and selling of agricultural products under public intervention[10] stipulates that cereals must be "sound, fair and of marketable quality" in order to be eligible for public intervention. This demand is fulfilled if the cereals are free from abnormal smell and live pests (including mites) at every stage of their development, if they meet certain minimum quality requirements, and if their levels of contaminants, including radioactivity, do not exceed the maximum levels permitted under Union legislation. The minimum quality requirements mentioned above are established and include inter alia a maximum level for ergot of 0.05 % in durum and common wheat i.e. 0.05 % or 500 mg/kg w/w sclerotia. No maximum levels are specified for EAs.

Undesirable substances in feed are regulated by Directive 2002/32/EC[6]. EAs are not listed as such in the Annex of this Directive. However, the Directive sets a maximum content for rye ergot (*Claviceps purpurea*) of 1 000 mg/kg in all feed containing unground cereals. The maximum content relates to a feed with a moisture content of 12 %.

Based on the information contained in the FAO Food and nutrition paper 81 "Worldwide regulations for mycotoxins in feed and food in 2003",[11] following regulatory levels have been established for ergot and/or egot alkaloids in a few countries outside the European Union:

- In Australia and New Zealand a maximum level of 500 mg/kg of ergot sclerotia in cereal grains is applied.

- In Canada, guideline levels for ergot sclerotia have been determined for the grading of cereal grains ranging from 0.01 % for the highest quality grades, up to 0.1 % for the lowest quality

[7] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–3.
[8] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.
[9] Commission Recommendation 2012/154/EU of 15 March 2012 on the monitoring of the presence of ergot alkaloids in feed and . OJ L 77, 16.3.2012, p. 20-21.
[10] Commission Regulation (EU) No 1272/2009 laying down common detailed rules for the implementation of Council Regulation (EC) No 1234/2007 as regards buying in and selling of agricultural products under public intervention OJ L 349, 29.12.2009, p. 1-68.
[11] http://www.fao.org/docrep/007/y5499e/y5499e00.HTM

grades. Furthermore, maximum levels have been established for EAs of 6 mg/kg in pig feed, of 3 mg/kg in feed for cattle, sheep and horses and of 9 mg/kg in feed for chicks.

- In Uruguay, a guidance level for EAs in animal feed was issued, providing that EAs should not be detectable in feed for pigs and female rabbits and a guidance level of 450 µg/kg in other feed.

3. Methods of analysis

3.1. Stability of reference compounds and stock solutions

The analysis should be carried out under reduced light influence to avoid the formation of "lumi"-derivatives. In addition epimerisation of EAs may occur at room temperature (Scott, 2007). Epimerisation is also facilitated at low and high pH-values. Therefore, special precautions should be taken to control the quality of the stock solutions. Stock solutions of EAs in pure organic solutions are not stable and it has been recommended to keep aliquots as thin films at -18 to -30 °C, which should be re-dissolved immediately before use and were reported to be stable for more than 12 months (Lauber et al., 2005). Ware et al. (2000) used a special stabilization solution for standards consisting of ethylene glycol, 1,2-propandiol and tartaric acid in 25 % ethanol. This is similar to the ethanol/tartaric acid solution used in the official §64 method of the German Food and Feed Law (LFGB, 2012). A comprehensive study of the main *C. purpurea* EAs demonstrated that EAs were stable against epimerisation and degradation in various solvents if stored below -20 °C. In chloroform, all investigated ergopetide alkaloids were stable even at 20 °C for more than 6 weeks. Summarising, to prevent degradation (formation of "lumi"- and "aci"-derivatives) and epimerisation, stock solutions of EAs should be kept in non protic solvents and temperatures at or below -20 °C (Hafner et al., 2008). In addition, EA stability and epimerisation should be confirmed for the applied extraction procedure, individual sample matrix, solvents (e.g. compatibility with HPLC-system) and other conditions like cooled autosampler trays to process large sample sets (Smith and Shappell, 2002; Hafner et al., 2008; LFGB, 2012).

3.2. Extraction and sample clean-up

The techniques of EA-sample preparation most recently in use will be summarised in this paragraph.

Extraction

Most methods developed so far are for samples that are grain based and of high dry matter content. Hence, liquid extraction using solvent mixtures is a commonly applied method to extract EAs from ergot sclerotia or grain-based foods. Two general routes are common. First, extraction in rather non polar solvents and mixtures like dichloromethane, ethyl acetate, and small amounts of methanol in combination with ammonium hydroxide to raise the pH and improve the solubility of the EAs in the organic phase has been applied (Lauber et al., 2005; Bürk et al., 2006; Müller et al., 2009; Franzmann et al., 2010a; Diana Di Mavungu et al., 2011). For example, Müller et al. (2009) used ethyl acetate/methanol/ammonium hydroxide 25 % (75/5/7; v/v/v). The second route applies rather polar solvents like methanol, acetonitrile in combination with buffers or diluted acids to lower the pH, to extract EAs into a polar-aqueous phase (Krska et al., 2008; Storm et al., 2008; Kokkonen and Jestoi, 2010, Mulder et al., 2012). For example, Storm et al. (2008) used methanol/0.013 M aqueous phosphoric acid (70/30; v/v).

Sample clean-up

Many methods use additional processing to purify and concentrate the crude EA-extracts. Nowadays, solid phase extraction (SPE) has largely replaced multi-step liquid-liquid extraction procedures (Scott and Lawrence, 1980). Several different principles and SPE materials were successfully applied: basic alumina cartridges (Müller et al. 2009; Franzmann et al., 2010a; LFGB, 2012) or dispersive PSA-SPE (Krska et al., 2008). PSA stands for primary and secondary amine; this is a modified silica

functionalised with ethylenediamine groups and providing a strong retention of free fatty acids and other polar matrix compounds. Storm et al. (2008) applied strong cation ion exchange cartridges; Reinhard et al. (2008) used mixed-mode cation exchange/reversed-phase sorbent cartridges; Lauber et al. (2005) used diatomaceous earth based cartridges, while various types of C18 cartridges were used by Shelby and Flieger (1997), Fajardo et al. (1995) and Mohamed et al. (2006a). A modified silica gel containing a specific binder, which allows the selective binding and recovery of the -ine epimers (*D*-lysergic acid derivatives) only, while the *D*-isolysergic acid epimers (suffix -inines) are not retained, has been applied as well (Shelby and Flieger, 1997). Recently, specially designed push-through SPE cleanup columns for EAs were used for sample clean-up, reducing the amount of time for this step to less than one minute (Kokkonen and Jestoi, 2010).

Another approach, "dilute and shoot" is of increasing importance and gains special attention when samples are analysed by HPLC-MS/MS or high resolving MS-analysers. For example, EAs can be extracted by dichloromethane/ethyl acetate/methanol/ammonium hydroxide 25 % (50/25/5/1, v/v/v/v), concentrated and reconstituted with the mobile phase used for the subsequent HPLC-MS/MS analysis (Bürk et al., 2006). Mulder et al. (2012) applied extraction with methanol/water/formic acid (60/40/0.4, v/v/v), followed by ultra-filtration over a 30 kD filter to remove co-extracted proteins and injection into the HPLC-MS/MS system. Similar approaches have been published for multi-residue or multi-family-mycotoxin analysis, which alongside other compounds also detect one or more EA(s) (Driehuis et al., 2008; Mol et al., 2008; Martos et al., 2010).

3.3. Spectroscopic methods: Colorimetry/spectrophotometry, fluorimetry and near infrared spectroscopy (NIR)

The oldest methods used for the detection of EAs are based on the colorimetric method discovered by van Urk (1929). Two molecules of EAs are allowed to react with *p*-dimethylaminobenzaldehyde in acidified solution, yielding after light exposure an intense blue colour, which can be assayed at 580 nm. Later, some improvements and modifications like the addition of ferric chloride or sodium nitrite and combinations were introduced (Jegorov, 1999). Infrared (IR), TLC and colorimetric assays are still part of the European Pharmacopoeia to test for identity and purity of salts of ergometrine and ergotamine (European Pharmacopoeia, 2008). Reaction with ninhydrin was recommended to detect EAs in a colorimetric assay (Zakhari et al., 1991). In general, most EAs are covered by these reactions. Hence, the result usually reflects a sum parameter determination of EAs and provides little information on the individual alkaloids present. A drawback of these colorimetric methods is that also non-EAs such as tryptophan may react, leading to an overestimation of the EA content (Lorenz, 1978).

A number of EAs (especially $\Delta^{9,10}$-ergolenes) show fluorescence upon excitation, which can be used for the EA determination. However, although quite simple in terms of practical implementation, these methods lack specificity.

In addition, NIR was introduced to quantify EAs in tall fescue (*Festuca arundinacea*) (Roberts et al., 1997). It was demonstrated that several wavelengths correlated consistently with the ergovaline content of freeze tried tall fescue but no limits of detection (LODs) or quantification (LOQs) were reported. Instead, the applied range for the method was given ranging from 51 to 495 µg ergovaline per gram dry matter. In a later study, it was concluded that total EA content in tall fescue could be quantified by NIR spectroscopy, but that the calibration of the method required special attention (Roberts et al., 2005). Still, there are only scattered reports utilizing this technique and it remains to be seen whether this methodology can be generally applied to detect EAs in other matrices.

A recent study demonstrated the online detection and quantification of ergot contamination in cereals by hyperspectral images, which were collected using an NIR hyperspectral imaging and multivariate image analysis. The method was meant to fulfil the Commission fixed ergot concentration limits of 0.1 % for feedstuffs containing unground cereals, and the limit of 0.05 % in 'intervention' cereals destined for human consumption. For wheat samples with levels of ergot contamination as low as 0.01 %, it was possible to identify groups of pixels detected as ergot to conclude that the sample was contaminated. In

addition, no false positives were obtained. The LOD and LOQ were 145 and 341 mg sclerotia per kg grain, respectively. Additional studies were done to optimise the parameters in terms of number of samples analysed per unit of time or conveyor belt speed (Vermeulen et al., 2012).

3.4. Immunological methods

Immunological methods such as radioimmunoassays (RIAs) and enzyme linked immunosorbent assays (ELISAs) were developed for EAs from around the beginning of the 1970's when antibodies to *D*-lysergic acid were produced in rabbits and guinea pigs and a radioimmunoassay for the hapten was developed. The driving force was first of all the development of assays for determination of the semisynthetic hallucinogen LSD (van Vunakis et al., 1971). Flieger et al. (1997) reviewed the development up to 1995. More recently, ELISA methods have been used to detect and/or to determine the total concentration of EAs produced by endophytic fungi in plant material and rumen digesta (Tunali et al., 2000; Schnitzius et al., 2001; Ayers et al., 2009). While some of the early assays used polyclonal antibodies, Schnitzius et al. (2001) used monoclonal antibodies produced in the murine hybridoma cell line 15F.E5. Molloy et al. (2003) developed ELISA systems for the determination of dihydroergosine in sorghum ergot based on polyclonal as well as monoclonal antibodies. Both systems were able to detect concentrations above 0.01 mg/kg, quantification being most reliable at concentrations of 0.1 mg/kg or higher. Today different commercial ELISA kits are available. Such kits have among others been used for the determination of total EAs in tall fescue hay (Roberts et al., 2002, 2009), and for the determination of total EA's in urine samples from lambs fed tall fescue (Hopkins et al., 2010). According to the producer of the above mentioned kit, e.g. for the determination of total EA content in plant tissues the method has a limit of detection (LOD) around 2 µg/kg (Hill et al., 2001; Nicholas S., personal information). Such ELISA analysis for "total alkaloids" will be best suited for use in investigations with the aim of obtaining a rough estimate of the contamination of a given feed material.

3.5. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

The use of GC is limited to the analysis of low molecular weight clavines and simple lysergic acid amide EAs. Due to their higher molecular weights and the number of polar groups, the volatility of the ergopetides is low and these compounds cannot be volatilised without degradation (Flieger et al., 2007). Common derivatisation procedures were tested but did not yield uniform products and therefore are of limited use. Only in forensics, LSD abuse is regularly monitored by GC or GC-MS based methods including transformation into trimethylsilyl ethers or trifluoroacetylation at N-1. However, hydrolysis of ergopeptines and subsequent GC-MS analysis can help to identify the amino acids of the peptide-part. In addition, some direct methods, which are based on a controlled quantitative thermal decomposition of ergopeptines in the injection port, subsequent GC-separation of the resulting fragment molecules and MS identification were reported (Jegorov et al., 1997). Although less sensitive and accurate than HPLC methods, these methods were successfully used (Feng et al., 1992).

Lately, a new approach was published to estimate the total sclerotia content of rye (Franzmann et al., 2010a). Ricinoleic acid ((*R*)-12-hydroxy-(*Z*)-9-octadecenoic acid) was quantified by GC-FID after oil extraction and hydrolysis followed by silylation. *Claviceps* spp. contain a high amount of fatty oils (about 30-40 %), of which about 30 % (w/w) is made up by ricinoleic acid, a fatty acid usually not present in grains. Hence, the presence of ricinoleic acid is a marker substance for sclerotia presence and in combination with 15-hydroxy pentadecanoic acid as internal standard, allowed the determination of ergot sclerotia impurities in rye products down to 0.01 %. Unfortunately, no correlation was obtained between the ricinoleic acid concentration and the EA content of sclerotia, therefore the method did not allow for the determination of the total EA content (Franzmann et al., 2010a).

3.6. High performance liquid chromatography (HPLC) and liquid chromatography-(tandem) mass spectrometry (HPLC-MS/(MS))

HPLC-UV and HPLC-FLD

Some comprehensive review articles covering the separation of EAs by liquid chromatography (LC) combined with analysis by spectroscopic methods will be summarized briefly (Flieger et al., 1997; Jegorov, 1999; Scott, 2007; Krska and Crews, 2008).

Starting in the early 1970s the different classes and subclasses of EAs could be separated by normal phase high performance liquid chromatography (NP-HPLC), but these methods fell short in separating individual members of these EA-groups. From the early 1980s, chromatographic separation on reversed-phase (RP), mainly C8 and C18-material, has replaced NP-HPLC and is the most commonly used technique today in the analysis of EAs. The elution on C18 depends on the hydrophobicity and is in ascending order: Clavines, lysergic acid amides, ergopeptines, ergopeptinines. Under alkaline conditions the individual ergopeptines always elute before the corresponding ergopeptinines. This can be used to confirm ergopeptine positive samples. Heating in 0.2 % acetic acid and re-examination for the corresponding ergopeptinines will verify for ergopeptines (Rottinghaus et al., 1993). All common reverse phase-based separations use methanol-water or acetonitrile-water mixtures adjusted to alkaline pH-values by adding modifiers like ammonium hydroxide, ammonium carbonate, ammonium carbamate or triethylamine. UV detection can be used at wavelengths of 310 nm for ergopeptines and ergopeptinines and at 280 nm for dihydroergopeptines. Most methods in use are isocratic.

Sensitivity and selectivity can be increased by fluorescence detection (FLD), which was successfully applied for ergopeptides, dihydroergopeptines, lysergic acid amides and simple ergolene derivatives (Jegorov, 1999). $\Delta^{9,10}$-ergolenes can be excited at a wavelength of 310 nm and then detected at an emission wavelength of 410 nm. $\Delta^{8,9}$-EAs and saturated D-ring EAs show maximal fluorescence with excitation at 272 nm and emission detection wavelength at 371 nm. Hence, double analysis or in-series connection of two fluorescence detectors are necessary to cover a full EA profile (Schardl et al., 2006). HPLC separation on C18 reversed phase columns in combination with FLD is still a state of the art technique for the detection and quantification of trace amounts of EAs in complex matrices and most of the available occurrence data was obtained with this analytical technique (Lauber et al., 2005; Reinhard et al., 2008; Storm et al., 2008; Müller et al., 2009; Franzmann et al., 2010a).

An official §64 method of the German Food and Feed Law (LFGB) which was also tested in an international ring trial with 26 laboratories has recently been validated and published for grain and flour (LFGB, 2012). This method is based on the method by Müller et al. (2006, 2009) and uses ethyl acetate/methanol/ammonium hydroxide 25% (75/5/7; v/v/v) for extraction of a 20 g sample, basic alumina cartridges for sample clean-up and HPLC separation on C6-phenyl or phenyl-hexyl columns and FLD detection. The LOQ for this method ranged from 0.08 to 3.30 µg/kg while the maximum LOQ was obtained for ergometrine (Müller et al., 2009).

HPLC-MS/(MS)

Recently, a variety of MS methods were developed as an alternative for HPLC-FLD to detect and quantify EAs in complex matrices. Due to their molecular structure, EAs are rather polar organic molecules, which makes them favourable targets for electrospray ionization (ESI) mass spectrometric (MS) detection. In many cases ESI-positive mode (ESI+) resulted in more intense protonated molecular ions ($[M+H]^+$) compared to ESI-negative mode (ESI-) producing deprotonated molecular ions ($[M-H]^-$) (Mohamed et al., 2006b).

Some recent studies elucidated the fragmentation patterns of common EAs in ESI+ mode of triple quadrupole and ion-trap analysers and facilitates the application of HPLC-ESI-MS/MS methods for EA-

analysis (Lehner et al., 2004; Mohamed et al., 2006b). Collision-induced dissociation (CID) of the $[M+H]^+$ ions of simple lysergic acid derivatives and ergopeptine alkaloids results in common fragmentation behaviour. One set of fragment ions (*m/z* = 208, 223 and 268) is class specific and common for all simple lysergic acid derivatives and ergopeptine alkaloids. Furthermore, the $[M+H-H_2O]^+$ and [peptide moiety-H_2O]$^+$ ions are generally observed and compound specific. These features can be used for precursor ion scans, using the marker ions *m/z* = 223 and/or 208 to screen for unknown or unexpected EAs in complex matrices (Mohamed et al., 2006b). Sensitive and selective multi reaction monitoring (MRM) MS methods on triple quadrupole instruments frequently use a combination of compound and class specific transitions, such as $[M+H]^+ \longrightarrow$ *m/z* = 268, 223 and/or 208 for quantification or as qualifiers, in combination with the additional criterium of a fixed ion ratio for two simultaneously measured transitions for each individual compound (Mohamed et al., 2006a; Krska et al., 2008). In addition, it was demonstrated that ESI-MS/MS can be used as a tool to distinguish between the epimeric forms of ergopeptine alkaloids. It was observed that for *D*-lysergic acid peptide alkaloids fragment ions *m/z* 208 and 223 were produced in an almost 1:1 ratio, while for the *D*-isolysergic acid peptide alkaloids (suffix -inine) both fragment ions were produced in a ratio of 1:2 (Shelby et al., 1997).

Krska et al. (2008) reported an HPLC-MS/MS method for the main 12 EAs. The method comprises organic extraction, filtering and dispersive solid-phase extraction. The final validation was carried out for ten different matrices on six different days applying a special analytical scheme to limit the number of samples. In total, four different concentrations were analysed (blank, 5 µg, 50 µg and 100 µg of each of the 12 EAs per kg). On each of the six independent validation days five of the matrices at levels 5 and 100 µg/kg and five of the matrices at the levels "blank" and 50 µg/kg were analyzed. LOQs for the ergopeptine-isomers and ergometrine ranged from 0.45 to 2.8 µg/kg.

To check for comparability and for potential systematic errors, this newly developed method was compared in a mini-intercomparison study together with two other laboratories (one using a similar method, the other one using an HPLC-FLD method (based on that of Müller et al., 2006). There, three different sample types were analysed by the three different laboratories: a high-level barley containing an estimated level for individual EAs of 0.5–50 mg/kg, a mixture of barley with wheat yielding a reduced-level sample (5–500 µg/kg EAs), and a low level sample of rye flour containing less than 50 µg total EAs/kg. Good comparability of measurement results with almost all deviations within the measurement uncertainties of the respective methods could be demonstrated and only sporadically, exceptions for single analytes or matrices were found (Krska et al., 2008).

Recently, an HPLC-MS/MS method was used to monitor for the presence of the 12 major *C. purpurea* EAs in a large European survey on food and feed commodities (Diana Di Mavungu et al., 2011). The reported LODs for the individual compounds and matrices varied between 0.05 and 0.4 µg/kg and the LOQs between 0.2 and 1.3 µg/kg.

Combination of ultra high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) with dedicated EA-immunoaffinity columns resulted in low LOQs of 0.01 to 0.06 µg/kg for 5 ergopeptines in wheat (for ergometrine, the reported LOQ was 1.0 µg/kg) and of 0.01 to 0.1 µg/kg in rye for 10 different EAs (ergometrine: 10 µg/kg). The run time of the method was less than 5 min (Kokkonen and Jestoi, 2010).

A new trend in the HPLC-MS/MS analysis of EAs is the integration in multiresidue mycotoxin screening methods. For example, Sulyok et al. (2007) developed a method that was successfully applied for a semi-quantitative survey including all 12 major *C. purpurea* EAs in addition to 12 other EAs representing other *Claviceps* species like *C. fusiformis*. A simplified extraction work-up was incorporated in this method and LODs of 0.07 to 4 µg/kg were reported.

Eight EAs (ergosine, ergocristine, ergocornine, ergocryptine and their corresponding epimers) were included in a multi-method for 32 mycotoxins in beer using UHPLC-Orbitrap-MS (Zachariosova et al.,

2010). A simple work-up (extraction with acetonitrile, followed by evaporation of the solvent and reconstitution in mobile phase solvent) in combination with high resolution MS allowed for low detection limits, expressed as lower calibration levels, varying between 0.5 and 5 µg/l, depending on the alkaloid and type of beer analysed.

In conclusion, HPLC-MS/MS and HPLC-FLD methods show similar performance characteristics. Figure 9 shows an example of an HPLC-FLD analysis of the main EAs extracted from *C. purpurea* ergot.

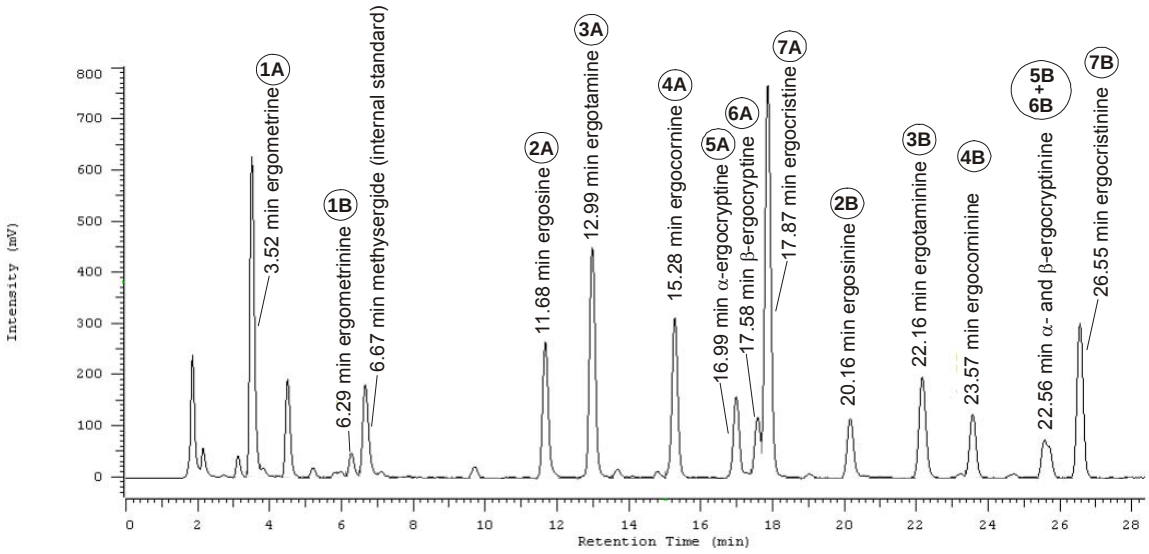


Figure 9: HPLC-FLD analysis of EAs from *C. purpurea* ergot. Each pair of EA-epimers shares the same number whereas -ine epimers are characterised by the capital letter A and the corresponding -inine epimers by the capital letter B (stationary phase C18 , mobile phase: acetonitrile/ammoniumcarbamate buffer gradient, excitation wavelength: 330 nm; emission wavelength 415 nm) (Figure according to Franzmann et al., 2010).

Referring to Figure 9 there are critical areas of overlapping peaks, which add some uncertainty to the identification and quantification of all 14 individual EAs. Particularly in HPLC-FLD, the two EAs β-ergocryptine (6A) and ergocristine (7A) are not always separated into two distinct peaks, which means, in those cases β-ergocryptine (6A) remains unnoticed and at the same time the values for ergocristine (7A) are misstated (including β-ergocryptine (6A)). Using HPLC-MS/MS approaches, β-ergocryptine (6A) and ergocristine (7A) although not separated in time, can be easily distinguished due to the difference in molecular mass (β-ergocryptine, 575 amu and ergocristine 609 amu). In addition, α- and β-ergocryptinine are not separated in state-of-the art analytical approaches (5B and 6B). Here, HPLC-MS/MS also reaches its limits, since α- and β-ergocryptinine (5B and 6B) are isomers, isobaric by nature.

In conclusion, state-of-the-art HPLC-FLD analysis of the main *C. purpurea* alkaloids will result in the determination of the concentrations of 12 EA, namely: ergometrine (1A), ergometrinine (1B), ergosine (2A), ergosinine (2B), ergotamine (3A) ergotaminine (3B), ergocornine (4A), ergocorninine (4B), α-ergocryptine (5A), ergocristine (7A; maybe elevated by a small amount of unresolved β-ergocryptine (6A)), ergocristinine (7B) and the sum of α- and β-ergocryptinine (5B+6B).

HPLC-MS/MS analysis, on the other hand, will be able to provide the quantitation of 13 EAs, including β-ergocryptine (6A) most likely accompanied with a more accurate determination of ergocristine (7A) but also containing only one value for the sum of α- and β-ergocryptinine (5B+6B).

Concerning these critical EAs, the reported data of EAs in the literature are not always precise and most of the time only subsets of the above mentioned 14 main *C. purpurea* alkaloids are quantified and presented. Especially α/β-ergocryptine (and the corresponding -inines) are sometimes presented as individual compounds, as the sum of both, or mentioned in a not clearly defined manner.

For example, Müller et al. (2009) demonstrated the HPLC-FLD separation of 12 EAs, namely, ergometrine, ergosine, ergotamine, ergocornine, α-ergocryptine, ergocristine and the corresponding -inines (12 EAs), while in the results section, α-ergocryptine is replaced by ergocryptine, leaving some uncertainty if only the final results would be used for data evaluation. Similar is true for a recent survey on EAs in cereals intended for human consumption and animal feeding (Diana Di Mavungu et al., 2011). There, also no differentiation between different ergocryptines (and -inines) is made and 12 EA values are reported.

For the currently used HPLC-FLD approach LODs ranged from 0.02 to 1.1 µg/kg and LOQs ranged from 0.08 to 3.3 µg/kg for 12 different EAs (6 -ines and the corresponding 6 -inine epimers, see above Müller et al. (2009)). Diana Di Mavungu et al. (2011) reported LODs of 0.05 to 0.4 µg/kg and LOQs of 0.2 to 1.3 µg/kg for the same set of compounds using HPLC-MS/MS.

3.7. Reference materials, validation and proficiency tests

This section lists and annotates relevant and available standard reference materials and sources as reported in the latest publications on EA-analysis (Table 1). Additional information can be obtained from a recently published similar list (Battilani et al., 2009). The following list does not claim to be exhaustive.

Currently only one rye matrix certified reference material is available showing rather high uncertainties for the 12 EAs tested.

Table 1: Available standard compounds cited in publications within the last five years.

Compound	CAS No.	Reference
Naturally occurring		
Ergometrine	60-79-7	1-4
Ergometrinine	479-00-5	1, 3, 4
Ergosine	561-94-4	1-4
Ergosinine	596-88-3	1-4
Ergotamine (salt)	113-15-5	1, 3, 4
Ergotaminine	639-81-6	1, 3, 4
Ergocornine	564-36-3	1-4
Ergocorninine	564-37-4	1-4
α-Ergocryptine	511-09-1	1-4
α-Ergocryptinine	511-10-4	1-4
β-Ergocryptine	20315-46-2	1
β-Ergocryptinine	19467-61-9	1
Ergocristine	511-08-0	1-4
Ergocristinine	511-07-9	1-4
Ergostine	2854-38-8	1
Ergostinine	3268-95-9	1
Semi-synthetic		
Methysergide (salt)	361-37-5	1
Dihydroergocristine	17479-19-5	
Dihydroergotamine (salt)	511-12-6	4
Bromocriptine (salt)	25614-03-3	5
D-lysergic acid	82-58-6	
Methylergometrine (salt)	113-42-8	4

[1] Reinhard et al., 2008
[2] Zachariasova et al. 2010
[3] Krska et al., 2008
[4] Diana Di Mavungu et al., 2011
[5] Bicalho et al., 2005

An HPLC-FLD method for the determination of the 12 EAs in grain and flour has been internationally validated in an international ring trial with 26 laboratories for grain and flour and published (LFGB, 2012). However, no fully validated or standardized methods are available for the separation and detection of EAs species by HPLC-MS/MS.

There is only one proficiency study provided on a regular basis.

3.8. Conclusions

Analytical methods available so far have mainly focused on EAs from *C. purpurea*. For other *Claviceps* spp. relevant to food and feed, data are sparse and no conclusions can be drawn.

Screening for the total ergot sclerotia content in (unground) grains (which does not necessarily correlate with EA-content) to meet current EU legislation for feed (Directive 2002/32/EC[6]) can be achieved by visual inspection, by determination of ricinoleic acid by GC-FID (Franzmann et al., 2010a) and possibly by using NIR-methods (Vermeulen et al., 2012).

HPLC-FLD and HPLC-MS/MS are currently the only two analytical methods that allow the unambiguous identification and quantification of the major *C. purpurea* EAs. HPLC-FLD permits the determination of 12 analytical values for the most frequent *C. purpurea* EAs namely ergocornine, ergocorninine, ergocristine, ergocristinine, α-ergocryptine, ergometrine, ergometrinine, ergosine,

ergosinine, ergotamine, ergotaminine and the sum of α- and β-ergocryptinine. HPLC-MS/MS enables, in addition, the determination of β-ergocryptine.

The degree of epimerisation is difficult to control during sample preparation and analysis. In addition, both epimeric forms can inter-convert. Hence, it is necessary to determine both epimers and specify the EA-content as a sum of both epimers for each EA. Major drawbacks, in particular for HLPC-FLD-based methods, are the labour-intensive sample preparation and rather long chromatographic runtimes to separate the epimers (Lauber et al., 2005). Recent developments in HPLC and HPLC-MS/MS technology (UHPLC) will most likely result in much higher throughput rates by reducing the sample preparation as well as separation time (Bürk et al., 2006; Kokkonen and Jestoi, 2010).

Based on HPLC-FLD, an official §64 method of the German Food and Feed Law (LFGB) which was also tested in an international ring trial with 26 laboratories has recently been validated and published for grain and flour (LFGB, 2012).

The availability and stability of standards still represents a problem. Especially β-ergocryptine and all -inine epimers are not always available and are costly. Isotopically labelled standards, useful for HPLC-MS/MS approaches are currently not available. Since EA-derivatives are used or tested as pharmaceuticals some analytical methods use semi-synthetic EAs as alternatives for internal or surrogate standards for quantification or as indicators for method performance.

In addition, since some of these reference compounds are used as starting materials for the synthesis of hallucinogenic compounds (LSD and derivatives) the handling and storage of these compounds is regulated in some Member States and special permissions are needed and regulatory requirements need to be fulfilled.

There is only one proficiency study provided showing relatively high standard deviation on the analytical results for the tested EAs in naturally contaminated rye.

4. Occurrence of ergot alkaloids in food and feed

4.1. Previously reported occurrence results

4.1.1. Food

A survey to identify the occurrence of mycotoxins in infant cereal foods in the Canadian market was conducted by Health Canada between 1997-1999 (Lombaert et al., 2003). Infant cereal food samples (comprising 273 North American and 90 European products) were collected from the Canadian retail market. Among other mycotoxins, ergotamine, ergosine, ergocornine, α-ergocryptine and ergocristine were monitored in 162 out of 363 samples. An HPLC-FLD method was used with a reported LOQ (S/N of 10) of 4 µg/kg for the individual EAs. EAs were detected in 41/162 samples, including the majority of barley-based cereal samples (31/55), 6/75 multi-grain cereal samples, 2/9 teething biscuits and 2/6 oat-based cereal samples. No EAs above the LOD were detected in 7 soy-based samples, 9 rice-based samples and 1 soy formula. Barley-based cereal samples showed also the higher levels (maximum content of 108 µg/kg and mean content of 18 µg/kg).

An investigation of 34 samples of rye flour (17 organic and 17 conventional) sampled between 2000 and 2005 was conducted in Denmark (Storm et al., 2008). The samples were analysed by a method including solid-phase cation-exchange and HPLC-FLD. Ten alkaloids (ergotamine, ergometrine, ergocornine, α-ergocryptine and ergocristine and their corresponding epimers) were analysed; the reported LODs for the individual compounds were in the range of 0.2 to 1.1 µg/kg. EAs were detected in 32 out of the 34 analysed samples, with ergotamin(in)e and α-ergocryptin(in)e being the most common alkaloids. The distribution between individual EAs was shown to be highly variable with some samples containing all five ergopeptines and their epimers (which could have been formed during analysis), while others contained only a few types, consistent with variations between ergot sclerotia

from different locations shown in the same publication. Mean total EA contents of 60 µg/kg and 32 µg/kg with maximum concentrations of 230 µg/kg and 100 µg/kg were observed in conventional and organic samples, respectively.

In total, 109 samples of bread, flour, infant formula and baby food samples (containing rye and wheat) were collected in Switzerland in 2001, 2003 and 2005 and analysed for the content of EAs (Reinhard et al., 2008). Samples from 2001 were analysed by means of HPLC-FLD and HPLC-MS/MS, whereas only the latter analytical technique was applied with the samples collected in 2003 and 2005. Reported LODs for the FLD method were: 0.4 µg/kg for ergometrine and 1-5 µg/kg for the other EAs and for the HPLC-MS/MS reported LODs were: 2 µg/kg for the -ines and 1 µg/kg for the –ininες. Results were reported as the sum of 16 alkaloids: ergometrin(in)e, ergosin(in)e, ergotamin(in)e, ergocristin(in)e, ergostin(in)e, ergocornin(in)e, ergocristin(in)e, α- and β-ergocryptin(in)e. Rye flour and bread showed the highest contents of EAs, although considerable fluctuations were noted among the different samples. A maximum concentration of 519 µg/kg was observed in rye flour collected in 2001 (median concentration of 172 µg/kg is reported in 13 samples). In rye bread, maximum levels of 248 and 477 µg/kg were detected in 2001 (median content of 87 µg/kg for 14 samples) and 2003 (median concentration of 120 µg/kg in 7 samples), respectively. Levels ranging from 5 to 45 µg/kg were measured in bread not containing rye (3 samples in 2001 and 3 samples in 2003). In wheat flours, a maximum total EA concentration of 211 µg/kg was found in 2001, with median levels ranging from 6 to 103 µg/kg in different wheat flour types over the 3 years. Lower levels were measured in infant formula and baby food samples containing rye (maximum level of 26 µg/kg in 9 samples, median level of 1 µg/kg).

Klug et al. (1988) investigated 118 cereal-based food products collected from German outlets on their EA content with an HPLC-FLD method (LODs varying from 1.5 µg/kg for ergometrine to 10 µg/kg for ergocristinine). In total, 29 samples contained EAs in the range of 12 to 199 µg/kg. No differences were noted between products containing conventionally or organically grown cereals.

Lauber et al. (2005) compared the levels of total EAs in rye meals and grains collected from German conventional and organic agriculture from 2 harvest years, 2003 and 2004. Samples were analysed for the 12 major *C. purpurea* EAs using an HPLC-FLD method, but no LODs or LOQs were stated. EAs were detected in all samples. Higher levels were detected in conventional and organic samples from 2003, reflecting the different climatic conditions of the 2 years. In 2003, higher EA levels were measured in the 18 samples grown with conventional methods (maximum content of 3280 µg/kg, median content of 850 µg/kg) in comparison with the 12 organically grown samples (maximum EA content of 1490 µg/kg, median content of 196 µg/kg). For the 2004 harvest a lower difference was noted for median EA concentrations (220 and 256 µg/kg calculated for 15 conventional and 6 organic samples, respectively.

Bürk et al. (2006) reported the results of a survey conducted by the Bavarian Health and Food Safety Authority in rye products between 2004 and 2006. The content of five alkaloids (ergometrine, ergocornine, α-ergocryptine, ergocristine and ergotamine) was determined in 66 samples by means of HPLC-MS/MS analysis (LOQ ranging between 0.1 and 1.0 µg/kg for the analysed alkaloids). Results were reported as total alkaloid content. The highest concentration of total alkaloids was measured in a sample of rye bread (258 µg/kg). A content > 10 µg/kg was measured in 14 out of 23 rye bread samples. The majority of the samples of pumpernickels and rye-bread rolls were below the LOQ. A maximum content of 47 µg/kg and 28 µg/kg was measured in pumpernickels (20 samples) and rye bread rolls (14 samples), respectively. Concentrations of 11, 31 and 91 µg/kg were detected in 3 out of 9 samples of rye crispbread, with the remaining samples being below the LOQ.

In a German study on the EA content (ergometrine, ergotamine, ergocryptine, ergocristine, ergocornine) of 58 rye samples collected in 2005, an average EA concentration of 397 µg/kg was obtained (Masloff, 2006). Ergotamine (39 %) and ergocristine (34 %) were the major EAs found. Twelve samples exceeded 1 000 µg/kg total EA content with a maximum of 2 350 µg/kg. The same study reported on the analysis of 103 samples collected in the period 1997-2005 from the production chains for human and

animal products (covering (feed) grains, bran, flour and bread). Highest average and maximum EA content was found for flours (n = 61, average 1 782 µg/kg, maximum 18 114 µg/kg). Most flour samples (37) had an EA content of less than 500 µg/kg. For bread (n = 12), the average amount was 415 µg/kg and the maximum 1 307 µg/kg for the sum of the 5 EAs studied. It should be noted that the report is not clear on the extent to which the samples investigated were obtained by random sampling or whether (in part) selective sampling was used.

Müller et al. (2009) investigated the EA content of 39 German rye and rye product samples from the harvest of 2006/2007. Using an HPLC-FLD method for the determination of 12 EAs, a mean total concentration of 137.5 µg/kg for rye flour (n = 22), 62.2 µg/kg for rye (n= 7), 157.7 µg/kg for coarse rye meal (n = 7) and 26.4 µg/kg for rye flakes (n = 3) were reported. The maximum amounts found were, respectively, 715, 197, 740 and 66 µg/kg. Ergocristine and ergotamine were the most prominent EAs present, followed by ergocornine and ergosine.

An investigation of 45 rye flours and bran (39 samples of conventional and 6 of organic production), collected in 2009 in Baden-Württemberg, Germany, revealed the presence of EAs in 43 samples (LODs for individual EAs: 15 µg/kg) (Neef et al., 2009). An average content of 422 µg/kg was found, with a maximum of 2 411 µg/kg.

The EA content was determined in 31 rye flour and wholemeal rye flour samples taken in 2009 from retail shops in Lower Saxony (Germany) (Reinhold and Reinhardt, 2011). The 12 major EAs of *C.purpurea* were analysed by HPLC-MS/MS with a reported LOD of 10 µg/kg. Sixteen samples (52 %) contained EAs above the LOD, with a mean total EA content of 213 µg/kg and a maximum of 1 063 µg/kg. Three samples exceeded 500 µg/kg. The average content for the individual EAs (each reported as sum of both epimers) varied between 42.4 µg/kg for the sum of ergometrin(in)e and 94.5 µg/kg for the sum of ergotamin(in)e.

Crews et al. (2009) analysed the content of EAs in 28 rye-based food samples purchased from a food retailer in the UK. The major EAs (ergotamine, ergometrine, ergocristine, ergocryptine, ergocornine and ergosine) and their corresponding epimers were analysed by HPLC-MS/MS. The LODs for the individual compounds were in the range 0.4-2.7 µg/kg and the LOQs were between 0.5 and 2.8 µg/kg. The analysed food products were both of conventional and organic origin and included samples of rye bread (11), rye crispbread (8), rye flakes (2), rye flour (1), crackers (2) and 4 samples of bread and bread mix (mixed cereals). EAs were detected in all but three of the samples. Highest values were recorded in the rye crispbread samples, with maximum total EA levels of 131, 183 and 340 µg/kg. A total EA content ≥ 20 µg/kg was detected in 6 out 8 samples of rye bread, with a maximum value of 121 µg/kg. In the other food products, levels were lower or non detectable. Co-occurrence of all the monitored EAs was generally observed in the rye bread samples, with ergosin(in)e, ergotamin(in)e and ergocristin(in)e mostly found at higher levels. In rye crispbread, ergotamin(in)e, ergometrin(in)e and ergocristin(in)e were always detected at higher levels compared to other alkaloids. Differences in the -ine to -inine ratio were noted between the rye crispbread and rye bread samples. In the latter, -inine levels were higher than -ine levels, whereas rye crispbreads showed a less consistent pattern, possibly reflecting the higher variability in the manufacturing processes. No clear differences were observed between organic and conventional food.

Recently, Diana Di Mavungu et al. (2011) reported on the presence of EAs in 182 rye and 127 wheat food samples collected from mills/silos in eight different European countries (Estonia (14 wheat, 34 rye), Finland (13 wheat, 28 rye), France (25 wheat, 12 rye), Germany (17 wheat, 30 rye), Italy (12 wheat), Poland (15 wheat, 30 rye), United Kingdom (15 wheat, 22 rye) and Sweden (16 wheat, 26 rye). Most samples originated from the harvest of 2010, a lesser part from 2009. Samples were analysed by an HPLC-MS/MS method (LOQ: 1 µg/kg) for the 12 main EAs (ergotami(ni)ne, ergosi(ni)ne, ergocristi(ni)ne, ergocrypti(ni)ne, ergocor(ni)ne and ergometri(ni)ne). For the rye food samples a mean total EA content of 94 µg/kg was found with a maximum of 1 120 µg/kg for a rye sample from the UK. In 95 % of the rye samples one or more EAs were detected above the LOQ. In approximately 75 % of the samples the total EA content was less than 100 µg/kg. Of the samples analysed, those from France

contained overall the highest contamination (between 29 and 928 µg/kg, median: 164 µg/kg, n = 12) and those from Sweden overall the lowest contamination (between 0 and 152 µg/kg, median 10 µg/kg, n = 26). The wheat samples were slightly less frequently contaminated (86 % contained EAs above the LOQ) and the maximum reported was 605 µg/kg for a wheat sample from France. The highest contamination was found for the French samples (all samples > 1 µg/kg, n = 25), the lowest for the Finnish samples (46 % < LOQ, maximum found: 2 µg/kg, n = 13).

In the same study also 182 food samples collected from Belgium shops were analysed (Diana Di Mavungu et al., 2011). The samples included 40 rye-based, 61 wheat-based and 81 multigrain-based products. With respect to the country of production, Belgian products formed the largest group (76 samples), but also products from France (n = 36) and the Netherlands (n = 34) were well represented. The group of rye-containing products consisted of 19 rye bread and 21 rye flour samples. The mean EA contamination in these samples was 20 µg/kg with a maximum of 95 µg/kg for a sample of rye bread. The group of wheat-containing products consisted of 21 wheat bread, 22 wheat flour and 18 wheat bran samples. The wheat bran samples revealed a relatively high mean contamination of 174 µg/kg (median 119 µg/kg) and with a maximum of 533 µg/kg. For the remaining wheat bread and flour samples the mean contamination was 9 µg/kg and the maximum 71 µg/kg. The group of multigrain-based products consisted of 17 bread, 14 flour, 12 flakes, 8 crispbread and 30 biscuit samples. The average contamination with EAs was 8 µg/kg with a maximum of 58 µg/kg for a multigrain flour sample. In 75 % of the samples the contamination was 10 µg/kg or less.

4.1.2. Feeds

In 2005, a survey was undertaken in the Netherlands to determine the occurrence of mycotoxins in feeds fed to dairy cows (Driehuis et al., 2008). Twenty-four farms were visited, and a total of 169 feed samples analysed. Ergotamine was not detected in any of the samples above the LOQ of 50 µg/kg.

Ruhland and Tischer (2008) reported on the EA content of 64 grain feeds and 64 mixed grain feeds, collected in Bavaria (Germany) between 2005 and 2007. The samples were analysed for five EA (ergometrine, ergocornine, ergocristine, ergocryptine, ergotamine) by HPLC-FLD and the reported LOD was 5 µg/kg. EAs were detected in 91 % of the grain and mixed feed samples, the majority (66 %) contained concentrations between 10 and 250 µg/kg. A median level of 70 µg/kg was found for the mixed feeds, with a maximum of 4 883 µg/kg for a mixed feed for pigs. In the grain feeds the median concentration was 54 µg/kg and the maximum found was 1 236 µg/kg for a rye feed.

Diana Di Mavungu et al. (2011) analysed by HPLC-MS/MS 148 rye feed, 137 wheat feed and 27 triticale feed samples collected from feed mills during 2009 and 2010 from 10 different European countries (Belgium (12 wheat, 6 rye), Czech Republic (26 wheat, 19 rye, 11 triticale), Denmark (11 wheat, 17 rye), Finland (17 wheat, 25 rye), France (1 wheat), Germany (14 wheat, 9 rye, 6 triticale), Poland (17 wheat, 33 rye, 4 triticale), Sweden (13 wheat), Switzerland (12 wheat, 7 rye, 6 triticale), the Netherlands (14 wheat, 32 rye)). For rye feed a mean content of 319 µg/kg was found with a maximum of 12 340 µg/kg. The contamination, however, was rather variable: in 48 % of the samples no EA could be detected above a LOQ of 1 µg/kg and in 75 % of the samples the contamination was less than 54 µg/kg. In 10 % of the samples the total EA content was 588 µg/kg or more. Significant differences were observed with respect to the country of origin: samples from Switzerland were mostly highly contaminated with EAs (6 out of 7 samples analysed contained between 1 082 and 12 340 µg/kg), while most of the rye samples (26 out of 32) collected in the Netherlands contained no measurable amount of EAs. For wheat feed a much lower mean EA concentration (19 µg/kg) and maximum (702 µg/kg) were reported. Also the percentage (68 %) of blank samples was higher than for rye feed and in 75 % of the samples the contamination with EAs was 1 µg/kg or less. Only 5 out of 137 samples analysed exceeded 100 µg/kg. For the triticale feed samples a mean EA concentration of 62 µg/kg was found and a maximum of 1103 µg/kg. However, only 27 samples were analysed, 14 of which (52 %) did not contain EAs.

Mulder et al. (2012) reported on the analysis of 184 feed samples collected in the Netherlands during 2007-2010. The study included 134 cereal samples (69 rye, 45 triticale, 18 wheat, 2 barley, 2 mixed grains) and 48 compound feeds, analysed by HPLC-MS/MS (LOD: 2 µg/kg) for the 12 major EAs of *C. purpurea* together with β-ergocryptin(in)e and some minor EAs, such as agroclavine, chanoclavine-1, elymoclavine, ergine and festuclavine. An average EA concentration of 134 µg/kg was found for the rye feeds, with an maximum of 1231 µg/kg, while no or only trace amounts (<10 µg/kg) were found in 49 % of the samples. The average EA concentration for triticale was 33 µg/kg with a maximum of 297 µg/kg, and 67 % of the samples were (practically) free of EAs. For wheat, the average was 54 µg/kg, the maximum 529 µg/kg and 61 % contained no or almost no EAs. The average EA concentration for all cereal samples was 93 µg/kg. Regarding the compound feeds, a much higher incidence of EAs was found (83 % contained ≥ 10 µg/kg EAs), with a maximum of 583 µg/kg and a mean concentration of 77 µg/kg. It was noted that compared to the cereal feeds, the compound feeds had a relatively high average EA content, which was unexpected taking into account that grains make up only part of the compound feeds.

Kim et al. (2007) reported levels of ergovaline ranging from 21 to 782 µg/kg in 25 samples of tall fescue hay infested with *Neotyphodium coenophialum* that were imported into Korea (country of origin not given). Other EAs potentially present in the tall fescue hay were not analysed.

4.2. Current occurrence results

4.2.1. Data collection summary

The Dietary and Chemical Monitoring Unit (DCM) call for data on the presence of EAs in food and feed[12] was launched in July 2010. European national food authorities and similar bodies, research institutions, academia, food and feed business operators and any other stakeholders were invited to submit analytical data on EAs. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a). In addition, data on the occurrence of EAs in 803 samples in different cereals intended for human consumption and animal feeding were obtained through an Article 36 grant (CFP/EFSA/CONTAM/2010/01) awarded to Ghent University (Diana Di Mavungu et al., 2011).

Figures 10 and 11 show the countries where the samples producing the different analytical results were collected and the distribution of these analytical results over the sampling years, respectively. More than 80 % of the samples were collected between 2009 and 2011.

[12] http://www.efsa.europa.eu/en/data/call/datex101217.htm

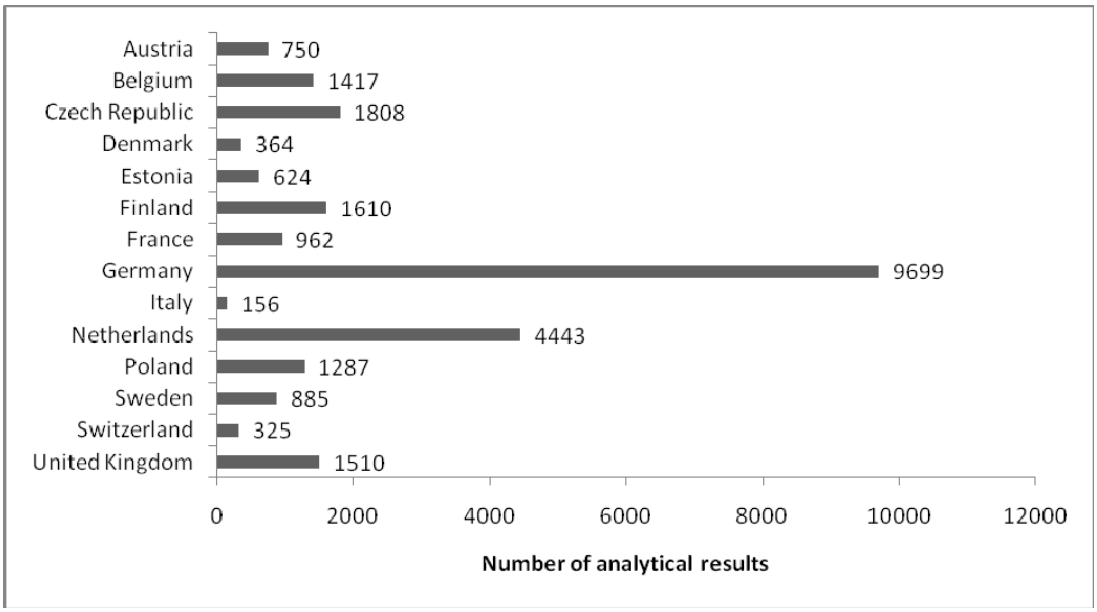


Figure 10: Distribution of analytical results by European sampling country. Results were reported for food, feed, and unprocessed grains of unknown end-use.

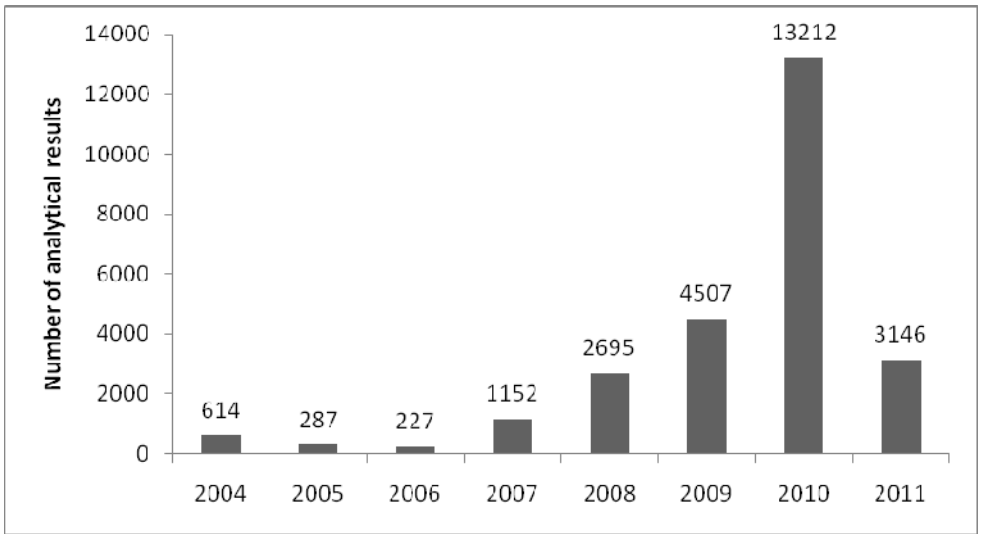


Figure 11: Distribution of the analytical results by sampling year. Results were reported for food, feed, and unprocessed grains of unknown end-use.

To ensure the quality of data included in the assessment, several data cleaning and validation steps were applied. Analytical results with incomplete or incorrect description of the relevant variables (e.g. parameter type, food classification, result value, LOD or LOQ) were not included in the data sets used in this assessment. The data sets were checked for duplicates (same sample transmitted twice or repeated analysis of the same sample) and all duplicates were excluded. In addition, several imputations were carried out. The two isomers of ergocryptinine are not separated by liquid chromatography and, therefore, in the samples that reported α-ergocryptinine this was considered as the sum of α- and β- isomers. Likewise, α- and β- isomers of ergocryptine were considered together. An average ratio of 1.3:1 (α:β) was calculated among those samples where both isomers of ergocryptine were reported. This ratio was applied to calculate the occurrence value of the missing isomer in the samples where only one isomer was analysed and quantified. By using this approach, 465 results were added to the data set as

the sum of α- and β-ergocryptine. Samples where only α-ergocryptine or β-ergocryptine were reported without being quantified (< LOD or < LOQ) were excluded from the dataset since no ratio could be applied. In order to avoid a bias of the outcome of the exposure assessment, analytical results with LOD > 10 µg/kg and LOQ > 20 µg/kg were excluded (a total of 3 031 results). The CONTAM Panel emphasizes that this truncation does not entail a recommendation for future performance criteria of monitoring programmes as analytical state of the art methods allow considerably lower LODs/LOQs. The truncation was only applied to avoid a bias due to unrealistically high upper bound values in the exposure assessment.

The analytical results excluded due to incomplete or incorrect description of relevant variables as well as those excluded due to the selected cut-off for LOD and LOQ values are referred to hereafter as non-qualifying data.

Finally, from an initial number of 25 840 analytical results a total of 20 558 were used in this assessment, which included food and feed samples and unprocessed grains of unknown end-use. This number of analytical results represents a total number of 2 279 samples, of which 1 716 corresponded to food, 496 to feed and 67 to unprocessed grains of unknown end-use (Figure 12). These samples were collected in 13 different European countries (12 Member States and Switzerland) between 2004 and 2011.

Considering the imputations described above, the analysed samples included in the final dataset contained between one and twelve of the selected EAs (ergocornine, ergocristine, ergocryptine (α- and β-isomers), ergometrine, ergosine, ergotamine and the corresponding epimers).

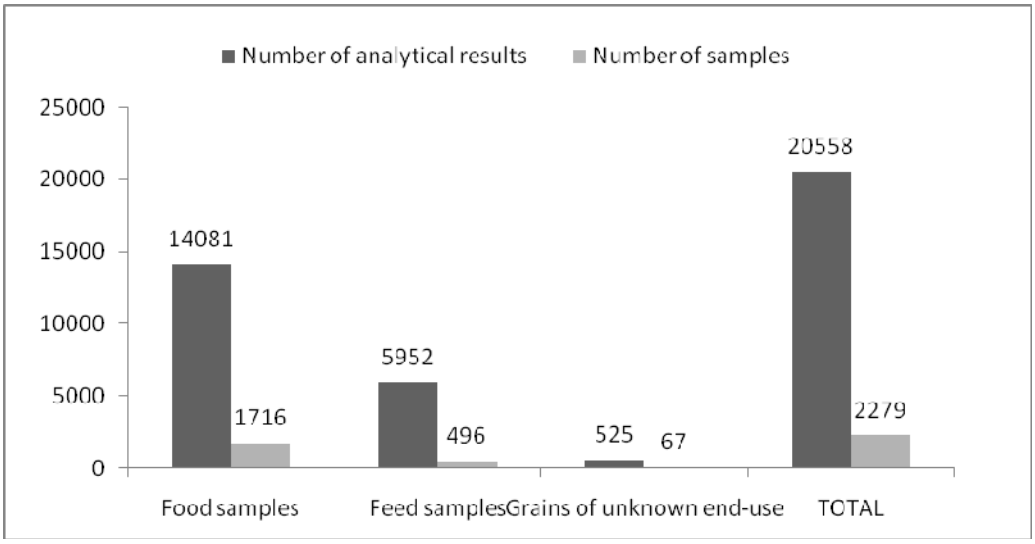


Figure 12: Number of analytical results for individual ergot alkaloids and number of samples used in the evaluation of the occurrence data (after excluding non-qualifying data). Analytical results and sample numbers are shown for three different categories: food, feed, and unprocessed grains of unknown end-use.

4.2.2. Data collection on food

After excluding non-qualifying data, a total number of 14 081 analytical results on food were available which corresponded to 1716 samples, most of them analysed between 2009 and 2011. The food samples were collected in 11 different countries, with Germany being the country with the highest number of samples.

Among the food samples, there was a great variation in the number of EAs for which data were reported as can be seen in Figure 13. Samples where data were reported for the 12 EAs represent the highest proportion with almost 35 % of the total food samples.

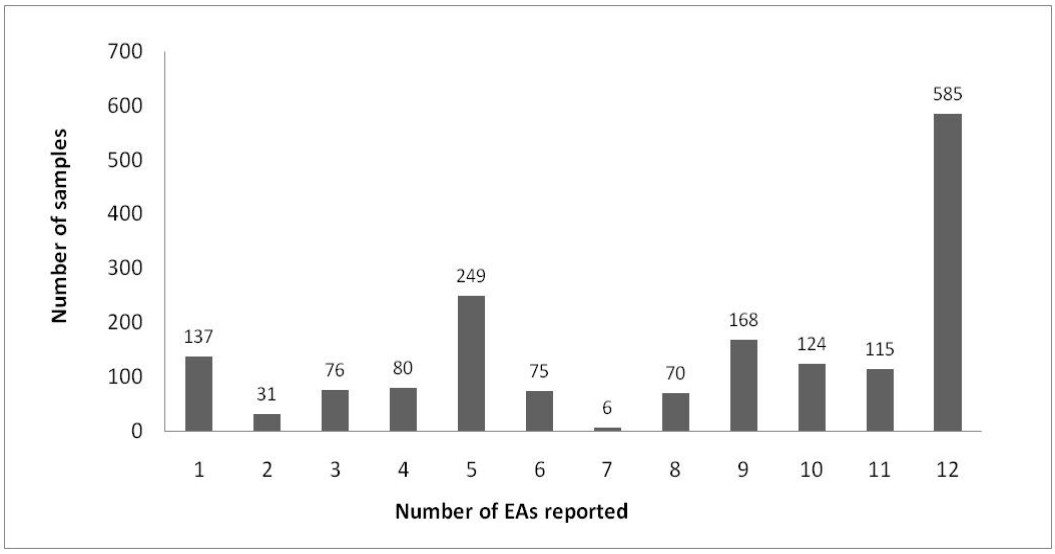


Figure 13: Distribution of food samples by the number of ergot alkaloids for which data were reported (after excluding non-qualifying data).

4.2.3. Data collection on feed

After excluding non-qualifying data, a total of 5 952 analytical results grouped in 496 feed samples were available, all with the 12 EAs reported. Feed samples were collected in ten countries across Europe, with main contributions from the Netherlands (230 samples) followed by Czech Republic (56 samples), Poland (54 samples) and Finland (42 samples). Most of the samples were collected in 2010 (73.5 %), although samples were also collected in 2007 (34 samples), 2008 (51 samples) and 2009 (46 samples).

4.2.4. Data collection on unprocessed grains of unknown end-use

In principle, this group of samples is not considered either food or feed because their final use is unknown. In this group, the sampling years were exclusively 2009 and 2010 and only 67 samples from just two countries, Finland and Sweden, were reported. The number of EAs reported in these samples was diverse, ranging from two to eleven EAs (Figure 14). For most of the samples (60) analytical data for eight EAs were reported.

Three groups of grains are represented in this category, with rye grain samples being reported in 36 cases, barley grain samples in 23 cases and wheat grain samples in 8 cases.

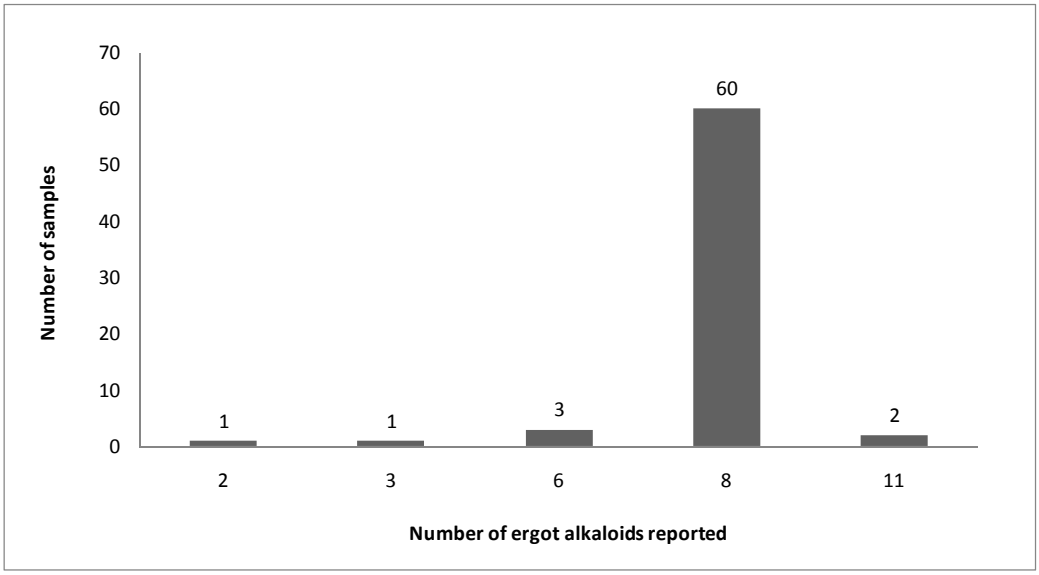


Figure 14: Distribution of EAs for which data were reported in samples with unprocessed grains of undefined end-use (after excluding non-qualifying data).

4.2.5. Distribution of samples across food groups

The food samples were classified according to the FoodEx classification system (EFSA, 2011a). FoodEx is a food classification system developed by the DCM Unit in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1 261 items at the third level and reaching about 1 800 end-points (food names or generic food names) at the fourth level.

At FoodEx Level 1, most of the submitted occurrence data belonged to grain and grain-based products. Going further down to FoodEx Level 2, the majority of the samples belonged to grain milling products (1 193 samples), predominantly consisting of samples of rye milling products that accounted for a total of 952 samples out of the total 1 716 (Figure 15). Samples of wheat milling products were also well represented with a total of 201 samples analysed. Among the grains for human consumption (180 samples) most of the samples reported were of rye grain, with a small number of samples analysed for the other grains (wheat, barley, spelt, etc...). For more than half (119 samples) of the 215 reported samples of bread and rolls, the grain used in their manufacturing was unknown, while bread and rolls made of rye or wheat were represented with 30 and 29 samples, respectively. Finally, among the 46 reported samples of cereal-based food for infants and young children 30 samples corresponded to simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids. A more detailed distribution of food samples in a less aggregated manner (at FoodEx Level 1, 2 and 3) is presented in appendix C.

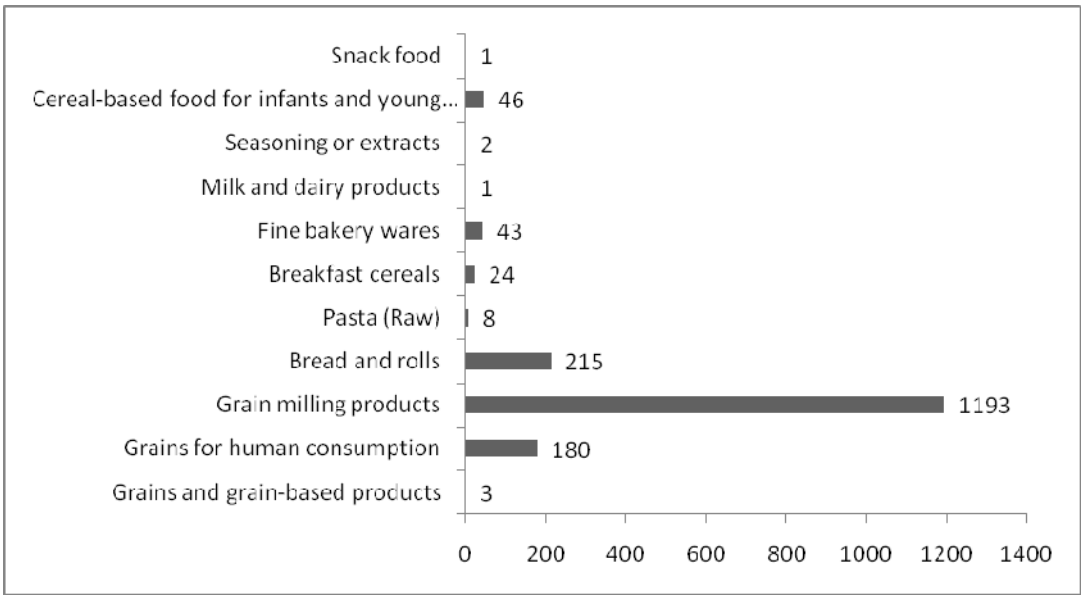


Figure 15: Distribution of food samples across different food groups (at FoodEx Level 2).

4.2.6. Analytical methods used for food

Data reported for EAs in food were obtained by HPLC with different detection systems (~ 70 %), while in the remaining cases (30 %) no analytical method was reported. Among the samples analysed by HPLC, 767 samples were detected by MS/MS, 123 samples by FLD, and for 352 samples a generic standard detection was reported without any further detail.

Reported LOD and LOQ values varied depending on the detection method. Detection by MS/MS allowed attaining minimum values of 0.1 µg/kg and 1 µg/kg for LOD and LOQ, respectively. Minimum LOD and LOQ values reported by FLD were slightly higher than those obtained by MS/MS, with values of 3.1 µg/kg and 6.25 µg/kg respectively. As commented in Section 4.2.1., analytical results with LOD > 10 µg/kg and LOQ > 20 µg/kg were excluded. As can be seen in Figures 16 and 17, different food categories such as vegetable-based meals (one sample, nine analytical results) or baking ingredients (one sample, four analytical results) were excluded due to the high LOD/LOQ reported. In total, 2 647 analytical results were excluded based on the selected LOD/LOQ cut-off.

Figure 18 shows the analytical results reported for the different food samples once those with LOD > 10 µg/kg and LOQ > 20 µg/kg were excluded. It can be seen in this figure that an important part of the selected analytical results (more than 60 %) were left-censored data (i.e. below LOQ or LOD). For some important food categories such as bread and rolls the percentage of left-censored data was even higher representing 80 % of the total reported data in that specific food category.

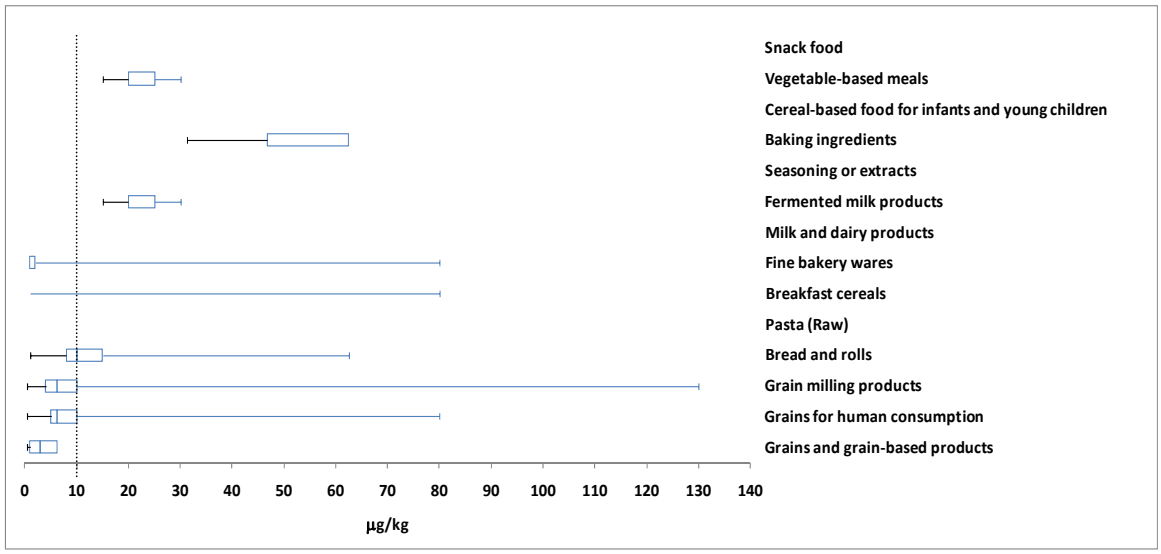


Figure 16: Distribution of LOD values among the analytical results across the different food categories (Box-plot: whiskers at minimum and maximum, box at 25th percentile and 75th percentile with line at 50th percentile).

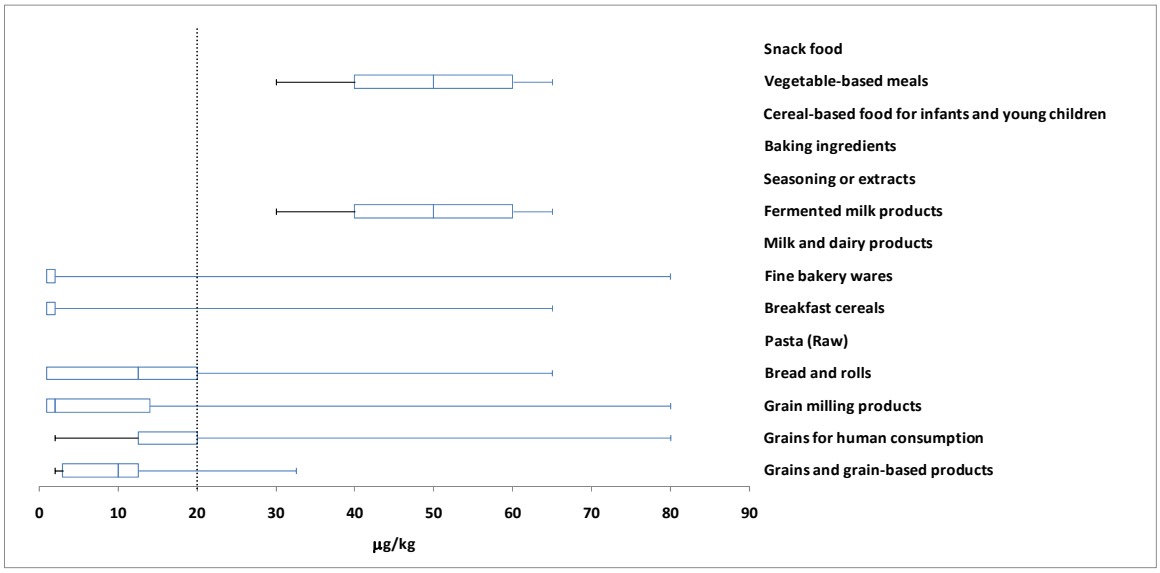


Figure 17: Distribution of LOQ values among the analytical results across the different food categories (Box-plot: whiskers at minimum and maximum, box at 25th percentile and 75th percentile with line at 50th percentile).

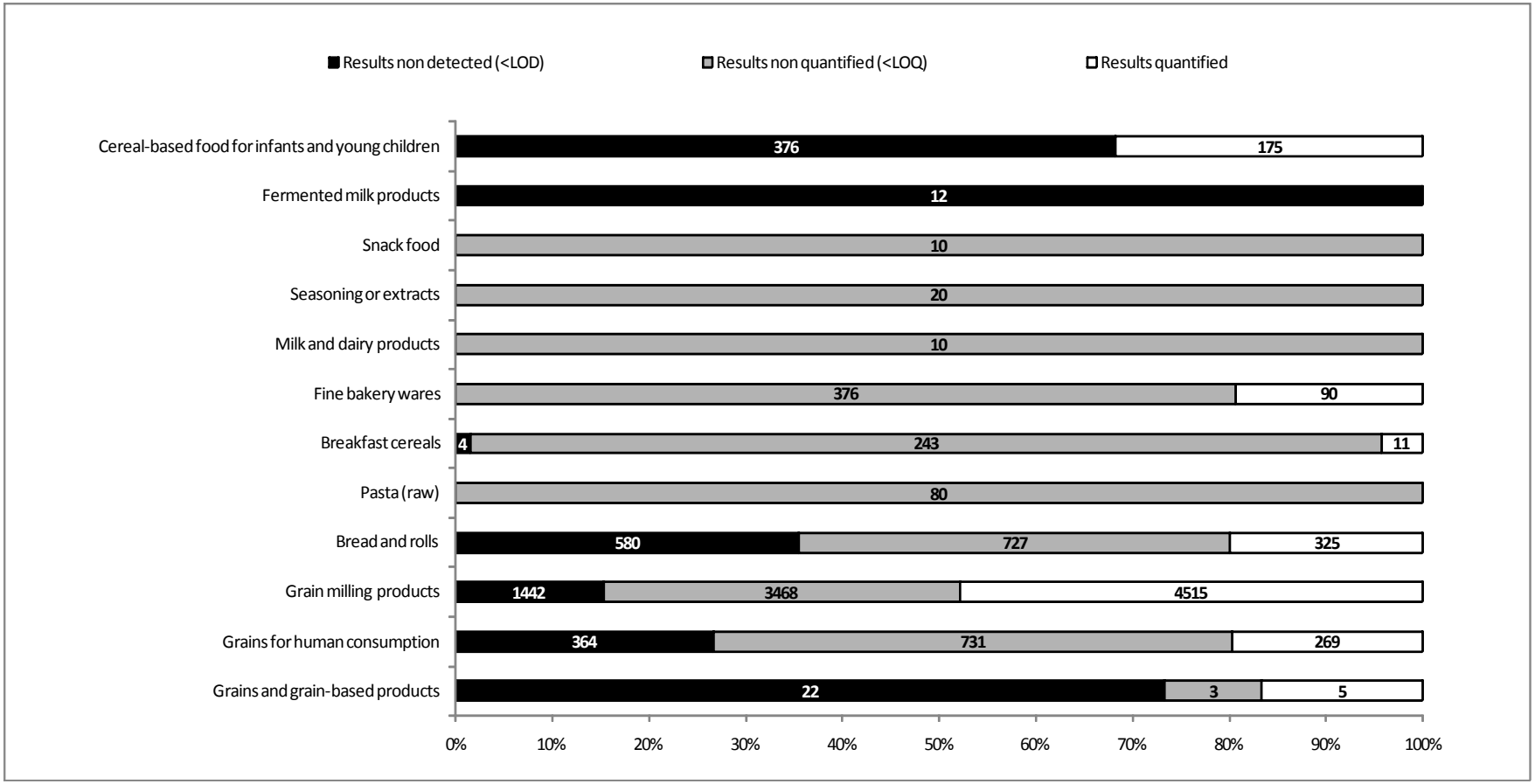


Figure 18: Number of analytical results below LOD, below LOQ and quantified samples across the different food categories.

4.2.7. Occurrence data on food

As shown in Figure 13, the distribution of the number of EAs reported in the different food samples varied between only one EA and twelve EAs. Out of the total 1 716 food samples, 573 samples, reported values with less than six alkaloids analysed. It was considered that including all 1 716 reported food samples would imply an unacceptable underestimation of the occurrence of EAs. The occurrence data were analysed in detail in order to find an appropriate approach that includes both a representative number of EAs/sample and sufficient number of samples. It was concluded that four EAs (ergotamine, ergocristine, ergocornine and ergosine) should be included in all samples selected for inclusion in the analysis of occurrence and exposure, based on their reporting frequency and their contribution to the concentration reported. These four EAs represented on average more than 40 % of the total EA concentration calculated for each of the 585 samples that reported the 12 EAs. A fifth EA, ergometrine, was not considered important for inclusion in the analysis of occurrence and exposure since, although its reported frequency was similar to the selected EAs, its contribution to the total concentration was lower (6 %) compared to the others, and it was not detected in more than 50 % of the samples.

Based on these facts, and with the aim of minimising the overestimation in the UB due to the substantial percentage of left-censored data, it was decided to explore the possibility of using these four EAs to calculate the occurrence value in each sample. In principle, since these 4 EAs represented on average more than 40 % of the total EA content, the occurrence values of the four EAs would be multiplied by a factor of 2.5 to calculate the occurrence values in all the samples, and similarly the same to calculate the UB using their LOD/LOQ in the case of left-censored data. However, although the average contribution of these four EAs to the total occurrence in each sample was rather similar amongst unprocessed and processed foods, in the case of feed samples there were differences in the contribution of the four EA among unprocessed and processed samples. Eventually, since the concentration of the four EAs in a specific sample can be influenced by different factors such as processing or the sample preparation, this approach was rejected.

Three other different scenarios were explored whereby samples fulfilled two conditions: they always contained the above mentioned four EAs together with a minimum number of at least six EAs, at least eight EAs or the twelve EAs. Among the three scenarios evaluated, that with at least six EAs included a total of 1 049 samples compared to 974 samples and 585 samples for the scenarios with at least eight and with the twelve EAs, respectively. The scenario with the at least six EAs containing ergotamine, ergocristine, ergocornine and ergosine (hereafter referred to as “at least six EAs”) provided an acceptable number of samples (more than 60 % of the total) and represented more than 80 % of the total analytical results available for food after the removal of non-qualifying data. As can be seen in Figure 19, the selected four EAs represented 60.7 % of the total concentration reported for the selected 1 049 samples.

The occurrence values for each food category were very similar in each of the three scenarios regardless the number of EAs analysed (Appendix D), indicating that no underestimation is taking place when selecting “at least six EAs” scenario. Therefore, this scenario was selected for carrying out the dietary exposure estimations.

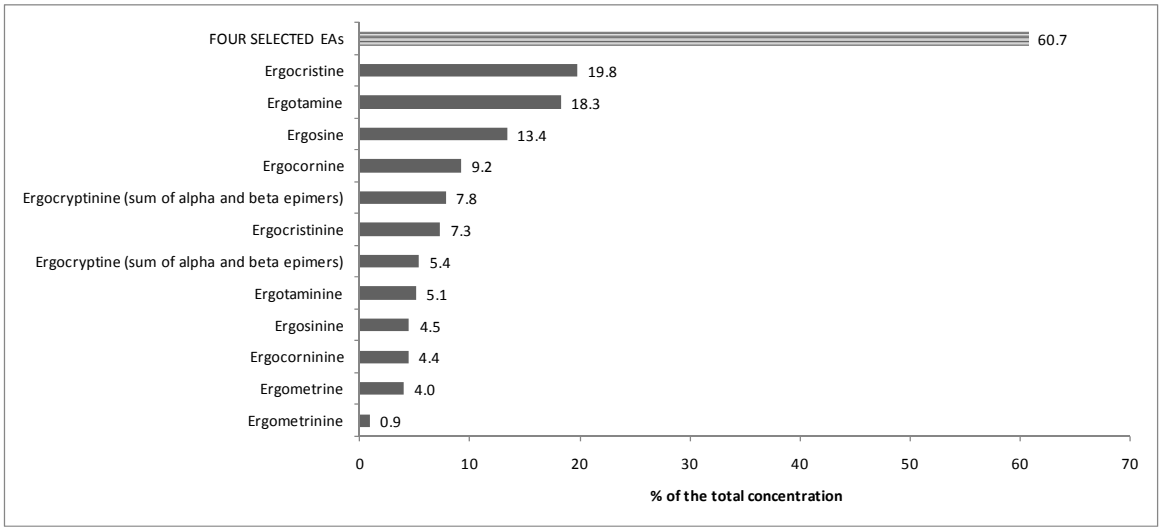


Figure 19: Contribution of the different EAs to the total concentration (%) in the 1 049 food samples under the selected scenario "at least six EAs". The four selected EAs are: ergotamine, ergocristine, ergocornine and ergosine.

The analytical results were submitted by the data providers as either corrected or not corrected for recovery. Where results were not corrected by the data provider a correction was applied by using the reported recovery rate. Where recovery was not available, no correction was applied.

The left-censored data were treated by the substitution method as recommended in the "Principles and Methods for the Risk Assessment of Chemicals in Food" (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report "Management of left-censored data in dietary exposure assessment of chemical substances" (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower-bound (LB) and upper-bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins).

In order to calculate the total concentration of EAs in each of the samples, the individual occurrence values of each EA were summed up. The LB of each sample (sumLB) was calculated by summing-up the individual LB of each EA, assigning a value of zero (minimum possible value) to all records reported as lower than the LOD (< LOD) or LOQ (< LOQ). The UB of each sample (sumUB) was obtained by summing-up the UB of each EA analysed in that sample, assigning the numerical value of the LOD to values reported as < LOD and the LOQ to values reported as < LOQ (maximum possible value). Most of the samples containing the 12 EAs reported the use of very sensitive methods (LOQ of 1 µg/kg and LOD of 0.1 µg/kg). Therefore, including left-censored data did not lead to overly conservative estimates for the UB.

Table 2 shows the selected samples (scenario "at least six EAs") with their occurrence values (LB and UB approach, in µg/kg) as used for evaluation of the dietary exposure assessment to EAs. More detailed summary statistics of the 1 049 food samples selected under this scenario are given in appendix E together with their food classification based on the FoodEx system.

When possible, the 95th percentile occurrence values (at LB and UB) were also calculated in the different food groups. This was only applicable for four food groups: rye grains, wheat milling products, rye milling products and bread and rolls. For the other food groups, the 95th percentile occurrence value was not statistically robust due to the low number of samples. Instead, the average values calculated from the last quartile are described (using those samples with equal or higher value than the 75th percentile).

Prior to the evaluation of the dietary exposure to EAs, the selected samples and food groups were carefully assessed. Compared to appendix E, Table 2 shows a different number of food samples and a different sorting. The reason is that some food groups were not considered for the exposure assessment since only one or two samples were reported. Accordingly, two samples of grain and grain-based products, one of milk and dairy products, two of malt extracts and one of snack food were discarded. Moreover, in some cases the reported food samples were grouped at FoodEx Level 2. This is also useful to cover incomplete consumption data that are not described at lower levels and that, otherwise, would have been overlooked during the exposure assessment. As a result, a group of grains for human consumption is described at FoodEx Level 2 containing 14 samples of different grains but rye. A similar approach was taken for the group grain milling products other than wheat and rye milling products. In this category, two samples for which information on the type of grain was not reported were moved to rye milling products based on their exceptionally high levels of contamination compared to the other samples.

For the group of bread and rolls, an additional food category at FoodEx Level 2, named bread and rolls is described, containing all the types of bread and rolls. Six samples belonging to bread and rolls and mixed wheat and rye bread and rolls (see appendix E) were left out of this group due to their low number of samples and the fact that all analytical results were left-censored data. Although pasta samples also presented all their analytical results as left-censored data, it was decided to include them in the exposure evaluation due to the importance of this food category in certain countries and the use in pasta manufacturing of grains susceptible of EA contamination. The eight different pasta samples were grouped at FoodEx Level 2 as Pasta (raw).

Among the 23 reported samples of breakfast cereals, one sample showed a particularly high concentration compared to the rest of the samples (LB = 155 µg/kg and UB = 157 µg/kg). This cereal sample was made of rye (rye flakes). Since the remaining samples were mainly multigrain cereals, all of them but two with non detected EAs and these two with just 1 µg/kg, it was decided to exclude this sample from the exposure assessment to avoid a bias of this food category.

The 40 samples of biscuits (cookies) were grouped under the more generic name of fine bakery wares (at FoodEx Level 2), which also includes pastries and cakes.

The 46 samples of cereal-based food for infants and young children were taken as one simple group (unspecified) to cover unspecified consumption data on this type of food, except for the food groups pasta for children where the occurrence values for pasta (raw) were applied. Additionally, those food samples in this group consisting of cereals to be reconstituted were grouped to make a separated food category independent of 'biscuits, rusks and cookies for children'.

A comparison of the occurrence levels of EAs between foods from organic and conventional farming was not possible due to the low number of samples in the same food category that reported information on the farming method. Likewise, a comparison of the occurrence over sampling years was not possible. In this particular case, the main limitation, in addition to the limited number of samples (only two sampling years, 2010 and 2011, where the 12 EAs were reported), was that no information was available on whether the samples entered into the food chain the same year of harvest or they were stored for a period of time and then used.

Table 2: Main food groups selected under the scenario "at least six EAs" (at FoodEx Level 2 and 3). Mean concentration values, and average of the last quartile and 95th percentile (P95) are expressed in µg/kg. In bold are listed the food categories at Foodex Level 2, grouping similar foods to cover incomplete consumption data when evaluating exposure assessment. Values are rounded off to the nearest whole number (0 decimal places).

	At least 6 EAs						
	N	**Mean LB**	**Mean UB**	**P95[(a)] LB**	**P95[(a)] UB**	**Average last quartile[(a)] LB**	**Average last quartile[(a)] UB**
Rye grain	92	101	183	638	638		
Grains for human consumption (excluding rye grain)	**14**	7	43			32	114
Wheat milling products	191	30	39	141	141		
Rye milling products	511	124	155	556	575		
Grain milling products (excluding wheat and rye milling products)	**28**	13	34			57	131
Rye bread and rolls	24	30	45			94	178
Wheat bread and rolls	29	3	15			16	20
Multigrain bread and rolls	18	17	23			47	48
Unleavened bread, crisp bread and rusks	13	3	16			10	20
Bread and rolls	**84**	14	25	63	92		
Pasta (raw)	**8**	0	20			0	20
Breakfast cereals	**22**	0	16			1	20
Fine bakery wares	**40**	3	15			14	20
Cereals to be reconstituted with nutritious liquids/water or other protein free liquid	35	7	8			38	38
Biscuits, rusks and cookies for children	11	19	20			79	79
Cereal-based food for infants and young children	**46**	10	11			61	61

(a) The 95th percentile was only calculated for rye grain, wheat milling products, rye milling products and bread and rolls due to the low number of samples available for each category. For the rest of food categories the average value of the last quartile was calculated;

4.2.8. Distribution of samples across feed categories

Feed was classified according to the catalogue of feed materials specified in the Commission Regulation (EU) No 575/2011.[13] Compound feedingstuffs were grouped according to the species/production categories for which the feed is intended. When information was provided, all samples reported the results as whole weight with a dry matter content that varied between 82 % and 91 %. Based on this moisture content it was decided that a conversion to a common basis (88 % dry matter) was not necessary.

Most of the feed samples, after the LOD/LOQ cut-offs were applied (see Section 4.2.9.), were rye and wheat grains (372 out of 496), with some samples of triticale (72 samples) and 49 samples classified as compound feedingstuff. Despite the importance of barley as a feeding material, occurrence data for this grain were only available for two samples. One sample corresponded to a mix of different grains.

4.2.9. Analytical methods used for feed

All the reported feed samples were analysed by HPLC-MS/MS. The minimum reported LOD was 2 µg/kg in several matrices (e.g. compound feedingstuffs or rye) while the minimum LOQ reported by some laboratories was 1 µg/kg also in different matrices (e.g. rye or wheat). As commented in Section 4.2.1., analytical results with LOD > 10 and LOQ > 20 were excluded (a total of 384 analytical results) For feed samples this resulted in 32 samples of barley and 32 samples of wheat excluded from the final dataset. The importance of barley as feed led to a detailed examination of the submitted occurrence data for these samples, which revealed that all the analytical results were left-censored, i.e. non quantified. Based on this, it was decided to exclude these samples to reduce the variation between the values of LB and UB that could bias the outcome of the exposure assessment. Therefore, after excluding non-qualifying data and as commented already in Section 4.2.3., 496 feed samples were initially available for the exposure assessment.

An important amount of the reported analytical results (76.2 %) was left-censored data. For the two main feed categories (rye and wheat), the percentage of left-censored data was 73.3 % and 91.6 %, respectively (Figure 20). Left-censored data in feed samples were handled by the substitution method as applied in food samples.

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

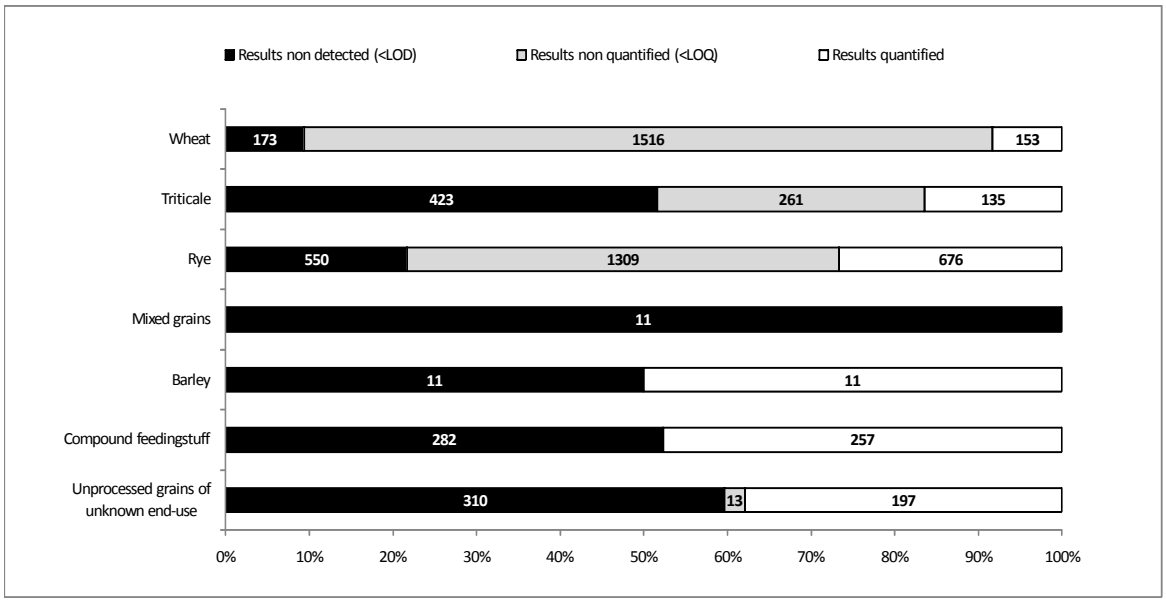


Figure 20: Number of analytical results below LOD, below LOQ and quantified in feed samples across the different feed categories and unprocessed grains of unknown end-use.

4.2.10. Occurrence data on feed including grains of unknown end-use

All the 496 feed samples complied with the requirements of the "at least six EAs" scenario, since the 12 EAs were reported for all of them. However, in the data set only two samples of barley were available for exposure assessment. Since barley is one of the most important feed crops, a total of 65 samples out of the 67 existing samples from grains of unknown end-use (23 barley grain, 36 of rye grain and 6 of wheat grain) were included as feed samples. Only two samples of wheat grains with two and three EAs reported were excluded. Among the new 65 samples included, barley and rye grain samples reported eight EAs while in the six wheat grain samples a diverse number of EAs (ranging between six and eleven) was reported. With the exception of these six samples of wheat grain, the selected EAs (ergotamine, ergocristine, ergosine and ergocornine) in the "at least six EAs" scenario were also reported in the 23 samples of barley grain and in the 36 samples of rye grain.

With the addition of the 65 samples of unprocessed grains of unknown end-use a total of 6 472 analytical results grouped in 561 samples were available to carry out animal exposure assessment (Figure 21). However, some of these samples were not considered for the exposure assessment. Those were samples that belonged to different types of compound feedingstuffs (equine, ovine, poultry and rabbit) where only one or two samples were available for each category. The same principle was applied to discard one sample of mixed grains. Finally, the concentration values of three samples of compound feedingstuffs for porcine were averaged to obtain a single value. These three samples were measurements for batches of feed that were produced containing highly contaminated barley as one of their ingredients and their inclusion as three individual samples would have biased the concentration values of this category. After excluding these eight samples a total of 553 samples were used for exposure assessment (Table 3).

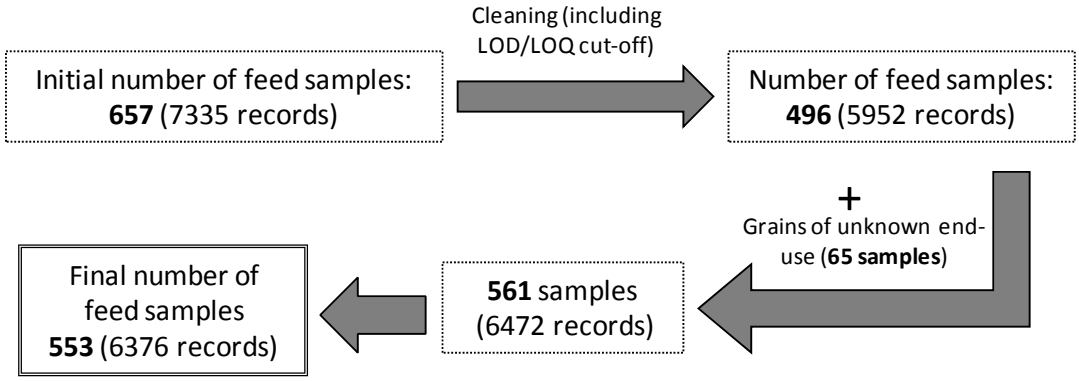


Figure 21: Scheme of the different steps carried out to select the samples available for animal exposure assessment.

The majority of the added analytical results coming from the 65 samples of unprocessed grains of unknown end-use were left-censored data (more than 60 % of the total, see Figure 20). However, unlike some feed samples that were excluded due to their high LOD/LOQ (see Section 4.2.9.), the analytical methods reported for the analysis of these 65 samples possessed low LOD/LOQ values (ranging between 0.15 µg/kg and 3.3 µg/kg).

As discussed below (Section 5.2.), compound feeds for livestock are manufactured from a range of feed ingredients which include cereal grains and their by-products. In some cereals (e.g. wheat and wheat by-products), the mean LB and UB values are markedly lower than for the compound feeds, particularly for pigs. The reasons for this are unclear, but the higher values in the compound feeds may have been influenced by many factors, including the origin of the feeds used, the year of sampling and the ingredients used in the manufacture of the compound feeds.

Table 3: EA concentrations (µg/kg) of the 553 feed samples across feed groups. Values are rounded off to the nearest whole number (0 decimal places).

	N	Variable	Mean	P50	P95[(a)]	Average last quartile[(a)]
Barley	25	SUM LB	72	0	-	225
		SUM UB	79	8	-	263
Rye and rye by-products	253	SUM LB	228	4	900	
		SUM UB	239	26	905	
Triticale	72	SUM LB	40	0	139	
		SUM UB	57	26	149	
Wheat and wheat by-products	161	SUM LB	22	0	83	
		SUM UB	34	12	99	
Compound feedingstuff BOVINE	18	SUM LB	40	25	-	105
		SUM UB	55	37	-	130
Compound feedingstuff PORCINE	24	SUM LB	79	27	-	242
		SUM UB	91	39	-	246

(a) The 95th percentile was only calculated for feed categories with more than 60 samples analysed (EFSA, 2011b). For the rest of feed categories, the average value of the last quartile was calculated.

4.3. Cleaning, milling, food and feed processing

Sorting/Cleaning/Milling

EAs are closely related to occurrence in cereal grains, therefore sorting and other cleaning methods early in grain processing will significantly reduce EA levels further down the food chain. The impact of food processing methods was recently reviewed by Scott (2009) and will be shortly summarised and updated.

Mechanical means and other conventional techniques of industrial grain processing like dockage removing, separators, air screens, density separators, colour sorting and their combinations can significantly reduce EA-levels in grain.

Milling processes, instead, result in redistribution of sclerotia particles in different milling fractions. So far, the reported results were variable and no general conclusions can be derived. Wolff et al. (1983) added various amounts of ergot to rye kernels and investigated the distribution in different milling fractions using ergometrine as an indicator for the ergot amount in the milling fractions. They found an amount of 20 - 30 % ergot in the bran and 60 - 70 % in the flours. Fajardo et al. (1995) reported a reduction of 75 % of the EA content in the straight-grade flours compared to that of the starting wheat grains contaminated with 0.03 % ergot. Increased levels were found for the lower grade ("clear") and millfeed fractions. Furthermore, levels were relatively low in the bran fractions, but high in the short fraction. In a recent model milling experiment using rye spiked with ergot, it was shown that the distribution of each individual alkaloid in the different milling fractions is slightly different (Franzmann et al., 2011). The main problem is ergot dust (simulating ergot abrasion) as it could not be effectively removed during cleaning. The amount of ergot correlates with the amount of the peripheral layers in the flour as the ergot abrasion adheres at the grain surface. Consequently, a peeling step could reduce the amount of ergot (Franzmann et al., 2011).

Processing

Food

Most studies on baking/pancake preparation with EA contaminated flours showed a reduction of EA-levels in the final product. For rye flour bread 54-85 % and for wheat flour 0-100 % losses are reported and usually a higher reduction in EA content is found in the bread crust when compared to crumb (Scott, 2009). Wolff et al. (1988) conducted bread baking experiments with rye meal contaminated with 1, 2 and 10 % ergot. An average loss of 50 % of total EA content was reported. The total amount of -ines was reduced by 62 %, and for the -ininies this was 5 % (calculated for the highest dose). The percentage of -inine epimers increased from 21 % in the flour to 40 % after baking. Fajardo et al. (1995) reported for 6 EAs (ergometrine, ergosine, ergotamine, ergocornine, α-ergocryptine, ergocristine) studied, a reduction of 22 - 55 % (average 36 %) in the crust of baked bread, but no reduction in the bread crumb was found. The fermentation process prior to bread baking did not affect the EA levels.

Baumann et al. (1985) reported losses of 20 to 74 % (average 48 %) for the sum of the epimeric forms of individual EAs during bread baking experiments with ergot contaminated rye flour. The total loss for the -ine epimers was 75 %, but for the –inine epimers a 31 % increase was observed, indicating a notable shift in ratio between the epimeric forms of the EAs. The total contribution of -inine epimers increased from 23.3 % in the rye flour to 61 % in the end product.

Similar results were reported by Franzmann et al. (2010b). During the baking process a steady decline of total EA content was noted (33 % loss after 20 min, 46 % loss after 60 min). However, the loss of the total amount of -ine epimers was 70 % after 60 min, while the –inine epimers increased by 96 %. The total contribution of -inine epimers thus increased from 15 % in the rye flour to 54 % in the end product. The lysergic acid derivatives ergine and erginine were detected as degradation products as well the aci-forms of ergotami(ni)ne, one of the major EAs present in the ergot used in the experiments. A slightly higher stability of the -ine forms was noted when sour dough was used for bread baking. The total amount of EAs present in the finished product was not different from experiments with regular dough, but the -inine forms accounted for 42 % of the total.

Preparation of different types of noodles or spaghetti from heavily ergot contaminated wheat flour reduced the total EA-content by 39 - 47 % (Fajardo et al., 1995). Further processing of these products by cooking and drying resulted in an over-all loss of 55 - 75 % of the total-EAs present in the starting flour (calculated based on the mean EA-values reported by Fajardo et al., 1995). A small part of the EA loss is due to leaking into the cooking water. It can be concluded that EAs are to some extent heat sensitive, but it depends on the EA-content of the raw material if significant amounts of EAs remain in the final product.

Beer is a product based on grain as an important ingredient and it was studied as potential source of EA-exposure of humans. Several steps of beer brewing containing sclerotia (0.1 % w/w) from barley and wheat ergot were analysed for its impact on EA-levels (Schwarz et al., 2007). Individual peptide alkaloids were determined by HPLC, and water-soluble alkaloids were determined by ELISA. Ergocristine was found to be the major EA in both ergots accounting for about 50 % of the total. Steeping of grains reduces smaller amounts of water soluble ergots, while kilning reduces EA-levels by about 30 %. In addition, mass balance calculations were performed to calculate the alkaloid concentration (ng/g or ng/mL) of different stages in micro-brewing experiments. These established that approximately 32 ± 6 %, 10 ± 3 %, and 2 ± 0 % of the total peptide alkaloids in the treated malt grist were recovered with the spent grain, wort, and beer, respectively. Recovery of the water-soluble alkaloids originally present in malt averaged 19 ± 10 %, 22 ± 10 %, and 14 ± 5 % for the spent grain, wort, and beer, respectively. Model experiments with different temperatures (also applied in the brewing process) demonstrated the impact of temperature on the found EA-loss. In summary, EA reduction in steeping appeared due to solubilising some alkaloids, while kilning resulted in a more significant reduction. Losses during kilning appeared to be due to thermal degradation which is also discussed as major reason for the losses observed during brewing (mashing and wort boiling) (Schwarz et al., 2007).

Recently, a multi-mycotoxin screening and quantitation method based on UHPLC high resolution and accurate mass measurements was introduced for the analysis of beer, including 8 EAs (ergosine, ergocornine, ergocryptine, ercocristine and the corresponding –inine epimers), but so far, no data set was presented for commercial beer samples (Zachariasova et al., 2010).

Grain based hard liquors are of no relevance since EAs are non-volatile and will not be transferred to the alcohol fraction during the distillation process.

Feed

Sclerotia can be removed from cereal grains by standard seed-cleaning techniques, although it is important to ensure that the screenings from ergot-containing grains are not used as feeds. Since the EAs are heat-sensitive, they may be reduced during compound feed manufacture, where pellets generally leave the die at temperatures ranging from 60 to 95 °C (Thomas et al., 1997).

Distillery by-products are widely used as animal feeds, particularly for ruminants. If contaminated grains are used in whiskey production, the sclerotia float to the top of the steeping vessel, and can be removed off the top of the steep. Where this is done, the risk of contamination of the malt culms is low.

Considerable quantities of forage crops are conserved by drying or by storing, under anaerobic conditions, as silage. The ensiling process involves the conversion of sugars, naturally present in the plant, to acids with an associated reduction in the pH of the crop, ideally to levels of pH 3.8 - 4.0. There is some evidence that certain EAs are not completely stable under ensiling conditions. Blaney et al. (2010) stored ergot-contaminated forage sorgum under conditions that were intended to simulate ensiling conditions. After six weeks, the mean dihydroergosine concentration had declined from 0.85 to 0.46 mg/kg.

Chestnut et al. (1991) investigated the effect of the treatment of *Neotyphodium coenophialum* infected (called "E+" for endophyte positive) tall fescue hay with anhydrous ammonia. On the basis of feeding experiments with lambs comparing non-ammoniated E+ and E- as well as ammoniated E+ and E- hays, they concluded that some detoxification took place. A more recent investigation showed the total content of EAs (on a dry weight basis) to decrease considerably (to about one third) as a result of hay-making, ensiling giving rise to no reduction. These authors found the EA content in ammoniated hay to be lower than in non-ammoniated, however the difference being non-significant (Roberts et al., 2002). A later two year study also showed an effect of storage of tall fescue hay, the authors concluding that producers should delay feeding of toxic tall fescue hay until at least 1 month after clipping in order to reduce toxicity (Roberts et al, 2009).

4.4. Identification of ergot alkaloids of relevance for food and feed

Based on the available literature and the occurrence data provided to EFSA, some conclusions regarding the most relevant EAs in food and feed can be made, and these are summarised below.

Food

C. purpurea is by far the most widespread *Claviceps* species in Europe and at the same time it is known to infect more than 400 plant species including main cereals like rye, wheat, triticale, barley and rarely oats. Based on these facts the most important EAs to be considered are the *C. purpurea* alkaloids.

Based on the EAs identified in sclerotia of *C. purpurea*, and recent literature data, chemical analysis should focus on the main *C. purpurea* EAs, namely ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (which is a mixture of α- and β- isomers), ergocornine, and the corresponding -inine epimers. It is known that α- and β-ergocryptinine cannot always be separated chromatographically and that with some analytical methods β-ergocyptine co-elutes with ergocristine, but these are not considered major issues because the quantification of total EAs is unaffected by the co-elution. The

above mentioned EAs were those monitored on a regular basis in the occurrence data on food submitted to EFSA. In general, in unprocessed grain/flour the –ine epimers are present in higher amounts (together responsible for up to 80 % of the EA-content). Although the composition of ergot is highly variable, in Europe on average ergocristine, ergotamine, ergosine and ergocornine may be present in higher amounts followed by the ergocryptine isomers and ergometrine.

Additionally, EAs specific for *C. africana* (frequently occurring on sorghum) and *C. fusiformis* (limited to pearl millet) might be of relevance for ethnic food, special diets or imports into the European Community. There are only limited literature data on EAs originating from *C. fusiformis* and *C. africana*. The EA-pattern in *C. africana* seems to be dominated by dihydroergosine, while *C. fusiformis* usually produces several clavine-type EAs such as agroclavine. No occurrence data specific to EAs typical for *C. africana* or *C. fusiformis* were provided to EFSA.

Feed

With regard to feed, because the same type of cereals or compositions containing cereals are used as for food, the EAs found in *C. purpurea* (ergotamine, ergocristine, ergosine, ergocornine, α- and β-ergocryptine, ergocornine and their corresponding -inines) are the most relevant.

5. Food and feed consumption

5.1. Food consumption

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built from existing national information on food consumption at a detailed level.

Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer. Results from a total of 32 different dietary surveys carried out in 22 different Member States covering more than 67 000 individuals are included in the Comprehensive Database version 1 as published (EFSA, 2011b). This included food consumption data concerning infants (2 surveys from 2 countries), toddlers (8 surveys from 8 countries), children (17 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries). Surveys on children were mainly obtained through the Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., 2011).

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, it should be pointed out that different methodologies were used between surveys to collect the data and thus direct country-to-country comparisons can be misleading.

5.1.1. EFSA's Comprehensive European Food Consumption Database

The CONTAM Panel considered that chronic and acute exposure to EAs had to be assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b), dietary surveys with only one day per subject were not considered for the calculation of chronic dietary exposure, as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as follows:

1. Infants: 2 countries; 2 dietary surveys
2. Toddlers: 7 countries; 7 dietary surveys
3. Other children: 13 countries; 15 dietary surveys
4. Adolescents: 10 countries; 12 dietary surveys
5. Adults: 14 countries; 15 dietary surveys
6. Elderly: 7 countries; 7 dietary surveys
7. Very elderly: 6 countries; 6 dietary surveys

Six additional dietary surveys with only one day per subject from six different countries (covering all age classes except infants) were considered for acute exposure assessment (Table 4). In this table the number of available days for each age class used in the acute exposure assessment are described beside the number of subjects available for the chronic exposure assessment.

Within the dietary studies, subjects were classified in different age classes as defined below:

1. Infants: < 12 months old
2. Toddlers: ≥ 12 months to < 36 months old
3. Other children: ≥ 36 months to < 10 years old
4. Adolescents: ≥ 10 years to < 18 years old
5. Adults: ≥ 18 years to < 65 years old
6. Elderly: ≥ 65 years to < 75 years old
7. Very elderly: ≥ 75 years old

Consumption records were codified according to the FoodEx classification system, which has been developed by the DCM Unit in 2009 (EFSA, 2011a). Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011b).

A few surveys reported consumption data at ingredient level, such as flour. In order to match those consumption data with occurrence data reported for processed food, a conversion factor of 1.5 was applied to convert flours into their respective bread and rolls categories, as previously used by EFSA (EFSA, 2011c).

Table 4: Dietary surveys considered for the chronic and acute dietary exposure assessment with the available number of subjects (for chronic exposure) and number of days (for acute exposure) in the different age classes.

Code[a]	Country	Dietary survey[b]	Method	Days	Age	Number of subjects[c] per days[d]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
AT	Austria	ASNS	24-hour recall	1	19-65					-/2123		
BE/1	Belgium	Diet National 2004	24 h dietary recall	2	15-105				584/1187	1304/2648	518/1045	712/1448
BE/2	Belgium	Regional Flanders	Food record	3	2-5		36[(e)]/108	625/1875				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1-5	860/1720	428/867	433/856				
BG/2	Bulgaria	NSFIN	24-hour recall	1	> 16				-/162	-/691	-/151	-/200
CY	Cyprus	Childhealth	Dietary record	3	11-18				303/909			
CZ	Czech Republic	SISP04	24-hour recall	2	4-64			389/798	298/596	1666/3332		
DE/1	Germany	DONALD 2006	Dietary record	3	1-10		92/276	211/633				
DE/2	Germany	DONALD 2007	Dietary record	3	1-10		85/255	226/678				
DE/3	Germany	DONALD 2008	Dietary record	3	1-10		84/252	223/669				
DE/4	Germany	National Nutrition Survey	24-hour recall	2	14-80				1011/2022	10419/20838	2006/4012	490/980
DK	Denmark	Danish Dietary Survey	Food record	7	4-75			490/3426	479/3398	2822/19722	309/2159	20[(e)]/140
EL	Greece	Regional Crete	Dietary record	3	4-6			839/2508				
ES/1	Spain	AESAN	24-hour recall	2	18-60					410/828		
ES/2	Spain	AESAN-FIAB	Food record	3	17-60				86/226	981/2748		
ES/3	Spain	NUT INK05	24-hour recall	2	4-18			399/798	651/1302			
ES/4	Spain	enKid	24-hour recall	2	1-14		17[(e)]/34	156/312	209/418			
EE	Estonia	NDS_1997	24-hour recall	1	19-64					-/1866		
FI/1	Finland	DIPP	Food record	3	1-6		497/1486	933/2773				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25-74					1575/3150	463/926	
FI/3	Finland	STRIP	Food record	4	7-8			250/1000				
FR	France	INCA2	Food record	7	3-79			482/3315	973/6728	2276/15727	264/1824	84/571
HU	Hungary	National Repr Surv	Food record	3	18-96					1074/3222	206/618	80/240
IE	Ireland	NSFC	Food record	7	18-64					958/6706		
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1-98	16[(e)]/48	36[c]/108	193/579	247/741	2313/6939	290/870	228/684
LV	Latvia	EFSA_TEST	24-hour recall	2	7-66			189/377	470/949	1306/2655		
NL/1	Netherlands	DNFCS 2003	24 h dietary recall	2	19-30					750/1500		
NL/2	Netherlands	VCP kids	Food record	3	2-6		322/644	957/1914				
PO	Polonia	IZZ_FAO_2000	24-hour recall	1	1-96		-/79	-/409	-/666	-/2527	-/329	-/124
SE/1	Sweden	RIKSMATEN 1997-98	Food record	7	18-74					1210/8466		
SE/2	Sweden	NFAn	24-hour recall	4	3-18			1473/5875	1018/4047			
SK	Slovakia	SK_MON_2008	24-hour recall	1	19-59					-/2763		
Sl	Slovenia	CRP_2008	24-hour recall	1	18-65					-/407		
UK	United Kingdom	NDNS	Food record	7	19-64					1724/12068		

(a): Abbreviations to be used consistently in all tables on exposure assessment; (b): More information on the dietary surveys is given in the Guidance of EFSA (EFSA, 2011b); (c): Number of available subjects for chronic exposure assessment in each age class; (d): Number of available days for acute exposure assessment in each age class; (e) 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b)

5.2. Feed consumption

Claviceps purpurea (ergot) only infects cereals and grasses, and therefore it is these feeds, or by-products derived from them, which are potential sources of the toxins. Amongst cereals, rye is the most commonly infected, but triticale, wheat and barley (in descending order of susceptibility) may also be contaminated. Rye is an important crop in the cooler parts of northern and central Europe, and although it is nutritionally inferior in several ways to the predominant cereal crops (wheat, rice, and maize), it is an important crop in these areas because of its winter hardiness and ability to grow in poor soils. Oats are rarely affected under European conditions (EFSA, 2005).

The amounts of cereal grains and cereal by-products consumed on a daily basis by livestock in the EU vary widely, both between and within species. For non-ruminant livestock (pigs and poultry), cereal grains and cereal by-products are important ingredients, and may represent as much as 70 % of the total diet. The actual amount fed on a daily basis is influenced by the size, type and age of the animal, and the level of productivity (Table 5). The choice of feed will be determined by a number of factors, including the feeds available and their cost, and their suitability in meeting the nutritional needs of the animal (McDonald et al., 2011). Typical feed intakes for a number of non-ruminant livestock categories are given in Table 5.

As mentioned above, rye is the most commonly infected cereal used in livestock feeds, but its use for this purpose is generally limited due to poor palatability at high levels in the diet and the possible presence of EAs. For ruminants, it may be included at levels of up to 25 % of the non-forage ration for dairy cows, and 30 - 40 % for finishing cattle. For fattening pigs (> 30 kg b.w.) rye can constitute up to 50 % of the total diets, and 40 % for lactating sows.[14] The use of rye in diets of pigs less than 25 kg b.w. is not recommended due to palatability problems. Rye grain is not recommended for growing chickens (broilers) and turkeys due to its high level of soluble fibre which affects growth and litter quality, although it may be fed to laying hens up to 40 % of the diet after the hens have reached peak production (40 weeks).[15]

Table 5: Examples of feed intake for pigs and poultry.

		Live weight (kg)	Feed intake (kg per day)	Feed intake (% b.w.)
Pigs	Piglets	7	0.32	4.60
	Pigs - growing	41	1.62	4.00
	Pigs - fattening	85	3.1	3.65
	Breeding sows - gestating	210	3.2	1.52
	Breeding sows - lactating	230	7.3	3.17
Broilers	Starter	0.2	0.03	15.96
	Grower	1.3	0.15	11.08
	Finisher ration 1	2.3	0.20	8.70
Layers	Starter (4 weeks)	0.3	0.026	10.20
	Grower (18 weeks)	1.3	0.068	5.35
	Breeding (week 20)	1.4	0.082	5.89
	Laying hen (32 weeks)	1.9	0.11	5.95

In contrast, diets of ruminant livestock (cattle, sheep, goats), rabbits and horses, consist predominantly of forages. The type of forage varies considerably throughout the EU, although grasses predominate. In northern Europe, *Claviceps purpurea* has a host range of more than 200 species of grasses. They include many of the important forages used as livestock feeds such as *Agrostis, Avena, Dactylis, Festuca, Hordeum, Lolium, Poa, Secale, Triticum. Claviceps paspali* (paspalum ergot) is only known to colonize

[14] Source: http://www.usask.ca/agriculture/plantsci/winter_cereals/winter_rye/Rye2.htm#livestock
[15] Source: www.usask.ca/agriculture/plantsci/winter_cereals

grasses of the genus *Paspalum*, which are important animal feeds in some areas, particularly in the Mediterranean countries. Ingestion may lead to paspali staggers, an intoxication caused by the content of indole-diterpenoid tremorgens - so-called paspalitrems - and not by the low content of EAs. Furthermore a wide variety of grasses used as feeds for livestock is also susceptible to infection with *Neotyphodium* species. Thus, Clay and Schardl identified 13 distinct plant species and further 5 plant genera as susceptible to infestation with either a species of *Neotyphodium* or with closely related species from the fungal genus *Epichloë* (Clay and Schardl, 2002). The plants include perennial rye grass (*Lolium perenne*), cocksfoot or orchardgrass (*Dactylis glomerata*), timothy (*Phleum pretense*), brome grass (*Bromus* spp.), and blue grass (*Poa* spp.). In addition, forage crops may be contaminated by weeds which are susceptible to parasitisation by EAs. For example, Naudè et al (2005) reported a number of cases of severe ergotism of dairy cows in South Africa which had consumed maize silage contaminated with ergotised nut sedge (*Cyperus esculentus*). This plant, which has a world-wide distribution, is a common weed in annual crops, particularly in southern Europe. As discussed above (Section 1.2.2.), however, intoxications of livestock associated with either *Claviceps purpurea* or *Neotyphodium* toxins are rare in grazing livestock in the EU. Furthermore, no data are available on levels of EAs in forage crops in Europe, and therefore data on consumption of forages are not included in overall estimates of feed intake or exposure.

Examples of feed intake levels for ruminants and horses are given in Table 6.

Table 6: Example intake levels of ruminant livestock and horses.

	Live weight (kg)	Growth rate or productivity	DM[1] intake (kg/day)	Forage % of total DM intake	Reference
Dairy cows, lactating	650	40 kg milk per day	21.4	60	AFRC, 1993
Fattening cattle[2]	400	1 000 g per day	9.6	80	AFRC, 1993
Fattening cattle: cereal beef	400	1 400 g per day	8.4	15	AFRC, 1993
Sheep: lactating ewes	80	Feeding twin lambs	2.8	65	AFRC, 1993
Goats: milking[3]	60	4 kg per day	3.3	60	NRC, 2007b
Goats: fattening	40	200 g per day	1.5	60	NRC, 2007b
Horses (active)	450	-	9.0	50	NRC, 2007a

[1]DM: dry matter.
[2]Housed castrate cattle, medium maturing breed.
[3]Months 2-3 of lactation.

Where non-forage feeds are fed to ruminants, cereal grains and cereal by-products may represent up to 70 % of the non-forage component of the diet. Barley, maize and oats, together with by-products derived from them, are most widely used although rye may be preferred in those areas where it is grown. Exceptions to these generalisations are cereal-beef, where cereals may account for up to 85 % of the daily feed intake, and veal calves.

In contrast to the situation for the human population (see Section 5.1.), there is no comprehensive database on feeds consumed by individual livestock categories in the EU. For all compound feeds manufactured in the EU, cereals represented 47 % of all feed materials used (FEFAC, 2009). Since pigs and poultry consume approximately two-thirds of all industrial compound feed produced, it would be reasonable to assume that these proportions are broadly typical for the majority of diets fed to these livestock also. However, in practice cereals and cereal by-products may account for up to 70 % of the

total ration in some situations, depending on the price and availability of other feed ingredients. Other feeds include oilseed meals, legumes, food and confectionary by-products, mineral and vitamin supplements and additives.

Commercial rabbit production takes place in at least 14 EU countries, but principally in Italy, France and Spain. Their diet consists predominantly of fibrous feeds; in commercial production they are usually fed a pelleted diet of cereals and vegetable proteins supplemented with minerals, vitamins and trace elements. Dried lucerne is usually the major ingredient, and levels of 50 % or more have been recommended for commercial rabbit diets (McNitt et al., 2000). Lebas and Renouf (2009) reviewed diet formulations used in experimental studies: in 58 diets, dried forage (lucerne) was included at levels of up to 65 %, while the proportions of cereals, cereal by-products (mostly wheat bran) and oilseed meals (mostly soya bean cakes and sunflower seed cakes) were 18 - 20 %, 18 - 20 % and 16 %, respectively.

Atlantic salmon is economically the most important farmed fish in Europe, although farmed fish also include rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. Given the wide range of species and environmental conditions for farmed fish, many different feeding strategies have been developed. However, given its predominance in EU aquaculture, feed intake and exposure to EAs have been estimated for salmon in this Opinion.

Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fish meals and fish oils. Since cold-water fish do not utilize carbohydrates as energy sources as well as warm-water species, there is less use of cereals. Berntssen et al. (2010) provided details of the composition of a diet for growing Salmonids, and the CONTAM Panel used this feed formulation to estimate exposure to EAs for salmon (2 kg) with a feed intake of 0.04 kg DM/day (EFSA, 2011d).

For companion animals (cats and dogs) food composition, including cereal content, varies considerably both between and within species, and is also influenced by the product range (economy, premium, super premium pet food products). Pet foods are formulated in order to meet the specific nutritional requirements of the animal and therefore different products will be used for different requirements. This inevitably results in a range of daily rations covering different conditions. However, for a typical dry pet food, the cereal content may be summarised as follows:[16]

- total cereal level between 30 % and 60 % (on 100 % recipe basis);
- for a super premium pet food the cereal content is likely to be nearer 30 %, while economy products will tend to the upper level of the range (60 %);
- corn gluten and wheat middling are used between 0 % and 15 % (on 100 % recipe basis);
- the remaining cereal part is full cereal grain (meal).

It should be noted that there is considerable variation in livestock feeding systems in the EU, and in particular the types and amounts of feedingstuffs that are used, and it is beyond the scope of this Opinion to attempt to describe them all. In order to estimate exposure to EAs, the CONTAM Panel has used general estimates of diet composition as reported by EFSA (2011d). These, and the estimates of the amounts of feed consumed described above, do not represent 'average' diets, nor do they necessarily reflect 'typical' feeding systems applicable to all of the EU. Instead, they are used to estimate levels of exposure to EAs that might not be atypical.

[16] G. Simone, Technical and Regulatory Affairs Manager, FEDIAF, personal communication.

6. Exposure assessment in humans and animals

6.1. Exposure assessment of ergot alkaloids in humans

Most of the reported food samples were on non or minimally processed food (mainly grain milling products). The results of these samples could not be used for the exposure assessment since the impact of processing on the final EA concentration in processed food could not be established. Therefore, the exposure assessment was based on the results from approximately 250 samples out of the 1 049 samples selected under the scenario "at least six EAs" (see Table 2).

6.1.1. Chronic dietary exposure to ergot alkaloids

For calculating the chronic dietary exposure to EAs, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.1.1.). Chronic exposure estimates were calculated for 26 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups and in some cases the same country provided more than one consumption survey.

The mean and the high (95th percentile) chronic dietary exposures to EAs were calculated separately for each dietary survey using consumption data recorded at the individual level and for both LB and UB mean concentrations for the selected scenario "at least six EAs" (see Table 2 for concentration values).

Minimum, median and maximum exposure estimates across dietary surveys and age groups are reported in Table 7. Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 26 dietary surveys are presented in appendix F (Table F1). In accordance with the specifications of the EFSA Guidance on the use of the Comprehensive database (EFSA, 2011b), 95th percentile estimates for dietary surveys/age classes with less than 60 observations are not considered since they may not be statistically robust.

Mean chronic dietary exposure values, across the different dietary surveys and age classes, ranged from 0.007 μg/kg b.w. per day (minimum LB) to 0.173 μg/kg b.w. per day (maximum UB). The 95th percentile dietary exposure ranged from 0.014 μg/kg b.w. per day (minimum LB) to 0.335 μg/kg b.w. per day (maximum UB).

6.1.1.1. Infants (< 12 months)

Only two dietary surveys are available for this age group, one of which had too few survey participants for calculation of a robust 95th percentile. Therefore, the dietary exposure estimate cannot be considered as representative of the European infant population. Bearing this limitation in mind, the mean dietary exposure was between 0.013 and 0.056 μg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure for the single qualifying study was 0.064 μg/kg b.w. per day (LB) and 0.188 μg/kg b.w. per day (UB).

6.1.1.2. Toddlers, other children and adolescents (≥ 1 to < 18 years old)

Toddlers and other children had the highest values of chronic dietary exposure to EAs. Together with the consumption of different foods containing EAs the high dietary exposure can be explained by the higher intake of food per kg b.w. in younger age groups. The highest mean dietary exposure was found for toddlers (≥ 12 months to < 36 months old) with 0.173 μg/kg b.w. per day and 0.335 μg/kg b.w. per day for the 95th percentile dietary exposure (both at the maximum UB).

6.1.1.3. Adults (≥ 18 to < 65 years old)

Based on the 15 dietary surveys providing consumption data for adults, the mean chronic dietary exposure to EAs ranged from 0.007 μg/kg b.w. per day to 0.078 μg/kg b.w. per day (minimum LB and maximum UB across European dietary surveys, respectively). The 95th percentile dietary exposure

estimates ranged from a minimum LB of 0.014 µg/kg b.w. per day to a maximum UB of 0.188 µg/kg b.w. per day.

6.1.1.4. Elderly and very elderly (≥ 65 years old)

Seven and six dietary surveys were available for elderly and very elderly, respectively. In general, both age groups showed slightly lower dietary exposure to EAs compared to other adults. The mean chronic dietary exposure ranged from 0.008 µg/kg b.w. per day to 0.066 µg/kg b.w. per day (minimum LB and maximum UB across European dietary surveys, respectively). The 95th percentile dietary exposure estimates ranged from a minimum LB of 0.015 µg/kg b.w. per day to a maximum UB of 0.140 µg/kg b.w. per day.

Table 7: Summary statistics of the chronic exposure assessment (µg/kg b.w. per day) to ergot alkaloids estimated across European dietary surveys.

Mean dietary exposure						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	0.013	-[(a)]	0.017	0.049	-[(a)]	0.056
Toddlers	0.032	0.056	0.075	0.118	0.153	0.173
Other children	0.017	0.042	0.075	0.105	0.132	0.170
Adolescents	0.009	0.019	0.038	0.050	0.077	0.104
Adults	0.007	0.011	0.039	0.039	0.052	0.078
Elderly	0.008	0.015	0.034	0.040	0.053	0.066
Very Elderly	0.008	0.011	0.034	0.041	0.056	0.066
95th percentile dietary exposure[(b)]						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	0.064	-[(c)]	0.064	0.188	-[(c)]	0.188
Toddlers	0.068	0.155	0.166	0.263	0.309	0.335
Other children	0.032	0.094	0.138	0.186	0.238	0.300
Adolescents	0.019	0.043	0.101	0.098	0.150	0.218
Adults	0.014	0.030	0.110	0.077	0.102	0.188
Elderly	0.015	0.041	0.072	0.088	0.092	0.127
Very Elderly	0.015	0.015	0.079	0.076	0.092	0.140

(a) Not calculated since estimates were only available from two dietary surveys; (b) The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table. (c) Not calculated since estimates were only available from one dietary survey.

In summary, it can be concluded that toddlers and other children have the highest chronic dietary exposure to EAs, probably largely due to the higher intake of food per kg b.w. in these age groups. A relatively high variation was found between the dietary exposure estimates across the different surveys within each age class.

6.1.1.5. Contributions of different food groups to ergot alkaloids chronic exposure

The contribution of the different food groups significantly varied among the different surveys, probably due to the different food consumption patterns across Europe. Overall, the main contribution to the exposure to EAs corresponded to different types of bread and rolls, especially to rye bread and rolls followed by wheat bread and rolls.

In those age groups with the highest chronic exposure (toddlers and other children) several food categories contributed to the exposure to EAs, among them rye bread and rolls, wheat bread and rolls,

unspecified bread and rolls as well as typical food for children such as biscuits and biscuits, rusks and cookies for children. However, it is important to mention that the countries with the highest dietary exposure in these age groups were those with relatively high consumption of rye bread and rolls.

For other children, rye bread and rolls accounted for a maximum of 54.2 % of the total exposure, with a calculated median value of 32 % of the total exposure across the different surveys. A similar maximum percentage of contribution was found for wheat breads and rolls (56 %) while the calculated median value (15 %) across the different surveys was approximately half of the value calculated for rye bread and rolls.

For toddlers, rye bread and rolls accounted for a maximum of 43 % of the total exposure, with a calculated median value of 37 % of the total exposure across the different surveys. In one of the surveys in this age group wheat breads and rolls contributed with 47 % of the total exposure, while the calculated median value across the different survey was 12 %.

6.1.2. Acute dietary exposure to ergot alkaloids

The acute dietary exposure to EAs was calculated on a per day basis, since individual meals are recorded only for a few countries in the consumption database. The preferred option is, therefore, to use individual consuming days. Consuming days offer a conservative estimate of the exposure, since it will sum the contribution of all meals during the same day.

For the calculation of the acute dietary exposure, individual consumption data for each selected food group was combined with the occurrence values of the corresponding food groups. For each survey, each age group and each individual the exposure is calculated by summing up the individual contribution of each food group within each day. It was agreed that either the 95th percentile occurrence value or the average of the last quartile should be used for those food categories identified as main contributors to the exposure (see Table 3). These categories were 'rye bread and rolls', 'wheat bread and rolls' and 'biscuits, rusks and cookies for children'. For the other food categories mean occurrence values were used. In addition to the mean acute dietary exposure, the 95th percentile dietary exposure (high consumers) was also calculated.

Table 8 shows the summary statistics of the acute exposure assessment (in µg/kg b.w. per day) to EAs calculated using 32 different dietary surveys carried out in 23 European countries. Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 32 dietary surveys are presented in appendix F (Table F2). Mean acute dietary exposure estimates across dietary surveys and age groups ranged from 0.02 µg/kg b.w. per day (minimum LB) to 0.42 µg/kg b.w. per day (maximum UB). The 95th percentile acute dietary exposure estimates across dietary surveys and age groups ranged from 0.06 µg/kg b.w. per day (minimum LB) to 1.03 µg/kg b.w. per day (maximum UB).

Similarly to chronic exposure, toddlers and other children also showed the highest acute dietary exposure to EAs. The highest mean dietary exposure was found for toddlers with a mean value of 0.42 µg/kg b.w. per day and 1.03 µg/kg b.w. per day for the 95th percentile dietary exposure (both in the UB). For adults, the mean acute dietary exposure to EAs ranged from 0.02 µg/kg b.w. per day to 0.23 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure ranged from 0.06 µg/kg b.w. per day in LB to 0.73 µg/kg b.w. per day in UB.

Table 8: Summary statistics of the acute exposure assessment (µg/kg b.w. per day) to ergot alkaloids estimated across European countries.

Mean dietary exposure						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	0.043	-[(a)]	0.049	0.047	-[(a)]	0.049
Toddlers	0.075	0.130	0.211	0.132	0.243	0.415
Other children	0.047	0.104	0.208	0.123	0.192	0.358
Adolescents	0.033	0.051	0.100	0.060	0.104	0.192
Adults	0.021	0.047	0.125	0.047	0.074	0.229
Elderly	0.028	0.053	0.126	0.049	0.076	0.232
Very Elderly	0.028	0.053	0.088	0.049	0.078	0.153
95th percentile dietary exposure[(b)]						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	0.161	-[(c)]	0.161	0.243	-[(c)]	0.243
Toddlers	0.210	0.374	0.567	0.45	0.546	1.030
Other children	0.115	0.243	0.431	0.238	0.387	0.820
Adolescents	0.082	0.128	0.350	0.148	0.237	0.669
Adults	0.055	0.103	0.384	0.105	0.160	0.734
Elderly	0.060	0.109	0.302	0.115	0.154	0.565
Very Elderly	0.079	0.113	0.149	0.109	0.160	0.383

(a) Not calculated since estimates were only available from two dietary surveys;
(b) The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(c) Not calculated since estimates were only available from one dietary survey.

6.1.2.1. Contributions of different food groups to ergot alkaloids acute exposure

The contribution of the different food groups to the acute dietary exposure showed important differences between the dietary surveys, even within the same age groups (e.g. from 0 to 85 % for the same food group). As in the case of chronic exposure, these differences can be probably explained through the different food consumption patterns across Europe.

As observed for the chronic exposure, in the age groups with the highest acute exposure (toddlers and other children) those countries with relatively high consumption of rye bread and rolls had the highest exposure levels. Although the use of high occurrence values (average of the last quartile) for three food categories might appear too conservative, in those countries with the highest acute exposure the contribution came basically from just one food category, 'rye bread and rolls'. Figure 22 shows the acute dietary exposure estimates for high consumers (95th percentile dietary exposure) in those age groups with the highest exposure. Estimates for high consumers of all food categories as well as high consumers of either only 'rye bread and rolls' or only 'wheat bread and rolls' were calculated. It can be seen that the high consumption of just 'rye bread and rolls' led to similar levels (grey bars) of acute exposure to EAs to those estimated in the high consumers of all foods (black bars). Therefore, it is clear that the major contribution to total acute exposure to EAs comes from consumption of 'rye bread and rolls'. At the same time, the contribution of 'biscuits, rusks and cookies for children' to acute exposure to EAs in the children age groups was negligible.

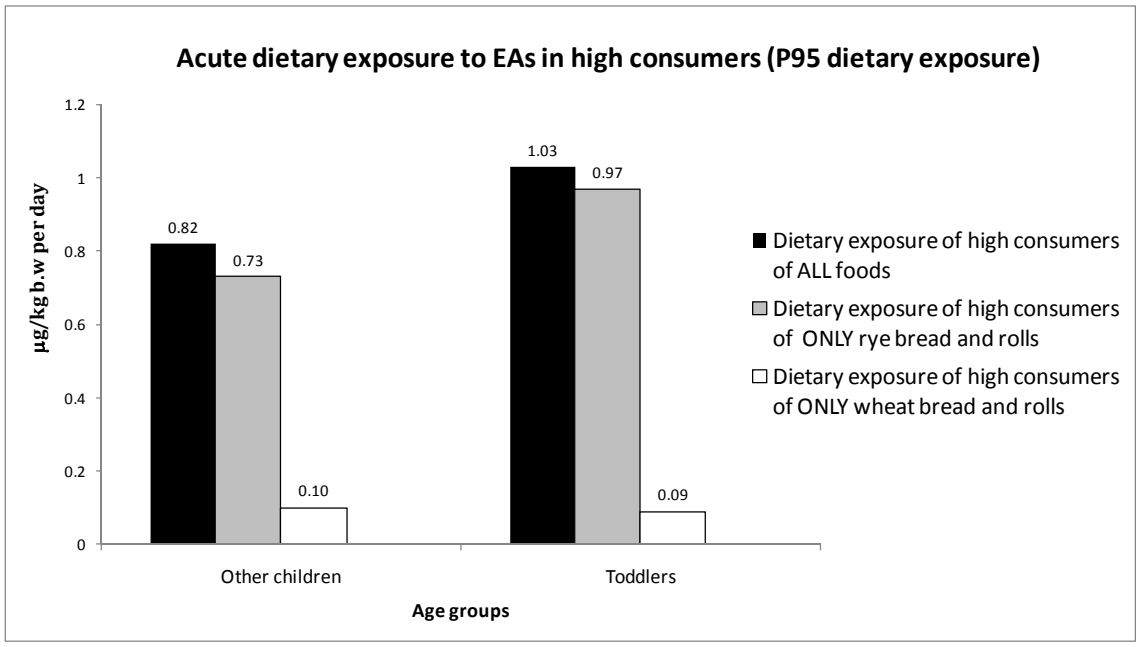


Figure 22: Acute exposure assessment to ergot alkaloids in high consumers considering consumption of: all food groups, only 'rye bread and rolls' and only 'wheat bread and rolls'. 'Biscuits, rusks and cookies for children' did not contribute to the exposure in the surveys shown. Data are shown for those surveys with highest exposure to ergot alkaloids (high consumers at the upper bound).

6.1.3. Dietary exposure to ergot alkaloids for specific groups

Vegetarians

In view of vegetarian consumption habits and the food categories affected by the presence of EAs, the CONTAM Panel decided to evaluate the chronic dietary exposure in this group of the population. The Comprehensive Database contains only limited data on food consumption of people who declared to be vegetarian at the time of the survey, and only dietary surveys with at least 15 adult vegetarians were selected. Following this approach, only five surveys were suitable to compare dietary chronic exposure in vegetarians with that in the general population (Table 9).

The low number of adult vegetarians included in the database makes it difficult to carry out an accurate comparison although, in general, the exposure to EAs observed for vegetarians was virtually the same as for the general population. However, in order to make a more appropriate estimation of the dietary exposure to EAs for vegetarians, more consumption data are needed for this specific group.

Table 9: Comparison of the dietary exposure to ergot alkaloids (µg/kg b.w. per day) between adult vegetarians and total adult population.

Country	Dietary survey	N Veget.	N All	µg/kg b.w. per day			
				Mean exposure		95th percentile exposure	
				Veget.	All	Veget.	All
Lower-bound							
Finland	FI/2	39	1 575	0.025	0.026	(a)	0.060
France	FR	15	2 276	0.009	0.009	(a)	0.018
Germany	DE/4	237	10 419	0.028	0.028	0.071	0.066
Sweden	SE/1	18	1 210	0.019	0.022	(a)	0.049
United Kingdom	UK	77	1 724	0.010	0.007	0.028	0.015
Upper-bound							
Finland	FI/2	39	1 575	0.051	0.052	(a)	0.108
France	FR	15	2 276	0.047	0.047	(a)	0.088
Germany	DE/4	237	10 419	0.067	0.061	0.152	0.125
Sweden	SE/1	18	1 210	0.059	0.062	(a)	0.119
United Kingdom	UK	77	1 724	0.053	0.042	0.115	0.078

N: number of subjects in the dietary surveys; Veget.: adult vegetarians; All: total adult population; b.w.: body weight.
(a): The 95th percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table

Consumers of raw grain products

The CONTAM Panel decided to evaluate the dietary exposure to EAs for consumers of raw grain products. Although these grains can be consumed in different ways, the typical manner and the one contributing the most to their consumption is probably as "homemade" muesli where the grains are milled or flaked, then soaked in milk, water or juice, and consumed accompanied by different fruits. These grains hardly undergo any processing as they are eaten raw, with just manual milling or flaking with domestic appliances. Therefore, consumers of raw grain products could, in principle, be exposed to higher concentrations of EAs than the general population.

Since neither consumption data nor occurrence data were available for exposure calculations in this group of the population, several assumptions were made. Consumption data were obtained from the Consumption Database based on the consumption of breakfast cereals across the different countries. The occurrence values were derived from the submitted data, creating a new category that grouped the two food categories that contain a mix of different grains and a mix of grain milling products. Since no standard composition of muesli is described in the literature, this mix of grains and grain milling products was considered adequate to represent a sample of muesli. The mean EA concentrations calculated for the mix category grain for human consumption and grain milling products, including a total of 42 samples covering various types of grain, are reported in Table 10.

Table 10: Occurrence values used to calculate the dietary exposure to ergot alkaloids for groups of population consuming raw grain products (μg/kg).

	N		N_T		Mean	Average fourth quartile
Barley, pearled	3	**Mix category grains for human consumption and grain milling products[(a)]**	42	**Lower bound**	11	40
Bulgur wheat	2					
Chapatti flour	1					
Corn flour	2					
Corn semolina	2					
Flour mix, wheat/rye/barley/oats	15					
Grain milling products	2					
Grains for human consumption	5					
Oat bran	2					
Sorghum flour	2					
Sorghum grain	1			**Upper bound**	37	85
Spelt flour, wholemeal	2					
Spelt grain	1					
Wheat germ	2					

(a) This category does not include rye grains nor rye/wheat milling products;
N: Number of samples available for each food group at FoodEx Level 4; N_T:Total number of samples of the created category.

Chronic and acute dietary exposure in this group of the population was calculated as for the general population but replacing the occurrence value of breakfast cereals by that of the new category of mix grains and mix grain milling products. The mean chronic exposure to EAs ranged from 0.008 μg/kg b.w. per day at the LB to 0.199 μg/kg b.w. per day for toddlers at the UB. For high consumers, the chronic exposure varied between 0.015 μg/kg b.w. per day at the LB and 0.381 μg/kg b.w. per day for other children at the UB. The mean acute exposure to EAs ranged from 0.024 μg/kg b.w. per day at the LB to 0.443 μg/kg b.w. per day for other children at the UB. For high consumers, the acute exposure varied between 0.067 μg/kg b.w. per day at the LB and 1.185 μg/kg b.w. per day for other children at the UB.

In conclusion, the calculated exposure to EAs in this group of the population was only slightly higher than for the general population. Unlike the general population, the age group other children showed the highest exposure due to the higher consumption of breakfast cereals compared to toddlers. Since no real data on consumption and occurrence were available, these results should be viewed with caution. However, special attention should be paid to eating raw grains as whole sclerotia could get into a food serving and consequently lead to comparatively high EA-exposure for consumers of those products

6.1.4. Non-dietary sources of human exposure

A medical report was published describing the impact of chronic inhalation of milling dust from heavily ergotised rye. Exposure to EAs was confirmed by HPLC-FLD determination of plasma ergotamine levels (9 ng/ml). Angiography demonstrated progressing narrowing of the lower leg arteries. By strictly avoiding the source of exposure (rye milling dust), the typical clinical signs of ergotism (and ergotamine plasma levels) reduced slowly over a period of 4 month and the person recovered (Stange et al., 1998).

Patients using ergotamine tartrate by oral administration for treatment of migraine are usually exposed to single oral doses of 1 to 2 mg of ergotamine tartrate, repeated, if necessary, half an hour later. Usually not more than 6 mg should be given in 24 hours (Brunton et al., 2006, Bracher et al., 2010; Martindale, 2010). More detailed information is given in Section 7.5.1.

Women may be exposed to oral doses of 0.2 to 0.4 mg ergometrine maleate 2 to 4 times daily for 2 7 days to reduce postpartum bleeding and puerperal uterine atony and sub-involution (Martindale, 2010). More detailed information is given in Section 7.5.2.

6.2. Exposure assessment of ergot alkaloids in animals

6.2.1. Estimation of ergot alkaloids intake in feed by farm livestock

The concentration of EAs in the diet is a function of the concentration in individual feeds and the amounts of those feeds consumed. Reported levels of EAs in cereal grains and cereal by-products are given above (see Section 4.2.4.), while diet compositions are as described by EFSA (2011d).

As discussed above (Section 1.2.2.), intoxications of livestock by EAs are rare in grazing livestock, and therefore exposure to these toxins in forage crops are not included in estimates of exposure.

Based on feed intake data described in Section 5.2., and the mean LB and UB values for EAs in cereals grains and cereal by-products (see Section 4.2.10.), estimates of the LB and UB concentrations of EAs in diets and exposure by pigs, poultry, fish and rabbits are given in Table 11.

These estimates are derived using typical inclusion rates of cereals and cereal by-products in livestock feeds, and their mean LB and UB values. As noted in Section 4.2.10., the mean LB and UB concentrations reported for compound feeds for pigs (79 and 91 µg/kg, respectively) are markedly higher than have been predicted using the values for individual cereals and cereal by-products, and their likely inclusion rate in the diet. If the values for compound feeds are applied to finishing pigs, then LB-UB exposure would be 237 - 273 µg per day, respectively, and 2.4 - 2.7 µg/kg b.w. per day.

Table 11: Estimated mean lower bound (LB) and upper bound (UB) concentrations and exposure by pigs, poultry, fish and rabbits to ergot alkaloids, based on at least six ergot alkaloids.[17]

	*	Diet concentration µg/kg	Intake µg per day	Intake µg/kg b.w.
Pig starter	LB	23	23	1.14
	UB	30	30	1.51
Pig finisher	LB	27	80	0.80
	UB	35	105	1.05
Lactating sow	LB	22	131	0.65
	UB	30	182	0.91
Chickens for fattening	LB	8	1.0	0.51
	UB	13	1.6	0.79
Laying hens	LB	6	0.8	0.39
	UB	10	1.2	0.61
Turkeys for fattening	LB	32	12.7	1.06
	UB	38	15.2	1.26
Ducks for fattening	LB	22	3.1	1.03
	UB	29	4.1	1.37
Salmonids	LB	3	0.11	0.06
	UB	4	0.18	0.09
Rabbits	LB	17	2.53	1.27
	UB	20	3.06	1.53

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

Similarly, mean LB and UB concentrations in the non-forage component of the ration of ruminants have been estimated and exposures calculated assuming typical feed intakes and live weights (Table 12).

Table 12: Estimated mean lower bound (LB) and upper bound (UB) concentrations and exposure by ruminants to ergot alkaloids.[16]

	*	Diet concentration µg/kg	Intake µg per day	Intake µg/kg b.w.
Dairy: high yielding	LB	20	164	0.25
	UB	24	201	0.31
Beef: intensive cereal	LB	44	377	0.94
	UB	49	420	1.05
Beef: fattening	LB	31	45	0.11
	UB	35	51	0.13
Sheep: lactating	LB	19	27	0.45
	UB	24	34	0.56
Goats: lactating	LB	20	52	0.86
	UB	23	59	0.99
Goats: fattening	LB	17	10	0.25
	UB	19	12	0.29

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

[17] LB and UB values calculated for feeds selection using Scenario 1, in which data for at least six EAs containing ergotamine, ergocristine, ergosine and ergocornine were used (Table 3).

Of the cereal grains used in livestock feeds, rye is the most susceptible to infection with *Claviceps* spp. under European conditions (EFSA, 2005). Where it is available at a competitive price, it may substitute for other cereals in rations for certain livestock, although its use is limited (as described in Section 5.2.). Based on the maximum inclusion rates in rations for pigs and ruminant livestock, exposure to EAs have been estimated in Table 13.

Table 13: Estimated mean lower bound (LB) and upper bound (UB) concentrations and exposure by pigs, poultry and ruminants to ergot alkaloids where rye and rye by-products are included up to their maximum recommended levels.[18]

	Inclusion rate*		Diet concentration µg/kg	Intake µg per day	Intake µg/kg b.w.
Pig finisher	50	LB	141	422	4.22
		UB	155	464	4.64
Lactating sow	40	LB	113	678	3.39
		UB	126	755	3.78
Laying hens	30	LB	75	9.0	4.49
		UB	82	9.8	4.91
Dairy cows: high yielding	25	LB	64	529	0.81
		UB	69	569	0.88
Beef: fattening	40	LB	93	134	0.34
		UB	99	142	0.36

* Inclusion rate of rye as a percentage of the non-forage part of the ration; b.w.: body weight; LB: lower bound; UB: upper bound.

6.2.2. Estimation of ergot alkaloids intake by companion animals

Using the approach outlined above, estimates of exposure for companion animals have been made (Table 14).

Table 14: Estimated mean LB and UB concentrations and exposure by companion animals to EAs.[17]

		Diet concentration µg/kg	Intake µg per day	Intake µg/kg b.w.
Cats	LB	7	0.25	0.06
	UB	17	0.56	0.14
Dogs	LB	7	1.75	0.07
	UB	17	3.94	0.16
Horses	LB	6	29	0.06
	UB	10	45	0.10

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

7. Hazard identification and characterisation

7.1. Toxicokinetics

Toxicokinetics have not been systematically studied for the majority of naturally occurring EAs. However, human data are available for the naturally occurring alkaloids used as pharmaceuticals, ergometrine and ergotamine.

[18] LB and UB values calculated for feeds selection using Scenario 1, in which data for at least six EA containing ergotamine, ergocristine, ergosine and ergocornine were used (Table 3).

Absorption

The absorption of **ergotamine** and a number of semi synthetic ergot derivatives (dihydroergotamine, dihydroergotoxine, dihydroergostine, dihydroergocornine, dihydroergovaline, dihydroergonine, 1-methyl-ergotamine, and bromocriptine) was studied in humans by Aellig and Nüesch (1977) using [^{3}H]-labelled compounds. Each drug was administered to six subjects in a randomized cross-over design as single oral and intravenous (i.v.) doses. The absorption rates for the compounds were calculated from the cumulative urinary excretion data after oral and intravenous administration. Ergotamine showed 60 % absorption, whereas most of the dihydrogenated alkaloids were less well absorbed (approximately 25-30 %), while bromocriptine was nearly completely absorbed. The peak-concentration was detected in plasma after about two hours after oral administration (range 1.0 - 2.7 hours).

Nimmerfall and Rosenthaler (1976) calculated an intestinal absorption of 31.5 ± 5.2 % in rhesus monkeys administered 0.25 mg/kg of [^{14}C]-ergotamine tartrate by gavage.

Ibraheem et al. (1983) treated two male and five female human volunteers with 2 mg of ergotamine tartrate by oral administration and monitored the blood concentration from 10 minutes up to 54 hours following the administration. No ergotamine was detected in blood (at a detection limit of 0.1 ng/ml) during the observation period. The authors concluded that the radioactivity measured in blood by Aellig and Nüesch (1977) was attributed to metabolites and not to the parent compound and estimated a maximum bioavailability of approximately 2 % for ergotamine tartrate.

De Groot et al. (1994) studied the pharmacokinetics of ergometrine in 6 male human volunteers exposed orally and intravenously to 0.200 mg and 0.075 mg ergometrine maleate (corresponding to 0.147 mg and 0.055 mg ergometrine), respectively. A large individual variation was observed in several pharmacokinetic parameters, such as the measured peak concentrations in plasma following oral exposure (ranging from 0.61 to 1.39 µg/l) or in the absorption half-life (ranging from 0.005 hours to 0.55 hours). The calculated bioavailability F (i.e. the percentage of the administered oral dose reaching the systemic circulation) was 0.76 and showed variability between the six subjects, ranging from 0.34 to 1.17.

Shappell and Smith (2005) studied the absorption of ergovaline and its epimer ergovalinine in an *in vitro* model of the intestinal epithelium (human Caco-2 cells). After six hours, 25 - 40 % of the administered concentrations (6.6 and 25 µM, respectively) crossed the intestinal cell layer. No major metabolites of ergovaline/ergovalinine were detected.

Evidence suggests that a specific absorption profile may exist in ruminants. Stuedemann et al. (1998) observed a rapid and extensive excretion via the urine in steers exposed to infested tall fescue (approximately 96 % of the estimated EA intake, measured by means of ELISA) and hypothesised that EAs are absorbed mainly in the forestomach of ruminants. Westendorf et al. (1993) observed a limited faecal excretion of 5 % of the EAs in sheep administered feed contaminated with EAs, suggesting that also in this case a high absorption took place. A higher faecal recovery (24 % mean recovery) and no urinary excretion (measured by HPLC at LODs of 5-10 µg/l) was measured by Schumann et al. (2009) in dairy cows fed a diet containing approximately 1.4 mg/kg EAs.

Hill et al. (2001) studied the absorption of a mixture of EAs (lysergol, lysergic acid, ergometrine, ergocryptine and ergotamine tartrate) in ruminant forestomach tissues. Rumen was the tissue with the greatest transport rate (25 % and 600 % more than the omasum and reticular tissues, respectively). For all tissues, the simple lysergic acid alkaloids were more efficiently transported than ergopeptine alkaloids. Lysergic acid was observed to have the highest transport rate regardless of the tissue tested.

Distribution

Following absorption, EAs are rapidly distributed into the body. Ibraheem et al. (1982) observed a rapid distribution from plasma following i.v. administration of 0.5 mg ergotamine tartrate to 10 human

volunteers, with a mean initial distribution half-life of 0.05 ± 0.1 hours and a calculated mean volume of distribution of 1 848 ± 771 ml/kg b.w. A similar rapid distribution was observed in 6 male volunteers administered 0.055 mg ergometrine by i.v. injection by De Groot et al. (1994), although a significant individual variability was noted (a mean distribution half-life of 0.18 ± 0.20 hours, a mean volume of distribution of 1 017 ± 317 ml/kg b.w.). The large volumes of distribution are consistent with extensive tissue distribution.

Little is known about the distribution of EAs in different tissues. Kalberer (1970) reported the distribution in different tissues following i.v. injection of 1 mg/kg b.w. [^{3}H]-ergotamine in rats. Two hours after the injection, higher radioactivity in liver, lungs, kidney and heart was measured in comparison to blood, whereas a low radioactivity concentration was observed in the brain. The ability of ergometrin(in)e, ergotamin(in)e and ergocristin(in)e to cross the blood-brain barrier was also assessed *in vitro* in porcine brain capillary endothelial cells (PBCEC) by Mulac et al. (2012). In this model, the three EAs showed the potential to cross the blood-brain barrier. The more lipophilic ergotamin(in)e and ergocristin(in)e were observed to diffuse more efficiently than ergometrin(in)e, for which a possible active transport through the blood brain barrier was identified. Furthermore, when pure ergocristinine was incubated with PBCEC, no transcellular diffusion was observed for this epimer, indicating that the transport could be selective for the 8-(R) form (-ine epimers) of the EAs (Mulac et al., 2012).

Some evidence in livestock indicates that ergopeptines may accumulate to a low extent in body fat (Strickland et al., 2011).

Metabolism

There is little information available on the metabolism of EAs, even for those used as therapeutic agents. Following oral exposure, more than 90 % of absorbed ergotamine is estimated to undergo pre-systemic metabolism in humans (Silberstein and McCrory, 2003). Similarly, kinetic studies of EA derivatives showed extensive pre-systemic metabolism following oral dosing (e.g. Ronca et al., 1996; Chen et al., 2002; Bicalho et al., 2005).

Although the metabolic pathway is not fully described for naturally occurring EAs, the *in vivo* metabolism has been elucidated for the synthetic derivatives bromocryptine and dihydroergotamine by Maurer and co-workers in rats and humans (Maurer et al., 1982, 1983; Maurer and Frick, 1984). For both substances, hydroxylation in position 8' at the proline ring of the peptidic moiety was observed as the primary metabolite formation, followed by further oxidation producing 8',9'- or 8',10'-dihydroxy-derivatives, and for dihydroergotamine, by the oxidation of the indole ring (Maurer and Frick, 1984). Several hydroxylated metabolites were observed to retain the biochemical activity and receptor binding potential of the parent compounds (Aellig, 1984; Maurer and Frick, 1984; Muller-Schweinitzer, 1984). A metabolic pathway similar to dihydroergotamine and bromocryptine was postulated for other ergopeptines (ergotamine and the semi-synthetic dihydroergotoxine) when tested *in vitro* in rat and bovine hepatic microsomes (Moubarak and Rosenkrans, 2000; Moubarak et al., 2002; Bicalho et al., 2008). Recently, the oxidative metabolic pathway was substantially confirmed for ergotamine and ergocristine in two immortalised human cell lines, HT-29 and HepG2, by Mulac et al. (2011). Cytochrome P450 3A4 (CYP3A4) was identified as the isoform subfamily involved in the formation of the (di)hydroxy derivatives. Ergometrine was also found to undergo oxidative metabolism in rats administered 3 mg/kg b.w. by i.v. injection. Hydroxylation of the ergoline ring to give 12-hydroxy-ergometrine (and its epimer 12-hydroxy-ergometrinine), followed by conjugation to glucuronic acid was observed as the main metabolism pathway. However, the unchanged parent compound and other metabolites possibly including *N*-demethylated products and unidentified glucuronide conjugates were observed when rats were injected ergometrine at a higher dose (45 mg/kg b.w.) (Slaytor and Wright, 1962; EMEA, 1999).

In ruminants, rumen microflora could play different roles in the metabolic fate of EAs. On one hand, fermentation in the rumen can liberate EAs from the plant tissues in case of endophyte-infested tall fescue, increasing thus the amount available for absorption (De Lorme et al., 2007; Ayers et al., 2009).

On the other hand, rumen microflora were shown to efficiently degrade ergopeptines to lysergic acid (De Lorme et al., 2007; Ayers et al., 2009).

Excretion

Following hepatic metabolism, biliary excretion represents the main elimination pathway of ergotamine and its metabolites, accounting for approximately 80 - 90 % of the absorbed dose in Rhesus monkeys and humans (Nimmerfall and Rosenthaler, 1976; Aellig and Nüesch, 1977) whereas urinary elimination of unchanged ergotamine was observed as a minor excretion pathway in humans (Aellig and Nüesch, 1977). A similar excretion profile can be extrapolated for other ergopeptides in humans.

On the other hand, urinary excretion of EAs, mainly as peptide-free lysergic moieties, appeared to be predominant in cattle grazing infected tall fescue (Stuedemann et al., 1998) and generally a lower faecal excretion was observed in ruminants (Westendorf et al., 1993; Schumann et al., 2009). The different excretion profile is likely attributable to the ruminant specific absorption profile of EAs, although the role of the ruminal microflora metabolism leading to the cleavage of the peptidic moiety cannot be disregarded (Stuedemann et al., 1998; Hill et al., 2001; Ayers et al., 2009).

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

Griffith et al. (1978) reported a series of LD_{50}s determined for several naturally occurring and (semi-) synthetic EAs by i.v., subcutaneous (s.c.) and oral exposure in mouse, rat and rabbit. An unreported number of mice (albino, MF2 strain), rats (albino OFA Sandoz) or rabbits (mixed breeds, hare-type or Silver-Fawn) of both sexes were used in those studies. Substances were suspended in 2 % gelatine for the oral administration. Animals were subjected to a 7 day-observation period during which clinical signs and mortalities were recorded.

The naturally occurring alkaloids tested show a low oral acute toxicity (Table 15). Oral LD_{50}s are always higher than i.v. LD_{50}s in the same species, reflecting the low absorption and high pre-systemic metabolism subsequent to oral administration. Moreover, marked differences in sensitivity exist between species, with the rabbit being the most sensitive.

Sub-lethal acute exposure to EAs induces signs of neurotoxicity in mammals, including restlessness, miosis or mydriasis, muscular weakness, tremor and rigidity. Tail gangrene was observed in rats 5 - 7 days after a single i.p. exposure to 25 mg/kg b.w. ergotoxine (a mixture including ergocornine, α- and β-ergocryptine, and ergocristine) (Griffith et al., 1978).

Table 15: LD_{50}s determined in mice, rats and rabbits by i.v. or oral exposure for EAs.

Substance	Species	Route	LD_{50} (mg/kg)
D-lysergic acid	Mouse	i.v.	240
	Rabbit	i.v.	100
Ergometrine	Mouse	i.v.	160
	Mouse	Oral	460
	Rat	i.v.	120
	Rat	Oral	671
	Rabbit	i.v.	3.2
	Rabbit	Oral	27.8
Ergotamine	Mouse	i.v.	265
	Mouse	Oral	3 200
	Rat	i.v.	38
	Rat	Oral	1 300
	Rabbit	i.v.	3
	Rabbit	Oral	550
Ergosine	Mouse	i.v.	33.5
	Rat	i.v.	30
	Rabbit	i.v.	1.23
Ergostine	Mouse	i.v.	125
	Mouse	Oral	1 700
	Rat	i.v.	47
	Rat	Oral	> 1 000
	Rabbit	i.v.	1.2
	Rabbit	Oral	~ 1 000

i.v.: intravenous

7.2.2. Repeat dose toxicity

Studies involving parenteral administration have shown that repeated dosing of various EAs cause ischaemia in some part of the body, such as the tails of rats, comb and wattles in cockerels or the margins of the external ear in dogs and rabbits, resulting from vasoconstriction (Griffith et al., 1978). Few studies have been conducted with oral administration.

Ergotamine tartrate (purity > 98 %) was fed to groups of 6 male and 6 female Sprague-Dawley rats at concentrations of 0, 4, 20, 100 and 500 mg/kg diet for 4 weeks (Speijers et al., 1992). Based on the records of feed consumption and body weight, these dietary concentrations equalled ergotamine tartrate doses of 0, 0.38, 1.9, 8.2 and 46 mg/kg b.w. per day for females and 0, 0.38, 1.8, 8.5 and 42 mg/kg b.w. per day for males (equivalent to ergotamine doses of 0, 0.34, 1.7, 7.3 and 41 mg/kg b.w. per day for females and 0, 0.33, 1.6, 7.5 and 37 mg/kg b.w. per day for males). There was a dose-related decrease in food intake, food conversion efficiency and body weight gain, with females appearing more sensitive than males. The authors considered the reduced food intake was due to decreased palatability of the diet at 100 mg/kg and above. Absolute weights of liver and spleen were decreased in both sexes, as were kidneys and thymus in females. Absolute and relative weights of the ovaries were increased at the top two doses. Relative weights of liver were increased in females, and of heart and brain were in both sexes. Other findings included changes in haematological parameters, serum thyroid hormones (not accompanied by histopathological changes) and, in the males, increased urine production. Histopathological examination revealed a slight increase in regenerative and degenerative changes in the kidneys and activation of the iliacal lymph nodes in the high dose group. Redness of the tail was seen in all animals of the high dose group, with necrosis in some cases. Based on significantly decreased body weight gain at higher doses, the no-observed-adverse-effect level (NOAEL) was 4 mg/kg **ergotamine tartrate** in the diet, which was equal to 0.3 mg/kg b.w. per day **ergotamine**.

Based on the results of Speijers et al. (1992), dietary concentrations of 0, 5, 20 and 80 mg/kg **ergotamine tartrate** (purity > 98 %) were selected for a subsequent 13 week study in groups of

10 male and 10 female Sprague-Dawley rats (Speijers et al., 1993). According to the authors, 20 mg/kg ergotamine tartrate in feed was equivalent to an ergotamine dose of 0.9 mg/kg b.w. per day. However the CONTAM Panel noted that the default factor for conversion from concentration in feed to dose in chronic studies appeared to have been used, and considered it more appropriate to estimate the doses based on the records of feed consumption and body weight. The calculated doses expressed as ergotamine were 0, 0.41, 1.7 and 6.5 mg/kg b.w. per day for the females and 0, 0.36, 1.4 and 5.4 mg/kg b.w. per day for the males. Body weight gain and food intake were decreased in females at the top dose group. Other findings in the top dose group of females, and sometimes also males, included slight changes in haematological and biochemical parameters, increased urine volume, decreased absolute weights of liver, kidneys, pituitary glands and uterus, increased relative brain weight and decreased relative pituitary weight. The only clear treatment-related histopathological finding was muscular atrophy in the caudal longitudinal muscles of the tail in 7 females and 7 males at 80 mg/kg, in 2 females and 1 male at 20 mg/kg, in 1 male at 5 mg/kg ergotamine tartrate in the diet, and in 1 male of the control group. The authors considered that the low incidence in the low dose and control group was the background level and concluded that the "no-toxic effect level" was 20 mg/kg diet ergotamine tartrate. However, based on the changes in the tail, the CONTAM Panel concluded that the NOAEL was 5 mg/kg diet **ergotamine tartrate,** equivalent to 0.4 mg/kg b.w. per day **ergotamine**. This conclusion was supported by modelling of the dose-response relationship (see Section 7.7.1).

In a study conducted in accordance with Good Laboratory Practice (GLP), groups of 6 male and female Sprague-Dawley rats were fed diets containing 0, 2, 10, 50 or 250 mg/kg synthetically produced **ergometrine maleate** (purity stated to be 98.0-101 %) for 4 weeks, with an additional control group pair-fed with the high-dose group (Peters-Volleberg et al., 1996). Based on the records of feed consumption and body weight, these dietary concentrations equalled ergometrine maleate doses of 0, 0.2, 0.9, 4.1 and 21 mg/kg b.w. per day for females and 0, 0.2, 0.8, 4.0 and 21 mg/kg b.w. per day for males (equivalent to ergometrine doses of 0, 0.1, 0.7, 3.0 and 15 mg/kg b.w. per day for females and 0, 0.1, 0.6, 2.9 and 15 mg/kg b.w. per day for males). There were no treatment-related effects on haematological or kidney function parameters. Plasma glucose and free or total thyroxine (T4) were decreased in some dose groups, but not in a clear dose-related manner. Similarly, relative and/or absolute weights of heart, liver, ovaries and kidneys were increased in some dose groups, but not in a clear dose-related manner. However, there were no treatment-related histopathological changes in these organs, except for the liver, where relatively large and occasionally swollen hepatocytes, with an appearance typical of glycogen storage, were observed in the high dose animals of both sex. Hepatocellular degeneration or necrosis was not observed. Necrosis of the tail tips was also not seen. Serum prolactin levels were markedly decreased in both sexes at 50 and 250 mg/kg diet (Peters-Volleberg et al., 1996). The authors reported that the NOAEL was 10 mg/kg diet, which was equal to 0.8 mg/kg b.w. per day ergometrine maleate (0.6 mg/kg b.w. per day ergometrine).

Groups of six male and female Sprague-Dawley rats were fed diets containing 0, 4, 20, 100 or 500 mg/kg **α-ergocryptine** (purity > 99.9 %) for 28-32 days, with an additional control group pair-fed with the high-dose group (Janssen et al., 2000a). Based on the weekly records of feed consumption and body weight, these dietary concentrations equalled doses of 0, 0.4, 1.7, 10 and 60 mg/kg b.w. per day for females and 0, 0.3, 1.4, 7 and 44 mg/kg b.w. per day for males. α-Ergocryptine treatment resulted in decreased feed intake, feed efficiency and bodyweight gain, and in a number of changes in haematological parameters, serum enzyme activities, kidney function, and absolute and relative organ weights, most of which were considered to be indirect effects of reduced feed intake. Findings not considered secondary to reduced feed intake included inflammation and muscular degeneration in the tail tips, which was observed only at the microscopic level and was assumed to be due to the vasoconstrictive properties of ergocryptine. Increases in absolute and relative ovary weight, and in relative liver weight, were also considered to be a direct effect of ergocryptine. In addition, the authors noted that the relative heart weight was increased in the high dose females, which did not appear consistent with body weight change, but there were no histopathological changes in the heart (Janssen et al., 2000a). Ergocryptine also affected thyroid and pituitary function, with dose-dependent decreases in serum total T4 and prolactin concentrations in both sexes, and carbohydrate and lipid metabolism,

demonstrated by changes in serum/plasma concentrations of insulin, glucagon, cholesterol, glucose and triglycerides. The authors postulated that the observed effects on food intake and metabolism were due to interaction of ergocryptine with central dopaminergic activities (Janssen et al., 2000b). The NOAEL in this study was 4 mg/kg diet, equal to 0.3 mg/kg b.w. per day α-ergocryptine.

From the results of the 28-day studies described above it can be concluded that there is no major quantitative difference in the toxicity of ergotamine, ergometrine and α-ergocryptine.

7.2.3. Developmental and reproductive toxicity

EAs have a number of well-established effects on the reproductive process including prevention of pregnancy by interfering with implantation, embryotoxicity, developmental effects and inhibition of lactation (Griffith et al., 1978). There is also some evidence of inhibition of ovulation, from a study in which 1 mg **ergocornine** was injected subcutaneously to rats (Kraicer and Strauss, 1970).

A single s.c. injection of (0.175 - 3 mg) **ergotoxine ethanesulphonate** into rats in early gestation resulted in termination of pregnancy and occurrence of proestrus and oestrus within 3 days. A 1:1:1 mixture of the three components (**ergocornine, ergocryptine** and **ergocristine**, as their methane sulphonates) was as active as ergotoxine ethanesulphonate. Individually, **ergocristine** appeared to be the least active, with termination of pregnancy at doses of 2 mg and above, whereas **ergocornine** and **ergocryptine** were effective at doses about an order of magnitude lower (Shelesnyak, 1957). Subsequent studies confirmed this effect occurred also in mice and hamsters and suggested that the alkaloids did not have a direct toxic effect, but interfered with implantation by inhibiting release of prolactin from the pituitary (Griffith et al., 1978).

Feeding of a diet containing 2 % ergot sclerotia to female BS/VS mice prevented pregnancy. Similarly, mice failed to become pregnant following oral administration of 250 µg **ergotoxine ethanesulphonate** or **ergosine/ergosinine** (60:40) on days 3, 4 and 5 after mating (which was confirmed by the presence of a vaginal plug) (Mantle, 1969). The potency for inhibiting implantation following oral dosing has been reported to be **ergocornine > ergonine > ergotamine > ergometrine** (unpublished data cited in Griffith et al., 1978). Following subcutaneous injection in rats, the relative potency was **ergocryptine > ergocornine > ergonine > ergotamine > ergometrine** (Flückiger et al., 1976). Floss et al. (1973) questioned whether or not prolactin plays a similar essential role in the process of ova implantation in humans as it does in rats, which raises doubts about the relevance of the observations of impaired implantation in rodents.

Sommer and Buchanan (1955) intraperitoneally injected 1 mg/kg b.w. per day of **ergotamine methanesulphonate** or **ergotoxine ethanesulphonate** to pregnant rats on gestational days (GD) 12 - 21. Of 24 animals receiving **ergotamine methanesulphonate**, five apparently failed to conceive or resorbed their foetuses at an early stage, 4 died during late pregnancy after fetal death had occurred and 4 gave birth to non-viable pups. About one third of the live-born pups died during the first 16 days. Similarly, of the 19 animals receiving **ergotoxine ethanesulphonate**, two failed to conceive or resorbed their foetuses, 4 died after fetal death had occurred and 3 gave birth to non-viable pups. The surviving pups gained weight more slowly than the pups of control animals for the first 10 days. Further groups of rats were injected with 2 mg/kg b.w. per day of **ergotamine methanesulphonate** or **ergotoxine ethanesulphonate** over the same period of pregnancy. They were culled within several hours of delivery, and showed decreased milk yield and decreased fat content of the milk compared to control animals.

Administration of 1 mg **ergocornine methanesulfonate** by subcutaneous injection to pregnant rats on gestational day 7, i.e. post-implantation, resulted in a high incidence of resorptions and visceral malformations. Injection of progesterone reduced these effects, suggesting that they were mediated by progesterone deficiency (Carpent and Desclin, 1969).

Ergotamine tartrate was administered orally to pregnant mice (0, 30, 100 and 300 mg/kg b.w. per day) and rats (0, 1, 3, 10, 30 and 100 mg/kg b.w. per day) on gestational days 6 - 15 and to rabbits (0, 1, 3, 10 and 30 mg/kg b.w. per day) on gestational days 6 - 18 in a study conducted in accordance with then current US Food and Drug Administration (FDA) guidelines for teratological testing (Grauwiler and Schön, 1973). The fetuses were removed near term by caesarean section (mice on day 18, rats on day 21, rabbits on day 20). In mice, there was a dose-related decrease in maternal weight gain and weight of fetuses, which was statistically significant compared to controls at all doses. Fetal loss was significantly increased and skeletal ossification was significantly decreased at 100 and 300 mg/kg b.w. per day, but not at 30 mg/kg b.w. per day and hence these observations could be secondary to effects on the dams. In rats, maternal weight gain was significantly decreased at 1 mg/kg b.w. per day, similar to control at 3 mg/kg b.w. per day and then markedly decreased in a dose-related manner from 10 mg/kg b.w. per day. Significantly decreased fetal weight and increased fetal loss and delayed skeletal ossification occurred at doses of 10 mg/kg b.w. per day and higher. Similar effects were reported in rabbits, but the changes were smaller and showed no clear dose-dependency. Overall, rats were the most sensitive of these three species to the maternal and fetal effects. There was no evidence of teratogenicity in any of the species (Grauwiler and Schön, 1973). The authors postulated that the fetal effects were mediated via pharmacodynamic action on the cardiovascular system of the dams.

In a subsequent study, pregnant rats were dosed orally with 10 mg/kg b.w. **ergotamine tartrate** on single days between gestational days 4 - 19. Dosing from day 11 onwards resulted in increased prenatal mortality, reaching a maximum on day 14. Dosing on gestational days 13 - 16 also resulted in characteristic anomalies (shortening or absence of nails, phalanges and digits), resembling some of the effects seen following interruption of uterine blood flow (Schön et al., 1975, available as abstract only). The potency for causing embryonic death following oral dosing has been reported to be **ergonine > ergoalanine > ergovaline > ergostine > ergotamine > ergometrine** (unpublished data cited in Griffith et al., 1978).

Elymoclavine (purity not stated) was injected i.p. into groups of 10-14 ICR mice on the 10th day of pregnancy at 0, 3, 30 and 60 mg/kg b.w. In animals maintained at 20 °C, the numbers of resorptions and malformed foetuses were increased at the top dose. Numbers of skeletal anomalies (fused ribs and abnormal thoracic vertebrae) were increased at 30 and 60 mg/kg b.w. (Witters et al., 1975).

The reproductive toxicity of EAs has been investigated in mink. Groups of 12 female mink were fed diets containing clean or *C. purpurea* contaminated oats, providing 0, 3, 6 or 12 mg/kg total EAs (**49.6 % ergocristine, 16.0 % ergocryptine, 14.7 % ergocornine, 12.9 % ergotamine, 6.8 % ergosine**) from 2 weeks before mating (with untreated males) until the kits were approximately 33 days old. There was a transient, dose-related decrease in feed consumption, which was statistically significant at all doses, but body weights were unaffected. The EAs resulted in a dose-related decrease in the number of mink whelping and kit survivability and an increase in gestation period at the top two doses. Decreased numbers of kits and serum prolactin levels were reported at all doses. No changes in organ weights or histopathological abnormalities were observed in the dams (Sharma et al., 2002). Insufficient information was provided to allow estimation of the dose of alkaloids in this study.

Effects on lactation

EAs are well known to inhibit milk production in humans, laboratory animals and livestock animals (Varga et al., 1972; Floss et al., 1973; Kopinski et al., 2007, 2008). This effect has been correlated by several authors to the decrease of prolactin secretion induced by EAs. Prolactin, also known as luteotropic hormone, is a protein hormone secreted mainly by the anterior pituitary gland (adenohypophysis).

The correlation between milk production inhibition and depressed prolactin secretion was first hypothesised by Zeilmacker and Carlsen (1962), who showed that the lactation inhibiting effect caused by i.p. injection of 1 mg **ergocornine** in female rats after parturition could be overcome by concurrent administration of prolactin.

Shaar and Clemens (1972) exposed lactating post-partum Sprague Dawley rats to 0.5 or mg **ergocornine hydrogenmalienate**, 4 mg **ergometrine maleate**, 1 mg dihydroergocornine, 4 mg **ergotamine tartrate** or 0.5 mg **ergocryptine mesylate** by subcutaneous injection on day 4-8 postpartum. A control group exposed only to vehicle (corn oil) was included. Adult and litter weights were taken on treatment days four, six and eight and blood samples were collected for measurement of serum prolactin levels at the end of the exposure period. In another experiment, lactating females and their litters were exposed to ergocornine hydrogenmalienate under the same experimental conditions applied in the first experiment. At the end of the exposure period adult rats were sacrificed, blood was taken and mammary glands were removed and weighed. Finally, hypophysectomised mature female rats were transplanted with anterior pituitary glands from two litter mate donors beneath the left kidney capsule and subsequently exposed to ergocornine hydrogenmalienate for two consecutive days, after which animals were sacrificed and blood was taken. In the first experiment, a significant decrease in litter weight, but no overt differences in nursing behaviour were observed in the groups injected with EAs in comparison to controls. Mean serum prolactin levels were significantly decreased in all adult groups treated with EAs in comparison to controls. The authors noted that ergometrine and ergotamine were less effective in comparison to the other alkaloids tested. Decrease in litter body weight and serum prolactin levels were confirmed in the second experiment, in which ergocornine hydrogenmalienate caused also a statistically significant decrease of the mammary gland weight in the lactating females. A significant decrease in serum prolactin levels was observed in hypophysectomised rats transplanted with anterior pituitary glands and treated with ergocornine hydrogenmalienate in comparison to untreated hypophysectomised and transplanted rats, showing that the inhibition of prolactin secretion can be caused by the direct action of ergocornine on the pituitary gland.

Although Shaar and Clemens (1972) showed a direct action of **ergocornine** to the hypophysis towards the inhibition of prolactin secretion, other studies indicated that an indirect action via the hypothalamus is also plausible and possibly EAs may act both on hypothalamus and hypophysis (Floss et al., 1973).

In both cases, the dopaminergic activity of the EAs is likely responsible for the inhibition of prolactin secretion since dopamine itself inhibits prolactin secretion (Ben-Jonathan and Hnasko, 2001; Fitzgerald and Dinan, 2008).

7.2.4. Genotoxicity

Naturally occurring ergot alkaloids

Studies investigating the genotoxicity of naturally occurring EAs have mostly been carried out on **ergotamine**.

No evidence was found for the mutagenicity of **ergotamine** tartrate in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, with or without metabolic activation with liver S9 from rat or hamster (Zeiger et al, 1987, TOXNET[19]). Ergotamine was not mutagenic in mouse lymphoma L5178Y cells with or without metabolic activation with rat liver S9 (Seifried et al, 2006, TOXNET). In the absence of metabolic activation **agroclavine** was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA1537, but showed weak mutagenic activity when activated with rat liver S9 (Glatt et al, 1987).

In the study of Dighe and Vaidya (1988), several EAs were tested for the induction of sister chromatid exchange (SCE) frequencies in cultured Chinese hamster ovary cells (range of concentration between 10^{-5} and 10^{-8} M). The results indicated that **ergotamine** and **ergometrine** are effective inducers of SCE, while **ergocristine** is a weak inducer, and **α-ergocryptine** had no effect.

[19] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS

Ergotamine was tested for its ability to induce chromosomal damage in human lymphocytes in culture (Roberts and Rand, 1977a). The aberration frequency was significantly increased after treatment of the cells with 0.1 µg/ml, 0.25 µg/ml and 0.5 µg/ml of the drug. However, the degree of damage was considerably less than that produced by 0.1 mg/ml to 0.5 mg/ml of caffeine, which was used as a positive control and is a known mutagen in this test-system. **Ergometrine** was also effective in inducing chromosome aberrations in human leucocytes *in vitro* (Kato and Jarvik, 1969).

The *in vivo* chromosomal damaging properties of **ergotamine** were investigated following its i.p. administration (2 doses separated by 24 hours) to mice and Chinese hamsters (mixed sexes) (Matter, 1976). No increase in micronuclei was observed in mice and hamsters following treatment with **ergotamine** tartrate up to 150 mg/kg b.w. per dose. No increase in chromosome aberrations was observed in Chinese hamsters following treatment with ergotamine tartrate up to 150 mg/kg b.w. per dose.

The effect of **ergotamine** was studied in bone marrow of male mice by Roberts and Rand (1977b). Ergotamine was injected in doses of 25, 50 and 100 mg/kg b.w. (i.p., 2 doses separated by 24 hours). Significantly increased numbers of aberrations were observed in bone marrow preparations after treatment with the highest dose. Almost all the damage was in the form of chromatid aberrations. No exchange figures were observed, neither were other anomalies, such as nondisjunction or anti-mitotic activity. This frequency of damage was about 10-fold less than that produced by the alkylating agent, cyclophosphamide. Thus this EA was concluded to have weak chromosomal damaging effects *in vivo* only in very high doses.

The dominant lethal test was also carried out in the mouse using **ergotamine** (Roberts and Rand, 1978). Dominant lethal effects cause embryonic or fetal death. Induction of a dominant lethal event after exposure to a test substance indicates that the substance has affected germinal tissue of the test species. Ergotamine, administered by i.p. injection, increased early deaths per pregnancy at a dose of 100 mg/kg b.w. It was not effective at a dose of 25 mg/kg b.w., and at 50 mg/kg b.w. the interpretation of the result was uncertain. Reduced numbers of implantations were not consistently observed following treatment with ergotamine, but some anti-fertility effects were noted. A heritable translocation test carried out by Matter (1982) on mice administered ergotamine by i.p. injection at a dose level of 125 mg/kg b.w. showed no induction of transmissible chromosome damage.

In conclusion, with the exception of ergotamine, only limited genotoxicity studies have been carried out on naturally occurring EAs.. No mutagenic activity of ergotamine has been detected *in vitro*. Early studies showed it had some chromosome damaging effects *in vitro* and *in vivo* although the latter were weak. After metabolic activation agroclavine was weakly mutagenic in bacteria, but no *in vivo* studies have been carried out. Data are also lacking on other naturally occurring EAs.

Semi-synthetic ergot alkaloids

A variety of semi-synthetic EAs have been subjected to genotoxicity tests. These compounds included the 1-propyl and 1-pentyl derivatives of agroclavine and 8 other cytostatic clavines (Glatt et al., 1987, 1992), methylergonovine[20] (Dighe and Vaidya, 1988), α-dihydroergocryptine (Adams et al., 1993), dihydroergocristine (Dubini et al., 1990), dihydroergotoxine (which is a mixture of the methanesulphonate salts of dihydrogenated EAs (dihydroergocristine, dihydroergocornine, and α- and β-dihydroergocryptine) (Matter, 1976; Matter et al., 1978; Roberts and Rand, 1977a, b, 1978; Tsuchimoto and Stadler, 1976; Tsuchimoto et al., 1979), and methysergide (Matter, 1976; Roberts and Rand, 1977a, b, 1978).

Few positive indications of mutagenicity have been reported. Mutagenicity in bacteria has been demonstrated for some synthetic clavine alkaloids (Glatt et al., 1987, 1992). Methylergonovine was an effective inducer of SCE frequencies in cultured Chinese hamster ovary cells (Dighe and Vaidya, 1988).

[20] Synonym for methylergometrine.

Some weak chromosome damaging effects were observed *in vitro* and *in vivo* for dihydroergotoxine and methysergide by Roberts and Rand (1977a, b), although this was not confirmed in other studies.

The genetic toxicology of **lysergic acid diethylamide** (LSD) has also been investigated, although most of the studies are early (reviewed in Dishotsky et al., 1971; Long, 1972; Griffith et al., 1978; Li and Lin, 1998). In the paper of Dishotsky et al. (1971) 68 studies and case reports were reviewed. The conclusions of these studies were not consistent. There were some indications that LSD caused chromosomal aberrations in human leukocytes (Cohen et al., 1967), and that illicit LSD users showed chromosomal damage (Irwin and Egozcue, 1967). However not all studies reproduced the *in vitro* findings and the potential for chromosome damage caused by use of LSD by humans seems not to have been robustly confirmed. LSD has however been shown to cause mutations in drosophila (Browning, 1968; Vann, 1969), *E. coli* (Vann et al., 1970) and barley (Singh et al., 1970), and is positive in dominant lethal tests in ICR mice (Šràm et al., 1974). Interaction of LSD with DNA has been investigated and it has been suggested that intercalation of LSD within the DNA helix occurs (Smythies and Antun, 1969; Wagner, 1969).

In view of the structural differences of the semi-synthetic compounds from the naturally occurring EAs, and the limited data in many cases, it is not possible to draw further conclusions on the genotoxic potential of the latter from these data.

7.2.5. Carcinogenicity

No long-term exposure studies on **ergometrine**, **ergotamine**, **ergosine**, **ergocornine**, **ergocristine** and **ergocryptine** and their related –inines were available. In an old study, crude ergot was fed to Osborne-Mendel rats for periods of up to 2 years (Fitzhugh et al., 1944). A high incidence of neurofibromas was observed on the ears, which regressed if the ergot was withdrawn and resumed growth when ergot was reintroduced. Incidences of neurofibromas, which were absent in control and 1 % ergot groups, were 15 % in the 2 % ergot group and 30 % in the 5 % ergot group. The ergot treatments producing ear neurofibromas also caused significant decrements in body weight gains and a low protein diet appeared to exacerbate the tumorigenicity. In a parallel fractionation study, ergotoxine ethanesulfonate was added to the basal diet at a level selected to approximate that present in the 5 % ergot diet (0.1 % ergotoxine by weight in ergot). In that case, the tumour incidence (6.3 %) was still greater than in untreated rats, but 6-fold lower than in the 5 % ergot group (38 %). In addition, the incidences of tumour types observed in untreated rats (e.g., lung lymphosarcoma and kidney embryonal sarcoma) were approximately doubled in ergot-treated rats. The CONTAM Panel considered that the available information indicated that the observed tumours were related to a non-genotoxic mode of action.

7.3. Adverse effects in livestock, fish and companion animals

Acute toxicity, as reflected in the LD_{50} value of ergometrine in rabbits, mice and rats and the adverse effects in pigs, cattle, sheep, poultry, and rabbits as a result of consuming infected rye, wheat or barley (*C. purpurea*) or sorghum ergot (*C. africana*) for a shorter or longer period, was reviewed by EFSA (2005). Adverse effects seen in horses grazing on pastures where the grass was contaminated with *Neotyphodium* spp., which produce ergovaline, was also reviewed (EFSA, 2005). Some of the experiments cited included determination of "total alkaloid concentrations" in the diets, and one cattle experiment reported on the outcome of the poisoning resulting from oral administration of 1 mg/kg b.w. per day for more than 10 days. Nevertheless, EFSA concluded that data on sensitivity of agricultural animal species towards EA were incomplete, and did not allow the establishment of tolerance levels for individual alkaloids or mixture thereof (EFSA, 2005). Since 2005 a number studies/case reports of interest have been published, including five regarding pigs/piglets (Mainka et al., 2005a, b, 2007b; Kopinski et al., 2007, 2008), two for cattle (Schumann et al., 2007a, b), one involving chickens (Mainka et al., 2005b) one concerning water buffaloes (Millar et al., 2010) and one reporting on presumptive gangrenous ergotism in free-living moose and roe deer (Handeland and Vikøren, 2005). In addition

some new information on the causative agents in the development of paspali staggers (*Claviceps paspali*), and of poisonings stemming from the intake of endophyte infected grasses (rye grass staggers and fescue foot) has been published and will be presented under Section 7.3.1. (ruminants). More recently, Strickland et al. (2011) have published a comprehensive review of ergotism in livestock.

7.3.1. Ruminants

Ergot toxicosis in ruminants is usually a chronic disease, and the result of continued ingestion of small quantities of the fungus on grass. The incidence of ergotism in the EU is unknown, but in the United States it is a serious problem in those areas where fescue grasses are the predominant forage (Hannaway et al., 2009, cited by Strickland et al., 2011). The first symptoms are usually diarrhoea, inappetance, lameness, stiffness of the lower joints of the legs, and coldness and insensibility of the extremities. Dry gangrene appears later, affecting the feet, ears and tail of the animal (Clarke et al., 1981).

In a 230 day study using Holstein Friesian bulls with a mean initial live weight of 227 kg, the animals were fed natural grown ergot in which **ergotamine** accounted for 25 %, **ergocristine** (15 %) and **ergosine** (13 %) of the total alkaloid content (Schumann et al., 2007a). Two dosing levels together with a control group were arranged with 12 or 13 animals in each treatment group. Throughout the experimental period the dosing levels were kept at approximately 1.4 µg/kg b.w. per day or 8.56 µg/kg b.w. per day for the low and high exposure groups, respectively. After the end of dosing, 7 days of fasting were allowed before the animals were slaughtered at a live weight of approximately 550 kg. Statistically no differences were detectable between the three feeding groups in live weight gain, carcass composition, relative weight of liver and kidneys, liver enzyme activities and total bilirubin. No alkaloids could be detected in the bile, urine, or in any of the tissue samples (liver, kidney, muscle and fat). The authors conclude that although more investigations were needed to derive a NOAEL, an intake of approximately 9 µg/kg b.w. per day of total alkaloids does not seem to give rise to concern.

In a study lasting for 84 days, Schumann et al. (2007b) divided 35 male Holstein calves with a start weight of around 50 kg into three groups fed cereal-based rations with 0, 1 000 and 5 000 mg total ergot/kg of feed concentrate, respectively. Live weight, certain health parameters and feed intake were monitored throughout the study. Blood samples were analysed at the beginning at the end of the study. Total dry matter (DM) intake, live weight gain and feed-to-gain ratio were not influenced by exposure to EAs. Neither were parameters such as total bilirubin, aspartate aminotransferase, glutamate dehydrogenase, gamma-glutamyl transpeptidase and creatine kinase in the serum. Concentrations of the individual EAs in serum were lower than the detection limits. Looking at the highest dosed group, the mean concentrate intake per animal started at around 1 kg DM per day ending at 1.75. The mean DM intake over the whole of the study was 1.464 kg DM per day. During the experimental period, the mean body weight increased to approximately 120 kg. From the growth curve and the reported intake of ration as a function of time, it was calculated that the total EA content of the high dosed ration was 1496 µg/kg (corresponding to a mean intake of 1 496 x 1/50 = 30 (µg/kg b.w. per day) during the first 28 days of the experiment, while during the period from day 57 to 84 there was an approximate intake of 1 496 x 1.75/100 = 26.2 (µg/kg b.w. per day). From this study it thus can be concluded that approximately 30 µg/kg b.w. per day did not affect the calves.

Thirty out of a herd of 200 water buffaloes (*Bubalus bubalis*) less than two years old and reared for meat production in the UK were affected with ergot poisoning during the autumn and winter of 2008. Symptoms were lameness, ill thrift and poor weight gain. The herd had been fed hay *ad libitum* and grass silage while housed. Ergot was identified in grass silage (Millar et al., 2010). No information was given on the concentration of sclerotia or alkaloid concentration.

From Norway, gangrenous ergotism was reported in ten free-living moose over a period from 1996 and to 2004. The affected moose included seven calves and three yearlings. Lesions in moose found during October and November presented as dry gangrene, whereas moose found between December and June

presented with loss of the distal part of the limbs or open lesions. No information on the exposure levels is available (Handeland and Vikøren, 2005).

Naudè et al. (2005) reported on two outbreaks of *Claviceps cyperi* ergotism that resulted from feeding maize silage[21] to dairy cows as part of a complete ration. The EAs – mainly **ergocryptine** – were detected in samples of maize silage at levels of up to 975 mg/kg DM. Another outbreak of ergotism was reported in dairy cows when teff grass (*Eragostis teff*), which had been contaminated with *Cyperus esculentus*, was conserved and fed as hay.

Another disease caused by intoxication, namely "paspali staggers" resulting from the intake of sclerotia from *Claviceps paspali* is frequently described in South Africa and America, but is rare in Europe, with the first description from Spain being published in 2010 (Moyano et al., 2010). In this case, 23 calves out of a total of 130 animals were affected with clinical signs characterised by tremor, hyperexcitability, incoordination, ataxia, depression and paralysis, signs that were aggravated when animals were subjected to certain types of exercises. Lesions were detected in the brain and consisted in microhaemorrhages diffused through the parenchyma of the brain, neuronal degeneration, satellitosis, neuronophagia, gliosis, and moderate neuropil degeneration in the peripheral zones of the brain (Moyano et al., 2010). In addition to low concentrations of EAs, *Claviceps paspali* also produces indole-diterpenoid tremorgens which have been shown to be the actual cause of "paspali staggers" in cattle. These include the compounds paspalitrem A, B and C, paspalin, paspalinine and paspalicine (Uhlig et al., 2009). The exact mechanism of action of the tremorgenic endole-diterpenes is not known but may include inhibition of the function of GABA receptors by binding at or near the receptor's site of chloride influx (Gant et al., 1987). The paspalitrems and related tremorgenic compounds have also been shown to be produced by *Claviceps cynodontis* which are known to cause "Bermuda grass staggers"; i.e. tremors in cattle grazing Bermuda grass (*Cynodon dactylon*, kweek) infected with this latter fungus. The highest concentration of a secondary constituent found in ergotised seed heads of *Cyodon dactylon* was that of paspalitrem B, at around 150 mg/kg. In comparison, the concentrations of ergometrine and ergine, together with their C8 epimers were found to be only about 10 µg/kg (Uhlig et al., 2009).

Tall fescue poisoning (fescue lameness) and perennial rye staggers, which can occur when cattle (and other ruminants) graze on endophyte infected tall fescue (*Festuca arundinaceae*) and perennial rye grass (*Lollium perenne*), respectively, are rare in Europe. Fescue lameness, which resembles ergot poisoning, is believed to be caused by EAs, particularly ergovaline, in infected tall fescue. The symptoms begin with lameness in one or both hind feet and may progress to necrosis of the distal part of the affected limb(s). The tail and ears also may be affected independently of the lameness. In addition to gangrene of these extremities, animals may show loss of body mass, an arched back, and a rough coat. Outbreaks have been confirmed in cattle and similar lesions have been reported in sheep. Tall fescue is a cool-season perennial grass adapted to a wide range of soil and climatic conditions. It is used in Australia and New Zealand for stabilizing the banks of watercourses, and it is the predominant pasture grass in the transition zone in the eastern and central USA. Fescue lameness was reported in Kentucky, Tennessee, Florida, California, Colorado, and Missouri, as well as in New Zealand, Australia, and in Italy. Two fungi from toxic pastures have been implicated in fescue lameness. The clavicipitaceous endophyte fungus *Neotyphodium (Acremonium) coenophialum* can synthesize EAs in culture (MVN, 2011a) and when present in its host plant (Malinowski et al., 1998). The EA ergovaline has been detected in toxic fescue and is strongly implicated in some of the fescue toxicosis syndromes. However, the complete aetiology of fescue foot remains unresolved (MVN, 2011a). In spite of the fact that it recently was shown that all samples of *Festuca arundinacea* collected in Italy, Spain and Denmark, respectively, produced ergovaline (Jensen et al., 2007), intoxications are nevertheless seldom seen in Europe. A recent *in vitro* study (Foote et al., 2011) has confirmed that EAs associated with toxic endophyte-infected tall fescue are vasoactive, and affect arterial blood supply and venous drainage from the bovine foregut. The study also confirmed that the vasoconstrictive effects were different for the individual

[21] The maize crop had been heavily contaminated with *Cyperus esculentus* and *Cyperus rotundus* which contained sclerotia of *Claviceps cyperi.*

alkaloids examined (ergovaline, ergotamine, ergocryptine, ergocristine, ergometrine, ergocornine, and lysergic acid).

Even though the endophyte of *L. perenne*, i.e. the fungus *Neotyphodium (Acremonium) lolii* does produce the ergopeptine alkaloid ergovaline, this is not the primary causative agent of perennial rye staggers. Rather, the condition is caused by the indole diterpene alkaloids tremorgenic neurotoxins called lolitrems, mainly lolitrem B. The neurotoxic tremorgens are believed to cause incoordination by interference with neuronal transmission in the cerebral cortex through production of a reversible biochemical lesion; no specific histologic lesion is recognized (MVN, 2011b).

7.3.2. Pigs

Kopinski et al. (2007, 2008) published two reports of experiments involving feeding of pigs with sorghum ergot (*C. africana*) with a known content of total alkaloids, of which the major alkaloid was dihydroergosine. In the first study, 51 pregnant sows from a continually farrowing piggery were sequentially inducted into the experiment each week in groups of four to seven, as they approached 14 days of farrowing. Diets contained sorghum ergot sclerotia within the range of 0 (control) to 1.5 % w/w (intermediate concentrations being: 0.3, 0.6, 0.9, and 1.2 %) and were randomly allocated and individually fed to sows. The highest level (1.5 % w/w) corresponded to 7 mg alkaloids/kg feed, including 6 mg **dihydroergosine**. Three sows fed a diet containing 1.5% ergot for 6 to 10 days preceding farrowing produced no milk, and 87 % of their piglets died despite supplementary feeding. Ergot inclusions of 0.6 % to 1.2 % had less of an effect on milk release and neo-natal piglet mortality was reported. The authors concluded that sorghum ergot should not exceed 0.3 % (1 mg alkaloid/kg) in diets of multiparous sows fed before farrowing, and should be limited to 0.1 % for primiparous sows (Kopinski et al., 2007). In the second experiment, diets containing 3 % w/w sorghum ergot (16 mg alkaloids/kg, including 14 mg dihydroergosine/kg) were fed to 12 sows from 14 days post-farrowing until weaning 14 days later. Ergot fed sows displayed a smaller weight loss during lactation (24 kg/head vs. 29 in control sows) despite feed consumption being less. However, ergot-fed sows had poorer weight gain of litters over the 14-day period (16.6 kg/litter vs. 28.3 kg/litter for controls; $p < 0.05$). Sow's plasma prolactin was reduced with ergot feeding after 7 days to 4.8 µg/l compared to 15.1 in control sows (Kopinski et al., 2008).

In a study published after the EFSA opinion (2005), Mainka et al. (2005a) examined at the influence of ergot-contaminated feed on growth, body composition and carry-over of EAs in growing-finishing pigs. Diets containing 0, 1 and 10 g of ergot (*C. purpurea*) per kg, corresponding to mean total EA contents of 0.05, 0.60 and 4.66 mg/kg, were fed *ad libitum* to pigs with an initial mean b.w. of 30.5 kg. On the day the feed was changed from grower to finisher diet, the pigs had an average b.w. of about 70 kg. At a b.w. of 115 kg, the animals were slaughtered. No information on feed consumption was given in the publication. The total fattening period for all three groups was approximately 110 days. The alkaloid content given was estimated as the sum of ergometrine, ergotamine, ergocornine, α-ergocryptine, ergocristine, ergosine and their –inine epimers as analysed by HPLC. Tendencies towards reduced feed intake and body weight gain were observed at the highest dose (4.66 mg total EAs per kg diet). Heart and spleen weights showed significant linear increases with EA intake. The contents of EAs in samples including serum, bile, liver, meat and back fat were in all cases lower than the detection limits for the respective EA (Mainka et al., 2005a). This work indicates a no-observed-effect level (NOEL) between 0.60 and 4.66 mg/kg feed when pigs are fed ad libitum, with the only effect – in this study – being reduced body weight gain. None of the adverse effects typically associated with ergot poisoning were observed in this study.

In another study, Mainka et al. (2005b) looked at the influence of ergot contamination of feed on the performance and health of piglets. Using crossbred piglets weaned at 28 days and included in the experiment at day 35 (initial mean weight at experiment start 8.1 kg) the authors established a control group together with 4 dose groups (0.5, 1, 2 and 4 g of ergot per kg of feed. Each group consisted of 8 castrated male and 8 female piglets. The alkaloids in the ergots used were identified as **ergocristine** (14.9 %), **ergometrine** (8.1 %), **ergotamine** (5.4 %), **ergocornine** (3.2 %) and **α-ergocryptine** (1.9 %)

– given as percentage of total alkaloid content, which was 2 775 mg/kg (the remaining 66.5 % alkaloid content was unidentified). Cumulative DM intake and live weight gain was recorded and a number of serum values were determined at the start and at the end of the experiment (total protein, serum albumin, aspartate aminotransferase, glutamate dehydrogenase, γ-glutamyltransferase, and porcine growth hormone). Feed intake and weight gain were significantly decreased in the highest dosed group. Serum albumin concentration showed a significant linear alteration while serum aspartate aminotransferase were significantly increased at the highest dose level. The authors concluded that the critical level of total EAs for piglets seemed to be in the range of 5.6 to 11.1 mg/kg diet (Mainka et al., 2005b). The authors also calculated the daily intake per kg mean live (body) weight of total and the five key EAs. These changed during the experiment (where animals were fed *ad libitum*) but were, for the highest and next highest dosed groups, 0.48 (0.16 – key EAs) and 0.26 (0.09) mg/kg b.w. per day, respectively, as calculated as a mean for the weeks 1-5 (Mainka et al., 2005b). This corresponds to a NOAEL of approximately 0.26 (total alkaloids) mg/kg b.w. per day.

In a more recent study, Mainka et al. (2007b) investigated the variation in total EA content and composition between different collections of ergots, and fed piglets with two such differing sources in order to determine the effects. A 35-day study was performed, which was characterised by different alkaloid patterns but standardised on equal total alkaloid contents in two concentrations (5.6 and 11.2 mg EAs/kg feed). The alkaloid patterns of the two ergot sources showed conspicuous differences, mainly in the ergotamine content which was nearly three times higher in source B as compared to source A. However, 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 high supplemented groups were significantly decreased relative to the control group and showed a linear dose-response. This could, at least to some extent, be explained by reduced feed intake. The serum protein content was significantly decreased in the high dosed groups as compared to the control. The authors concluded that the critical contamination level for piglets was 3.57 mg total EAs/kg feed (Mainka et al., 2007b). The exposure at the high dosed level was calculated to be 0.32 - 0.34 mg EAs/kg b.w. per day. The low exposure level showing no adverse effects except for reduced feed intake corresponded to 0.15 - 0.17 mg EAs/kg b.w. per day. The latter was therefore equal to the NOAEL in this experiment.

7.3.3. Rabbits

No new information has been published since the EFSA (2005) opinion.

7.3.4. Poultry

Poultry appear to be able to tolerate higher levels of ergot than other livestock (EFSA 2005). However, feeding rations with high levels of ergot sclerotia for an extended time can result in loss of appetite, increased thirst, diarrhoea, vomiting and weakness (Bailey et al., 1999). Convulsions, gangrene of the comb, wattles, or toes, paralysis and death may follow short-term feeding of ergot-contaminated rations. Because of the variation in both the quantity and type of the alkaloids present in ergot sclerotia, it has been difficult to establish safe levels, although safe dietary levels of ergot for chickens appear to be in the range of 0.3 - 0.8 % by weight, depending on the actual alkaloid concentration (Bailey et al.,1999).

Since the EFSA 2005, opinion Mainka et al. (2005b) have reported a study where they compared the effect of ergot contaminated feed on performance and health of piglets and chickens. For the chickens, five groups of seven 28-day old male chickens ("Lohmann Meat") were formed, the average initial weight of the chickens within each group being 43.2 ± 3.0 g. Feed and water was available *ad libitum*, the different groups being offered feed with a content of 0, 0.5, 1, 2 and 4 g of ergot/kg diet. The ergot was analysed to contain 2 775 mg of total alkaloids per kg, the EAs being **ergocristine** (14.9 %), **ergometrine** (8.1 %), **ergotamine** (5.4 %), **ergocornine** (3.2 %) and **α–ergocryptine** (1.9 %) (expressed as percentage of the total dry weight of the ergot, which in addition contained 66.5 % of unknown alkaloid residue). The total alkaloid contents of each of the diets were 0, 1.4, 2.8, 5.6 and 11.1 mg/kg. Serum activities of glutamate dehydrogenase (GLDH), γ-glutamyltransferase (γ-GT), alanine aminotransferase (ALT) were determined together with albumin and total bilirubin. After slaughter,

weights of liver, heart, spleen and bursa fabricii were recorded. Inner organs were examined and the proximal duodenum was scored for inflammation. No mortality was observed in the groups fed 0, 0.5, 1 and 2 g of ergot per kg feed; however, three chickens were taken out in the highest dosed group apparently due to difficulties not related to the experiment. Feed intake was not affected by the dietary composition, neither was the cumulative daily weight gain. The serum activities of GLDH and ALT were not affected. However, the γ-GT as well as bilirubin showed a significant linear increase, while albumin decreased also in a linear manner. The weight of hearts decreased in a linear manner while moderate inflammations were found in the proximal duodenum of the ergot fed groups from 2.8 mg/kg of alkaloids and upwards. Severe inflammation was only seen for two animals in the highest dosed group. The authors concluded that the highest dose did not reach a critical level for performance depression, but that the obvious adverse effect on the integrity of the mucosa needs to be further studied (Mainka et al. 2005b). The authors did not identify a NOAEL but it seems that this could be identified at 1.4 mg of EAs/kg feed. During the study the animals gained weight to reach a final weight of around 700 g.

The results of Mainka et al. (2005b) concerns young chickens and not adult birds. Hence, it is difficult to interpret the results in the light of the more general statement made earlier about poultry being les sensitive than many other animals. However, it can be noted that while some effects were seen for the young chickens at concentrations as low as for the piglets, no weight gain reduction was recorded for the young chickens at any exposure level used, which is in contrast to what was the case for the piglets.

7.3.5. Fish

No new information has been published since the EFSA (2005) opinion.

7.3.6. Companion animals

Horses

Cawdell-Smith et al. (2010) reported two cases of 'staggers' in horses as a result of ingesting feed contaminated with *Claviceps paspali*. In the first of these, three foals presented with ataxia in all limbs after consuming infected paspalum. One foal died and the other two recovered within one week of removal from the infected paddock. In the second case, two mares developed hindquarter paresis after grazing in an irrigation channel. After removal from the area, one of the affected horses continued to deteriorate. Despite treatment with antibiotics, fluids and electrolytes, one had to be euthanized. The second affected horse recovered after 2 days.

Pregnant mares are sensitive to ergopeptine alkaloids at levels as low as 50 - 100 µg/kg[22] in the diet. Ergopeptine alkaloids appear to interfere with the normal rise of progestagens (mainly 5-alpha-pregnanes) and prolactin in the latter stages of pregnancy, and foals born without the normal increases in maternal progestagens suffer hypoadrenocortical function and are small, weak or stillborn (Brendemuehl et al., 1995).

Cats and dogs

No new information has been published since the EFSA (2005) opinion.

7.4. Transfer from feed to livestock products of animal origin

EFSA (2005) reviewed the data on the carry-over of EAs into products of animal origin. The amount of useful data were scarce and, as a result, it had not been possible to estimate carry-over rates. However, the data that were available provided no evidence that EAs accumulate in edible tissue and therefore these are unlikely to be an important source of human exposure to EAs. Subsequently, Mainka et al. (2005a) reported a study in which growing and fattening pigs (30 - 115 kg b.w.) were fed diets

[22] http://www.omafra.gov.on.ca/english/livestock/horses/facts/info_ergot_alkaloid.htm

containing up to 4.66 mg EAs/kg, but they were unable to detect any alkaloids in meat and back fat. The European Mycotoxin Awareness Network (EMAN, 2005) also reported that EAs are not transferred to the milk of cows consuming ergot. In a study reported by Schumann et al. (2007a), Holstein Friesian bulls were fed diets with up to 421 µg/kg DM of total alkaloids for a period of approximately 230 days. No carry over into tissues could be detected. In another study reported by Schumann et al. (2009), dairy cows were fed diets contaminated with EA, resulting in an alkaloid concentration of the daily ration between 504.9 and 619.5 µg/kg DM. The actual alkaloid exposure varied between 4.1 and 16.3 µg/kg b.w. per day. When fed over a period of 4 weeks, no EAs were detected in the milk produced (LOD of 5 - 10 µg/kg).

7.5. Human pharmacological and toxicological data

Data on dose related pharmacological and toxicological effects available due to the medical use of ergotamine and ergometrine have been used as a basis for the risk assessment of ergot contamination of rye flours (BfR, 2004; Dusemund et al., 2006) and are presented below. Ergocornine methanesulphonate has been investigated in women in a clinical study to investigate some endocrinologic effects (Koi, 1966).

7.5.1. Ergotamine

Ergotamine is commonly used for medicinal purposes as ergotamine tartrate (Martindale, 2010; Bracher et al., 2010).

Pharmacodynamics

Ergotamine tartrate is used in migraine and cluster headache, and has been tried in the treatment of orthostatic hypotension. Since ergotamine tartrate may exacerbate the nausea and vomiting that commonly develops as a migraine attack progresses it is often necessary to give an antiemetic as well (e.g. Martindale, 2010).

It has marked vasoconstrictor effects and it has a powerful oxytocic action on the uterus (causing contractions). Its complex effects are due to its structural based affinities to the receptors of noradrenaline, dopamine, and 5-hydroxytryptamine (5-HT, serotonine) at which it acts as a partial agonist or antagonist (AHFS, 1995; Tfelt-Hansen et al., 2000; Marquardt and Schaefer, 2004; Brunton et al., 2006; Schardl et al., 2006; Forth et al., 2009; Bracher et al., 2010; Fachinformation, 2010; Martindale, 2010).

Migraine is thought to be due to vasodilatation of carotid arteriovenous anastomoses and ergotamine effects are explained by its agonistic action on adrenergic receptors and stimulation of serotonin-receptors (Silberstein and McCrory, 2003; Schardl et al., 2006; Forth et al., 2009).

Ergotamine was isolated from ergot by Prof. Arthur Stoll after his establishment of the pharmaceutical department of Sandoz (Switzerland) in 1918. It was introduced to the market under the trade name Gynergen in 1921 (Sandoz, 2010). Thus, this drug was introduced before any real effective regulations or authorities requiring controlled (randomized) clinical trials or safety evaluations were in place. This latter legislation process started with the 1938 US Food, Drugs and Cosmetics Acts as followed by the British Medical Research Councils first randomized experiments on streptomycin (IPC, 2012). The outcome of studies regarding its effectiveness is ambiguous, some authors doubting that it is more effective than placebo (Tfelt-Hansen et al., 2000).

Its high emetic potency is due to its stimulation of central dopamine receptors (Brunton et al., 2006; Schardl et al., 2006).

For details on mechanism see Section 7.6.

Therapeutic applications and dosage

Ergotamine tartrate is usually given orally or rectally, but has also been given sublingually and by inhalation. Ergotamine tartrate is used in migraine unresponsive to non-opioid analgesics. However, its adverse effects limit its use (Martindale, 2010).

The usual oral dose in the treatment of migraine is 1 to 2 mg of ergotamine tartrate, repeated, if necessary, half an hour later. Usually not more than 6 mg should be given in 24 hours (Brunton et al., 2006; Bracher et al., 2010), although some licensed product information recommends not more than 4 mg in 24 hours (Fachinformation, 2010) and others not more than 8 mg per attack (Martindale, 2010). The recommended minimum interval between successive 24-hour courses is 4 days, and the total weekly dose is limited to a maximum of 12 mg (Martindale, 2010), although again some recommend a lower weekly limit of 10 mg (Brunton et al., 2006; Bracher et al., 2010) or 6 mg (Fachinformation, 2010). It is also recommended that patients should receive no more than two 24-hour courses per month. According to earlier literature the maximum total dose should not exceed 20 mg ergotamine tartrate per month (Rote Liste, 2003). Similar doses may be given sublingually (Martindale, 2010).

Ergotamine tartrate may be used similarly in cluster headache to treat individual headaches during a cluster period. Ergotamine tartrate is also used in low doses given orally or rectally for limited periods of up to 2 weeks in the prophylaxis of headache attacks during a cluster period. Regimens that have been tried for such prophylaxis include 1 to 2 mg of ergotamine tartrate given 1 to 2 hours before an expected attack or 1 to 2 hours before bedtime for nocturnal attacks. The total maximum dose of ergotamine tartrate that may be given weekly for the prevention of cluster headache is less well established than for the treatment of migraine. Ergotamine is often given for only 5 to 6 days in each week, which allows the patient to assess whether the cluster period has ended (Martindale, 2010).

Since absorption of ergotamine from the gastrointestinal tract is poor and bioavailability is reduced by a high pre-systemic hepatic metabolism, ergotamine tartrate is also given rectally in an attempt to overcome these effects, with some improvement in absorption, but bioavailability is still about 5 % or less. Absorption of sublingual ergotamine is very poor. Caffeine is sometimes included in oral and rectal preparations of ergotamine to improve the latter's absorption, although whether it does so is not clear (Tfelt-Hansen et al., 2000; Silberstein and McCrory, 2003; Martindale, 2010).

It has to be taken into consideration that information on dosage does not apply to pregnant women and individuals with health conditions for which therapy with ergotamine tartrate is contraindicated (see below).

Adverse reactions

The adverse effects of ergotamine tartrate may mainly be attributed either to its effects on the central nervous system (CNS), or to vasoconstriction of blood vessels and possible thrombus formation. The following adverse effects have been reported in the indicated frequency associated with the oral use of ergotamine tartrate in the recommended therapeutic dose range as described in the foregoing section (Brunton et al., 2006; Forth et al., 2009; Bracher et al., 2010; Fachinformation, 2010; Martindale, 2010).

Frequently (≥ 1/100 up to < 1/10): After therapeutic doses nausea and vomiting commonly occur as a result of the direct emetogenic effect of ergotamine tartrate; some patients may also experience abdominal pain and diarrhoea.

Occasionally (≥ 1/1000 up to < 1/100): Weakness and muscle pains in the extremities and numbness and tingling of the fingers and toes may occur. Particularly in the beginning of treatment, central nervous effects such as dizziness, disorientation, headache and cerebral convulsions have been observed. Chronic, intractable headache (rebound headache) may also be a major withdrawal symptom following the development of ergotamine tartrate dependence. There may occasionally be localised oedema and itching in hypersensitive patients.

Rarely (≥ 1/10000 up to < 1/1000): Precordial distress and pain suggestive of angina pectoris, as well as transient tachycardia or bradycardia have been noted after chronic use, presumably as a result of coronary vasospasm induced by ergotamine tartrate. Hypertension has also been reported. Myocardial infarction has occurred rarely.

Very rarely (< 1/10000): Fibrotisations (retroperitonal, myocardial or cardiac valvular) have been observed.

Dependence: Dependence can develop insidiously when ergotamine tartrate is used for more than 2 days each week, even if total daily or weekly dosage recommendations are observed (Saper, 1987). Individual reports indicate a state of addiction characterised by a predictable and irresistible pattern of drug usage, the development of tolerance to adverse effects, and a syndrome of withdrawal on stopping the drug (Martindale, 2010).

Effects on ability to drive and operate machinery: Dizziness and feelings of anxiety have been reported (Fachinformation, 2010; Martindale, 2010).

Intoxication and adverse effects associated with overdosage

In cases of overdosage of ergotamine tartrate not in compliance with the recommended doses or accidental intake the following observations have been made.

Acute: Symptoms of acute overdosage include nausea, vomiting, diarrhoea, extreme thirst, coldness, tingling, and itching of the skin, a rapid and weak pulse, hypertension or hypotension, shock, confusion, convulsions, and unconsciousness. Symptoms of peripheral vasoconstriction or of cardiovascular disturbances, as seen in chronic ergotamine tartrate poisoning, may also occur but may be delayed. Fatalities have been reported. Death occurs due to respiratory arrest and circulatory failure (Bracher et al., 2010; Martindale, 2010).

Chronic: regular intake exceeding the recommended maximum dosage of 20 mg ergotamine tartrate/month may lead to permanent headache (Fachinformation, 2010). In chronic poisoning or ergotism, resulting from therapeutic overdosage or the use of ergotamine tartrate in susceptible patients, severe circulatory disturbances may develop. The extremities, especially the feet and legs, become numb, cold, tingling, and pale or cyanotic, with muscle pain. There may be no pulse in the affected limb. Eventually gangrene develops in the toes and sometimes the fingers. Other adverse effects include confusion and convulsions. On rare occasions symptoms of vasoconstriction of blood vessels in the brain, eye, intestines, and kidneys occur (Deviere et al., 1987; Galer et al., 1991; Lazarides et al., 1992; Redfield et al., 1992; Zavaleta et al., 2001; Bracher et al., 2010; Martindale, 2010).

Case reports after oral, rectal or sublingual application of ergotamine tartrate

In the following paragraphs, examples of cases of adverse effects associated with ergotamine tartrate use are described, in which the recommended daily therapeutical dose seems not to be exceeded, even though overdosage in some cases resulted from prolonged uses.

A 48-year-old man was hospitalized for pain and weakness in both upper extremities. Ergotism with ischemia in all four extremities was diagnosed. He had a long history of migraine and had taken 3 mg of ergotamine daily for more than 21 years (no indication of the way of administration and if taken in the form of tartrate). During the week prior to his admission, he had increased his daily ergotamine intake as a result of severe headache related to psychological stress (no indication of exact dose). He was a current smoker for 30 years. Angiography demonstrated vasospasm involving all four extremities. The patient stopped smoking and stopped taking ergotamine in an attempt to counteract the vasospasm. Follow-up computed tomography angiogram revealed that both brachial arteries had normalized (Jeong et al., 2006).

Oral application

A 29-year-old HIV-positive man receiving antiretroviral therapy was referred for circulatory evaluation because of paresthesiae and coldness of the left upper extremity. For two days, he had noticed difficulties with writing, and his left hand felt clumsy. Antiretroviral treatment with emtricitabine–tenofovir and lopinavir–ritonavir had been started two months earlier. Because of severe migraine headache he took 1 mg ergotamine tartrate per day orally for two weeks until the day before admission. The patient denied consumption of illicit drugs, including cocaine and cannabis, or intake of any additional medications. There were no clinical signs suggestive of HIV-related illnesses. Physical examination showed that the left hand was pale and arterial pulses were absent at the left wrist. The heart rate was 60 beats/minute, and arterial blood pressure was 105/65 mm Hg on the left arm and 115/65 mm Hg on the right arm. Oscillography showed significant reduction of perfusion of the left forearm. The results of duplex ultrasonography were consistent with high-grade, long-segment stenosis of the distal axillary artery. The authors noted that ergotamine has numerous potential interactions with HIV medications. Ergotamines are substrates of the cytochrome P450 3A4 isozyme (CYP3A4). As a result, coadministration with certain antiretroviral drugs and other potent CYP3A4 inhibitors could cause a pronounced increase in ergotamine levels, with potentially severe toxic effects. The authors concluded that the patient was experiencing ergotism caused by such an interaction (Fröhlich et al., 2010).

The CONTAM Panel noted that similar interactions of ergotamine tartrate with a variety of other drugs are known, see section "Specific groups - Interaction" below.

In a 48-year-old Nigerian woman extra-hepatic portal hypertension was diagnosed coincident with a chronic overdosage of ergotamine tartrate. She had suffered with severe classical migraine for 8 years, and her medication included oral ergotamine tartrate with caffeine for the acute migraine episode. She had however been taking 6 mg/day for at least the 3 months immediately previous to her admission. Examination at presentation revealed a left-sided hemiparesis. Ergotamine was therefore withdrawn from her treatment regimen, and the hemiparesis completely resolved within one week (Fisher et al., 1985).

Evans et al. (1980) reported on a woman who developed a pain, typical of an autonomic dysaesthesia. It first appeared at her ankles and over eight months progressed upwards to severely affect the whole of both legs as far as the hips. It was a constant burning sensation that she likened to being on fire. Since she had been prone to attacks of migraine she was taking tablets containing 2 mg ergotamine tartrate, 50 mg cyclizine hydrochloride, and 100 mg caffeine hydrate. She took them at night prophylactically and more during the day if headaches occurred. She regularly took up to 12 tablets (24 mg ergotamine tartrate) a week, which was considered to be excessive consumption of ergotamine. After withdrawal of ergotamine tartrate she had improved. Intense vasoconstriction was not a feature of her ergotism although she frequently complained of cold legs. She had no other sequelae such as ischaemia or gangrene.

Rectal application

A 39-year-old woman suffering from migraine took two suppositories, each containing 2 mg ergotamine tartrate, for the first time again after an abstinence of two years. The second suppository was administered two hours after the first. She developed symptoms of decreased peripheral blood flow in all four limbs 24 hours after administration of the second suppository. Walking distance without pain was reduced to 100 m, but the most severe changes affected the right arm, with livid discolorations and complete immobility 16 hours after the onset of symptoms. Despite administration of morphine derivatives the pain progressively increased. Angiography demonstrated spastic narrowing of all arm arteries below the axillary artery. No vessels were visualized below the lower-arm bifurcation. A residual deficit, incomplete lesion of the median nerve, persisted but gradually regressed during the following two months (Mumme et al., 1991).

A 42 year-old woman with a long history of migraine was presented with burning pain of the limbs and reduced walking distance. No risk factors for peripheral arterial occlusive disease were present. Over 20 years her daily medication included administration of an ergotamine-containing suppository (2 mg ergotamine tartrate, 100 mg caffeine daily). On examination both limbs were found to be cool and pulse-less below the knee. The peripheral Doppler pressure indicated a bilaterally reduced ankle-brachial index. Colour-coded duplex sonography showed constricted vessels and long stenosis with a decreased echo from the wall of the left and a distal occlusion of the right femoral artery without atherosclerotic changes. A diagnosis of ergotism was made (Bogun et al., 2011).

A 39 year-old woman was admitted to the hospital with an eight-month history of progressive intermittent claudication. During the previous year her usual episodic migraines had been turning into a chronic daily headache. She had been taking 4 mg of ergotamine tartrate rectally each day. No pulses were detected in the lower limbs, and bruits were heard over the femoral arteries. An angiogram showed a marked reduction of the lumen of medium-sized vessels of the extremities. Both echocardiographic and carotid ultrasonography-Doppler studies revealed no abnormalities. Ergotamine was discontinued. Six months later she was non-symptomatic and a repeat angiogram demonstrated a normal calibre of the femoral and popliteal arteries (Varona et al., 1996).

Two patients with manifestations of iatrogenic ergotism are described. One 61 year-old female patient presented with ischemia of all extremities and bilateral foot drop probably due to ischemic damage to the common peroneal nerves. The patient had been using two suppositories, each containing 2 mg ergotamine tartrate and 100 mg caffeine, daily for the preceding three weeks for headache. The foot drop totally resolved in several months following the discontinuation of ergotamine. A second 34 year-old male patient suffered from unilateral leg ischemia and transient monocular blindness. The patient had a five-year history of temporal headaches, for which he had been taking two or three suppositories, each containing 2 mg ergotamine tartrate and 100 mg caffeine, daily for several months. Prior to this he had taken 8-10 suppositories weekly for two years. Symptoms resolved after discontinuation of ergotamine. Both patients displayed typical angiographic findings of ergotism (Merhoff and Porter, 1974).

Sublingual application

Galer et al. (1991) reported a case of a 31 year-old man with a 9 year history of cluster headaches who developed severe, prolonged myocardial ischemia. He had been successfully treated with 2 mg methysergide three times a day. His medication was changed and with the initial dose of 2 mg ergotamine tartrate administered sublingually he immediately developed left substernal chest pain and heaviness with radiation into his left arm and numbness of the fingers. After experiencing these symptoms intermittently for two days he went to an emergency room, where myocardial ischemia was diagnosed on medical examination and electrocardiogram. Given the pharmacologic similarity of methysergide and ergotamine tartrate, the authors suspected that they may have synergistic cardiac toxicity and might have caused coronary vasospasm in the patient.

Specific groups – Interactions

Ergotamine tartrate is contraindicated in individuals with severe or uncontrolled hypertension, shock, severe or persistent sepsis, peripheral vascular disease, ischaemic heart disease, temporal arteritis, hyperthyroidism, anaemia, porphyria or hepatic or renal impairment (Tfelt-Hansen et al., 2000; Fachinformation, 2010; Martindale, 2010).

The vasoconstrictor effects of ergotamine are enhanced by sympathomimetics such as adrenaline. There is also an increased risk of peripheral vasoconstriction during use of ergotamine with beta blockers (Fachinformation, 2010; Martindale, 2010).

Because ergotamine is metabolised by the cytochrome P450 isoform CYP3A4, its toxicological/pharmacological effects can be altered by coadministration of other therapeutic agents that

are also CYP 3A4 substrates (e.g., azole antifungals, macrolide antibacterials such as erythromycin and clarithromycin, HIV-protease inhibitors including indinavir and ritonavir). Accordingly elevated ergotamine concentrations sufficient to cause ergotism may occur as a result of such drug-toxicant interactions (Tfelt-Hansen et al., 2000; Martindale, 2010). According to Wooltorton (2003) also grapefruit juice presents the theoretical possibility of interaction with ergotamine by inhibition of CYP3A4.

Ergotamine interacts with serotonin (5-HT_1) agonists resulting in an additional risk of prolonged vasospastic reactions (Martindale, 2010).

Pregnancy

Ergotamine tartrate is contraindicated during pregnancy. Women who may become pregnant or are pregnant are at risk from therapeutic doses of ergotamine tartrate because of its oxytocic effect on the uterus (causing contractions) and its vasoconstrictor effects. Thus during the entire pregnancy an increased risk for diminished placental blood circulation and premature labour is seen due to intake of ergotamine tartrate (AHFS, 1995; Tfelt-Hansen et al., 2000; Bracher et al., 2010; Fachinformation, 2010; Martindale, 2010).

Accidental dosage of ergotamine tartrate in the form of a suppository (ergotamine tartrate 2 mg and caffeine 100 mg) to a patient at 39 weeks of pregnancy caused uterine contractions and fetal tachycardia. Because of suspected placental abruption an emergency caesarean section was undertaken but no clear signs of retroplacental haemorrhage were found. The neonate recovered quickly after delivery and developed normally during the next 10 years (de Groot, 1993).

Hughes and Goldstein (1988) described a case in which the mother was exposed during gestation week 0 - 14 to suppositories containing 2 mg ergotamine and 100 mg caffeine (dose: 1 - 4 suppositories/week), and during gestation weeks 0 - 20 to propranolol (dose: 2 x 40 mg/day) and also took additional medication (e.g. codeine, acetaminophen). At birth the infant showed evidence of early arrested cerebral maturation and paraplegia. The authors noted that the nature of these defects suggested a primary vascular disruptive aetiology and hypothesised that ergotamine, acting either alone or in synergy with propranolol and caffeine, produced fetal vasoconstriction resulting in tissue ischaemia and subsequent malformation.

A fatal case of jejunal atresia has been reported in an infant born after a 35-week gestation to a woman who had taken ergotamine tartrate 6 to 8 mg daily, as tablets, throughout her pregnancy (Graham et al., 1983). Two cases of Moebius syndrome (a condition characterised by facial paralysis as a result of hypoplasia of cranial nerve nuclei) have been associated with exposure to ergotamine tartrate during the first trimester of pregnancy (Graf and Shepard, 1997; Smets et al., 2004). In the first report the mother had inadvertently been given three suppositories containing ergotamine tartrate within a period of 1 to 2 hours and at the time had experienced uterine cramping and a bloody vaginal discharge. The second mother had used 2-mg ergotamine tartrate suppositories on a regular basis during the first 8 weeks of pregnancy (Graham et al., 1983; Graf and Shepard, 1997; Smets et al., 2004; Martindale, 2010).

Acs et al. (2006) evaluated the possible association between ergotamine and congenital abnormalities in two studies based on the data set of the population-based Hungarian Case–Control Surveillance of Congenital Abnormalities (HCCSCA). The use of the mean daily dose of 1.5 mg ergotamine during the 2nd month of pregnancy showed a higher risk for neural-tube defects. This possible association was based only on three cases. However, out of 24 case and 55 control mothers, 22 (91.7 %) and 47 (85.5 %) had prospective and medically recorded ergotamine uses, respectively. A similar association was not found between intake of a migraine medication, containing ergotamine in combination with caffeine, aminophenazone and belladonna leaf (mean daily dose of 0.3 mg ergotamine) and neural-tube defects. According to the authors these differences may indicate a dose dependent teratogenic effect of ergotamine. The authors considered the possible associations between the migraine medication and

renal a/dysgenesis in the 2nd and 3rd months of gestation only as signal, because it was based only on two cases.

Based on the HCCSCA Bánhidy et al. (2007) reported that of 38 151 newborn infants with medically recorded gestational age and birth weight, 77 were born to mothers who had received ergotamine treatment during pregnancy. A statistically significant decrease was found in the mean gestational age (0.7 weeks) and birth weight (196 g) among exposed relative to unexposed infants, though these differences were not obstetrically significant. However, there was a significant increase in the proportion of low birthweight newborns (16.4 % vs. 5.7 %) and preterm births (16.4 % vs. 9.2 %) after the use of ergotamine (mean daily dose 1.5 mg) during pregnancy. The effect of ergotamine was more obvious in male newborn infants, particularly after treatment in the third trimester.

Breast feeding

Although the American Academy of Pediatrics includes ergotamine tartrate among those drugs that may be given with caution to breast-feeding mothers, it notes that maternal use in doses equivalent to those given for the treatment of migraine has been associated with vomiting, diarrhoea, and convulsions in nursing infants (American Academy of Pediatrics, 2001). Product information say that ergotamine tartrate is contraindicated during breast feeding, since the distribution of unchanged drug and metabolites into breast milk presents a risk of adverse effects (cardiovascular disorders, diarrhoea, vomiting, cerebral convulsions) in the infant. Furthermore, repeated doses of ergotamine tartrate may impair lactation via inhibition of prolactin release from the pituitary gland (Bracher et al., 2010; Fachinformation, 2010; Martindale, 2010).

7.5.2. Ergometrine

Ergometrine is used for medicinal purposes as ergometrine maleate. Ergometrine tartrate was formerly used (Bracher et al., 2010; Martindale, 2010).

Pharmacodynamics

Ergometrine maleate has a much more selective action on the uterus than most other EAs, especially on the puerperal uterus (Blaschek et al., 2006). Its main action is the production of intense uterine contractions, which at higher doses are sustained, in contrast to the more physiological rhythmic uterine contractions induced by oxytocin. Its action is more prolonged than that of oxytocin. Ergometrine maleate is used in the active management of the third stage of labour, and to prevent or treat postpartum or post-abortal haemorrhage caused by uterine atony. By maintaining uterine contraction and tone, blood vessels in the uterine wall are compressed, and blood flow reduced (AHFS, 1995; Martindale, 2010).

For details on mechanism see Section 7.6.

Therapeutic applications and dosage

Ergometrine maleate, which is used to produce uterine contractions in the active management of the third stage of labour and in the prevention or treatment of postpartum haemorrhage, is commonly administered parenterally. When given orally dosages of 0.2 to 0.4 mg are used 2 to 4 times daily (Bracher et al. 2010; Martindale, 2010).

Parenteral treatment of haemorrhage may be followed by ergometrine maleate 0.2 to 0.4 mg orally 2 to 4 times daily until the danger of atony and haemorrhage has passed, which is usually 48 hours (Martindale, 2010). The drug may be given in this dose orally for 2 - 7 days to reduce postpartum bleeding and puerperal uterine atony and sub-involution. Severe uterine cramping may be reduced by decreasing the dosage (AHFS, 1995).

Adverse reactions associated with drug use in the therapeutical range

Nausea and vomiting are the most common effects of ergometrine maleate. Furthermore abdominal pain, diarrhoea, headache, dizziness, tinnitus, chest pain, palpitations, bradycardia and other cardiac arrhythmias, coronary artery vasospasm, myocardial infarction, dyspnoea, and pulmonary oedema have been reported after use of ergometrine maleate. Hypertension may occur, particularly after rapid intravenous dosage; hypotension has also been reported. Hypersensitivity reactions, including shock, have occurred. Ergometrine maleate shows a lower tendency to produce gangrene than ergotamine tartrate, but ergotism has been reported and symptoms of acute poisoning are similar (AHFS, 1995; Bracher et al. 2010; Martindale, 2010).

Intoxications and adverse effects associated with overdosage

In cases of overdosage of ergometrine maleate not in compliance with the recommended doses or accidental exposure, the following observations have been made:

The principal manifestations of severe ergometrine maleate overdosage are seizures and gangrene. Other manifestations include vomiting, diarrhoea, dizziness, increase or decrease of blood pressure, weak pulse, dyspnea, loss of consciousness, numbness and coldness of the extremities, tingling, chest pain, hypercoagualability, and gangrene of the fingers and toes.

Ergometrine maleate has been given accidentally in adult doses to neonates sometimes instead of vitamin K (Whitfield and Salfield, 1980; Pandey and Haines, 1982; Mitchell et al., 1983; Donatini et al., 1993; Dargaville and Campbell, 1998; Martindale, 2010). Symptoms have included peripheral vasoconstriction, encephalopathy, convulsions, respiratory failure, acute renal failure, and temporary lactose intolerance. Recovery occurred after intensive symptomatic treatment including assisted ventilation and anticonvulsants. The long-term outcome of ergometrine maleate overdosage has been reported for six infants. Their ages at follow-up ranged from 18 months to 5 years; all had normal physical and behavioural development and neurological outcomes (Dargaville and Campbell, 1998).

One death was reported in an infant who received 0.2 mg of ergometrine maleate orally (AHFS, 1995).

Specific groups - Interactions

Similar high risks and similar precautions as for ergotamine tartrate are described. Individuals suffering from certain health deficiencies, such as hypertension, heart disease, venoatrial shunts, mitral valve stenosis or obliterative vascular disease, sepsis or hepatic or renal impairment, are at higher risks to develop adverse effects when exposed to ergometrine maleate compared to healthy persons (AHFS, 1995; Martindale, 2010).

Pregnancy

Ergometrine maleate causes intense uterine contractions in the range of the therapeutic doses described above. Thus it is evident that during pregnancy, apart from the use in the active management of the third stage of labour (medical indication), oral intake of ergometrine maleate in these doses may cause adverse effects. Ergometrine maleate is contraindicated for the induction of labour or for use during the first stage of labour. Its use should also be avoided in patients with preeclampsia, eclampsia or threatened spontaneous abortion (Martindale, 2010).

7.5.3. Ergocornine

Inconsistent results are reported with regard to effects of ergocornine on female sex hormones and their metabolites.

As reviewed by Floss et al. (1973), Shelesnyak (1957) speculated that ergocornine might affect the metabolism of progesterone. A preliminary study in women, in which the change in urinary levels of

various steroids in response to ergocornine was measured, resulted in the observation that ingestion of a single dose of 2 mg ergocornine in the immediate postovulatory phase markedly decreased urinary levels of pregnanediol (metabolite of progesterone) and estrogens and increased those of 17-ketosteroids and 17-hydroxycorticosteroids (Shelesnyak et al., 1963**).** This finding, which was confirmed by Sterba (1968), led to the more specific suggestion that the alkaloid might act by blocking 3-beta-hydroxysteroid dehydrogenase (Shelesnyak et al., 1963). An extended reinvestigation measuring the plasma and urinary levels of a number of steroids in normal and ergocornine-treated women failed to reproduce the decreases in urinary pregnanediol levels observed earlier and provided no evidence for a specific blockade of the 3-beta-hydroxysteroid dehydrogenase (Lindner et al., 1967).

Within the frame of investigating the potential of ergocornine as an oral inhibitor of implantation, the urinary excretion of steroids was measured, following oral administration of ergocornine methanesulphonate to thirty eight women in the post-ovulatory period. They were treated orally with four different doses of ergocornine: i) 2 mg, ii) 8 mg, iii) 10 mg for 5 days and iv) 20 mg for 10 days. Each of these treatments started from the second day of high temperature phase (post–ovulatory phase of the menstrual cyclus). Seven out of ten women, who received 2 mg, showed a fall in pregnanediol excretion after ergocornine administration, while the remaining three showed a slight rise in its value. In addition, seven out of ten subjects treated with 10 mg of this EA revealed a drop in urine pregnanediol level. Three out of five cases who received 20 mg of this agent disclosed a similar result. The mean value of urinary pregnanediol excretion (0.97 mg per day) in women given 8 mg was lower than that of the control group (1.64 mg per day). In four women, who received 10 mg, and three given 20 mg, measurement of urinary excretion of estrogens was carried out using specimens of urine collected on 3 different occasions described above. All of the seven women treated had an apparent increase in urinary oestrogen excretion following ergocornine administration. Estimation of urinary 17-ketosteroids (17-KS) excretion and of urinary 17-hydroxycorticosteroids (17-OHCS) excretion was carried out for three days for seven women given 10 mg and three treated with 20 mg. Six out of the ten women showed increased urinary excretion of 17-KS following the ingestion of ergocornine and four out of ten a rise in 17-OHCS excretion. Five out of the thirty-eight women were in early pregnancy (6 - 8 weeks) and had been granted artificial termination for financial reasons. In one out of five pregnant women treated (on the 22nd day of high temperature phase, immunological pregnancy test became positive) spontaneous abortion did occur after oral administration of 10 mg of ergocornine. The authors concluded that more studies seemed to be necessary to investigate the potential of ergocornine as an oral inhibitor of implantation (Koi, 1966).

7.5.4. Ergot - the sclerotia of *Claviceps* spp.

The toxicity of ergot is well known due to large outbreaks of human poisoning by grain crops, contaminated with sclerotia of fungal species within the genus *Claviceps*, in particular *C. purpurea* (WHO-IPCS, 1990).

Human data are available only for intoxications from sclerotia from *C. purpurea* and *C. fusiformis*. Humans are not exposed to sclerotia from *C. paspali,* which grows on grass that may be fed to farm animals (WHO-IPCS, 1990).

Knowledge about effects in humans also exists because in the past preparations of sclerotia of *C. purpurea* have been used in human medicine for obstetric and other purposes until these products were replaced by those containing isolated EAs.

7.5.4.1. Therapeutic use of the sclerotia of *C. purpurea* in human medicine

Sclerotia of *C. purpurea* in pulverised or extracted form were formerly used in tablets or liquids as an oxytocic. Preparations containing these extracts have also been used to treat migraine or nervous disorders. Nowadays these uses are obsolete because of difficulties in alkaloid standardisation. Furthermore a combined intake of EAs is not considered to be reasonable due to the differences in pharmacological effects of the different alkaloids, and thus lack of specificity for the desired pharmacological effect and associated risks (Kommission E, 1986; Blaschek et al., 2006).

In former times pharmacopoeias described preparations of powdered and defatted or ethanol/water extracted sclerotia of *C. Purpurea*, which were standardised for total alkaloid concentrations of 0.1-0.2 % (Martindale, 1972; Blaschek et al., 2006).

Regarding total alkaloid intake with these preparations, indicated single doses ranged from 0.2 to 3 mg/person and indicated daily doses were in the range from 6 to 7.5 mg/person per day (Martindale, 1972; Blaschek et al., 2006).

7.5.4.2. Intoxications associated with contamination with sclerotia of *C. purpurea* outbreaks

Numerous epidemics due to affected grain products, mainly rye bread, occurred in Europe between the 9th and 18th centuries mutilating and killing thousands of people. The latest outbreaks of ergotism in Europe occurred in 1926-28 in the United Kingdom and the former USSR. An outbreak reported in a French village in 1951 with clinical features of a neurological nature, which were not typical of convulsive ergotism, was first associated with ergot intake. Subsequently it was discovered that this outbreak was due to toxicity of organically bound mercury and not to ergot (WHO-IPCS, 1990; De Costa, 2002; Eadie, 2003).

Based on correlation of grain inspection and medical statistics it was estimated that the disease occurred when there was more than 1 % or more of ergot in the cereal grain, 7 % causing fatal poisonings. In many of the epidemics between the 10th and 18th centuries the amount of ergot in the grain exceeded 30 % (Lorenz, 1979). Similar figures have been reported by other authors referring to fresh ergot, which is supposed to be more toxic than aged ergot. They consider consumption of flour with ergot concentrations of 0.1 % to be harmless, of 1 % to bear risks of toxic effects and of 8 - 10 % to be associated with risks of fatality (Wirth and Gloxhuber, 1981).

In early reports two main types of disease were noted: the vasospastic gangrenous and convulsive form. The gangrenous and convulsive forms of ergotism could occur concurrently (WHO-IPCS, 1990; De Costa, 2002; Eadie, 2003). In France and other European countries west of the Rhine, outbreaks of ergotism were typically of the gangrenous type, whereas in central and eastern Europe and Scandinavia, outbreaks were normally of the convulsive type WHO-IPCS, 1990; De Costa, 2002; Eadie, 2003). The basis for the occurrence of these two general types and of toxic manifestations remains unclear. First, it was attributed to differences in diet, principally a deficiency in vitamin A as a factor in convulsive ergotism. Second, the presence of an unidentified EA with potent serotonergic properties and an increased ability to cross the blood-brain barrier in ergot from *Claviceps purpurea* strains east and north of the Rhine is discussed in literature (Floss et al., 1973; Eadie, 2003).

The gangrenous type is known as *ignis sacer* (sacred fire), Holy Fire or St Anthony's Fire. The initial symptoms are oedema of the legs followed by paraesthesias. This form is characterised by intense burning pain and gangrene of feet, hands and whole limbs, due to the vasoconstrictive properties of ergot. In severe cases, affected parts shrank, became dry and black, mummified and dropped off without loss of blood. Spontaneous abortion frequently occurred.

In convulsive ergotism, the whole body was attacked by general convulsion, which returned at intervals of a few days (WHO-IPCS, 1990; De Costa, 2002; Eadie, 2003). Convulsive ergotism was often accompanied by manic episodes and hallucinations. The convulsive form has not been reported in Europe for nearly a century.

Lactation inhibition by ergot was first reported in 1676**,** when it was noted that agalactia occurred among nursing women suffering from gangrenous ergotism (Floss et al., 1973).

In 1978, an epidemic was reported in Ethiopia (e.g. Demeke et al., 1979; King, 1979; WHO-IPCS, 1990). The locally grown barley, the staple food, had become dominated by wild oats heavily contaminated with *C. purpurea* sclerotia. The grain consisted of 70 % wild oats, 12 % barley, and 0.75 % ergot; ergometrine was detected in the sclerotia by thin layer chromatography. A total of

93 cases of ergotism were reported during the spring of 1978. More than 80 % of affected persons were between 5 and 34 years of age. In addition to the 93 cases, 47 deaths were reported as having been due to ergotism. Examination of 44 patients out of the 93 registered, revealed ongoing dry gangrene of the whole or part of one or more limbs (7.5 %), feeble or absent peripheral pulses (36.4 %), swelling of limbs (11.2 %), desquamation of the skin (12.8 %), and loss of one or more limbs (21.5 %). It was noted that 88 % of patients had involvement of the lower extremities. The most common general symptoms were weakness (78.5 %), formication (15 %), burning sensation (14.3 %), nausea (7.2 %), vomiting (5.6 %), and diarrhoea (6.8 %). In addition, 50-60 infants and young children died from starvation due to failure of the mothers to lactate. This may have been related to the effect of ergot on lactation (WHO-IPCS, 1990).

The last recorded outbreak of gangrenous ergotism occurred in the Arsi Zone, Ethiopia in 2001 and was attributed to the ingestion of barley containing ergotised wild oats (Urga, 2002). A review of the ergotism cases in the survey area indicated that 18 patients aged 5 to 30 years were affected by the disease with three reported deaths, children being the main victims. In the grain samples, which were collected from 7 households affected, ergotamine and ergometrine were determined photometrically. Concentrations ranged from 2.1 to 26.6 mg ergotamine/kg and from 0.9 to 12.1 mg ergometrine/kg.

Case reports

Since 1582 it has been documented that oral ergot was used by midwives in Europe to accelerate the course of labour. In the early 19th century, physicians began to use various solid and liquid preparations of ergot for obstetric purposes, but soon realised that the use of ergot during labour increased the risk of stillbirth (De Costa, 2002; Eadie, 2003).

Pfaender et al. (1985) reported the case of a 13-year-old girl who suffered for several months from headaches, impaired vision and spotting. Symptoms disappeared upon hospitalization during which she did not eat the muesli she usually had for breakfast at home. Analyses of the rye, which represented the major part of the muesli, revealed a contamination with 12 % sclerotia of *C. purpurea.* The authors attributed the headaches and the menstruation disorder to the intake of ergot.

According to Frohne and Pfaender (2004) 5-10 g of fresh sclerotia of *C. purpurea* are expected to be the acute lethal dose for humans. Regarding chronic intoxications they assumed a daily intake of 10 sclerotia of *C. purpurea* (total alkaloid content: 0.2 %) to be the threshold dose.

There is one reported case showing that chronic EA inhalation can cause ergotism affecting peripheral arteries. For 6 months a 42-year-old farmer without cardiovascular risk factors had been suffering from increasing pain in both feet and calves. Angiography two months apart had demonstrated progressive narrowing of all lower-leg arteries. Pain-free walking had become restricted to 50 m, there were no palpable pulses in the right foot and those in the left foot were markedly reduced. Enquiry of the patient revealed that he had been exposed to ergotamine-containing milling dust in the preparation of rye flour. Inhalational intake of ergotamine was proven by a high plasma ergotamine level, but there was no estimate of the inhaled dose reported. Complete avoidance of exposure to flour dust slowly decreased the plasma level of ergotamine within 4 months, after which all lower-leg arteries had almost completely re-opened. As the plasma ergotamine level fell only slowly the authors assumed that ergotamine had accumulated in a, so far unknown, body depot with slow release into the blood (Stange et al., 1998).

7.5.4.3. Intoxications associated with contaminations with sclerotia of *C. fusiformis*

Intoxication following ingestion of ergot from *C. fusiformis* in bajra (pearl millet, *Pennisetum typhoides*) has been reported from India (Krishnamachari and Bhat, 1976; Tulpule and Bhat, 1978; WHO-IPCS, 1990). Several outbreaks have been observed since 1958, when the first report was published; the latest occurred in the autumn of 1975 in the state of Rajasthan. 78 persons belonging to 14 households developed symptoms, characterised by nausea, repeated vomiting, and giddiness,

followed by drowsiness and prolonged sleepiness, extending sometimes to over 24 - 48 hours (Krishnamachari and Bhat, 1976). There were no signs or symptoms of vaso-occlusion. The disease generally developed 1 - 2 h following a single meal. The pearl millet from affected villages contained 15 - 174 g ergot/kg, resulting in a contamination of the grain with 15 - 199 mg total EAs/kg. The individual EAs were identified as agroclavine, elymoclavine, chanoclavine, penniclavine, and setoclavine. Pearl millet from villages with no cases of intoxication contained 1 - 38 g ergot/kg with a total EA content of 15 - 26 mg/kg. Analyses of alkaloids were performed by thin layer chromatography and spectrophotometrically. There were no deaths and no information on pathological changes is available. The number of households studied was too small for no-effect levels to be calculated, but the authors suggested that a daily intake of 28 µg total EAs/kg b.w. would be non-toxic (Krishnamachari and Bhat, 1976; WHO-IPCS, 1990).

7.6. Modes of action

In general, the effects of EAs result from their activity as ligands for adrenergic, serotonergic and dopaminergic receptors, which is conferred by the tetracyclic ergoline ring system common for this class of substances (Eich and Pertz, 1994).

The ligand activity of EAs is mainly related to the derivatives of lysergic acid (8*R*-epimers; suffix -ine) (Komarova and Tolkachev, 2001a), while isolysergic acid derivatives (8*S*-epimers, suffix –inine) are considered as biologically less or not active (Pierri et al., 1982). However, in view of the facile epimerisation equilibrium, leading to transformation of *D*-lysergic acid alkaloids (ergopeptines or ergopeptames; biologically active) into *D*-isolysergic acid alkaloids (ergopeptinines or ergopeptinams) and vice versa, both epimers are to be considered in the risk characterisation.

Because of their structural differences with the physiological monoamine neurotransmitters, EAs are generally characterised by a low specificity and selectivity in respect to the mentioned neuroreceptors and, depending on the individual structure, they can display a complex behaviour as receptor agonists, partial agonists or antagonists (Mantegani et al., 1999; Pertz and Eich, 1999). In addition to this, the high heterogeneity of the serotoninergic, 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 in principle a substance-specific profile for each EA. This notwithstanding, some general features have been determined, in particular for the naturally occurring and semi-synthetic EAs used as pharmaceuticals and these were reviewed by Pertz and Eich (1999).

An overview of the pharmacological modes of action for ergotamine and ergometrine is reported here below.

Ergotamine

Ergotamine acts as a partial agonist or antagonist with the receptors of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT, serotonine) (AHFS, 1995; Tfelt-Hansen et al., 2000; Brunton et al., 2006; Schardl et al., 2006; Forth et al., 2009; Bracher et al., 2010; Martindale, 2010).

Regarding reactions with alpha-adrenergic receptors ergotamine is a partial agonist and weak antagonist in blood vessels and various smooth muscles and mainly an antagonist in the CNS. Thus in therapeutic doses ergotamine causes peripheral vasoconstriction primarily by stimulating $alpha_1$- and $alpha_2$-adrenergic receptors. With higher doses ergotamine is also a competitive alpha-adrenergic blocker, but this effect is masked by its alpha-adrenergic agonist activity (Tfelt-Hansen et al., 2000; Brunton et al., 2006; Schardl et al., 2006).

Interacting with 5-HT-receptors (e.g. $5\text{-HT}_{1B,}$ $5\text{-HT}_{1D,}$ 5-HT_{2A}), ergotamine is a partial agonist in certain blood vessels and a poor agonist/antagonist in the CNS (Brunton et al., 2006; Schardl et al., 2006; Forth et al., 2009).

Stimulation of central dopamine $_{D2}$-receptors in the area postrema is the mechanism behind the emetic potency of ergotamine. Interaction with dopamine D_2-receptors in the anterior pituitary is also the key to the depression of prolactin levels (Brunton et al., 2006; Schardl et al., 2006).

Ergotamine effects in the treatment of migraine are explained by its agonistic action on alpha$_1$- and alpha$_2$-adrenergic receptors. Moreover the efficacy of ergotamine in migraine is associated with its stimulation of 5-HT$_{1B}$- and 5-HT$_{1D}$-receptors (Silberstein and McCrory, 2003; Schardl et al., 2006; Forth et al., 2009).

Ergometrine

Ergometrine is a partial agonist for alpha-adrenergic receptors in blood vessels (less than ergotamine tartrate) and has little antagonistic action. Through interactions with 5-HT-receptors, ergometrine is a partial agonist in human umbilical and placental blood vessels, a selective and fairly potent antagonist in various smooth muscles, and a partial agonist/antagonist in the CNS. Ergometrine is a weak antagonist of dopaminergic receptors in certain blood vessels and a partial agonist/antagonist in the CNS, being less potent than ergotamine tartrate in producing emesis (Brunton et al., 2006; Bracher et al., 2010).

7.7. Dose response assessment

7.7.1. Dose response data in animals

Different EAs may exert a series of different biological effects, depending on the substance specific affinity profile on the various neuroreceptors discussed in Section 7.6.

Decreased b.w. gain was seen in a number of studies, generally associated with depressed food intake, which is likely to be due to dopaminergic effects of EAs. Decreased serum thyroxine (T4) levels were observed in male and female rats treated with ergometrine (Peters-Volleberg et al., 1996). Decreased serum prolactin levels were observed in rats in the subacute study on α-ergocryptine (Janssen et al., 2000b). The results for these hormonal levels were variable and the CONTAM Panel concluded that they were not appropriate for establishing a health-based guidance value (HBGV). EAs have a number of effects on the reproductive process including prevention of pregnancy by interfering with implantation, embryotoxicity, and inhibition of lactation. These effects have generally been observed at higher doses than the LOAELs in the repeat dose studies.

Overall the CONTAM Panel concluded that the vasoconstrictive effect represented by tail muscular atrophy was the critical effect for the hazard characterisation. Dose-response analyses were performed on the available information from the subchronic study on ergotamine (Speijers et al., 1993), and the subacute study on α-ergocryptine (Janssen et al., 2000a, b) reported in Section 7.2.2. Dose response assessment was performed by means of the benchmark dose (BMD) analysis.

The results of the BMD analyses are reported in Table 16. The details for each BMD analysis, including for those endpoints not selected by the CONTAM Panel as critical for the hazard characterisation, are reported in Appendices (G-J). The lowest BMDL$_{10}$ of 0.33 mg/kg b.w. per day) was selected for use as a reference point in establishing HBGVs.

Table 16: Benchmark dose (BMD) analyses for ergotamine, α-ergocryptine and ergometrine. BMD and benchmark dose lower confidence limits (BMDL) were calculated by means of the software BMDS v2.1.2 (US EPA). Details on calculations and statistical analyses of the BMD models are reported in Appendices G-J.

					Results			
Substance	**Species/ strain/sex**	**Dosing period**	**Endpoint**	**Selected model**	**Benchmark response (BMR, %)**	**BMD (mg/kg b.w. per day)**	**BMDL [(a)] (mg/kg b.w. per day)**	**Reference**
Ergotamine	Rat/SD/f	13 weeks	Tail, muscular atrophy	Dichotomous - Gamma unrestricted	10	1.2	0.33	Speijers et al., 1993
α-Ergocryptine	Rat/SD/m	28-32 days	Tail, muscular degeneration	Dichotomous - Quantal-linear	10	1.5	0.75	Janssen et al., 2000a, b

(a): 95% confidence level.
b.w.: body weight; SD: standard deviation.

7.7.2. Dose response data in humans

Ergotamine

Ergotamine tartrate is prescribed in single oral doses of 1 - 2 mg, equivalent to 0.9 - 1.8 mg ergotamine (base), corresponding to 13 - 26 µg ergotamine/kg b.w. assuming a default b.w. of 70 kg (as indicated by EFSA, 2012), in the treatment of migraine. However, scientific evidence for therapeutic effects of these doses is ambiguous (Tfelt-Hansen et al., 2000). The oral therapeutic dose of ergotamine tartrate in the treatment of migraine is limited to a maximum intake expressed as ergotamine of 3.5 - 7 mg per day (corresponding to 50 - 100 µg/kg b.w. per day assuming a default b.w. of 70 kg) or of 5.3 - 10.6 mg per week (corresponding to 76 - 151 µg/kg b.w. per week or 11 - 22 µg/kg b.w. per day over a period of 7 days) to avoid possible severe adverse effects associated with overdosage such as peripheral vasoconstriction (Brunton et al., 2006; Bracher et al., 2010; Fachinformation, 2010; Martindale, 2010). According to older literature the maximum total monthly oral dose should not exceed 20 mg ergotamine tartrate, equivalent to 17.7 mg ergotamine per month (corresponding to 253 µg/kg b.w. per month or 8 µg/kg b.w. per day over a period of 30 days) (Rote Liste, 2003). In regular intake, exceeding this monthly maximum limit may lead to permanent headache (Fachinformation, 2010). It has to be taken into consideration that therapeutic doses below the recommended maximum limits may already be associated with less severe adverse effects such as nausea, vomiting or abdominal pain.

Excessive regular several-months-long oral intake of 24 mg ergotamine tartrate per week, equivalent to 21.2 mg ergotamine (base) per week (corresponding to approximately 300 µg ergotamine/kg b.w. per week or 43 µg ergotamine/kg b.w. per day over a period of 7 days) led to an autonomic dysaesthesia in a woman suffering from migraine (Evans et al., 1980).

These dose response data do not apply to individuals with contraindications, especially not to pregnant or lactating women in view of the oxytocic action of ergotamine, its inhibition of prolactin secretion (impairment of lactation) and possible adverse effects (ergotism) in breast fed infants (Fachinformation, 2010; Martindale, 2010).

Thus it has to be considered that ergotamine in oral doses lower than those mentioned above (single oral dose of 13 - 26 µg ergotamine/kg b.w.; maximum dose of 8 µg/kg b.w. per day over a period of 30 days) may lead to adverse effects especially regarding reproductive toxicity. No further details on

corresponding dose-response relationships are available, but the Panel assumes that the dose-response relationship for the uterine contracting effect of ergotamine will be similar to that of ergometrine.

Ergometrine

Ergometrine maleate has been prescribed orally in doses of 0.2 to 0.4 mg (equivalent to approximately 0.15 to 0.30 mg ergometrine) up to four times daily. The CONTAM Panel assumed a b.w. of 75 kg for pregnant women, taking into account the average b.w. of 67.2 kg for adult women reported by the EFSA Scientific Committee (EFSA, 2012). This led to a corresponding dose of 8-16 µg ergometrine/kg b.w. per day to produce uterine contractions (Bracher et al. 2010; Martindale, 2010). The lowest single dose to produce this effect is then 2 µg/kg b.w. The drug may be given orally for 2 - 7 days (AHFS, 1995).

7.8. Derivation of Health-based Guidance Values (HBGVs)

The genotoxic potential of EAs other than ergotamine has not been adequately investigated. The available data did not indicate bacterial or mammalian cell mutation. There is some evidence of clastogenicity, but the *in vivo* data are inconsistent. In a 2-year carcinogenicity study, crude ergot induced neurofibromas on the ears at 5 % and 2 %, but not 1 % in the diet, and these regressed if the ergot was withdrawn. The ergot treatments producing ear neurofibromas also caused significant decrements in body weight gains and a low protein diet appeared to exacerbate the tumorigenicity. The incidences of tumour types observed in untreated rats (e.g., lung lymphosarcoma and kidney embryonal sarcoma) were also increased in treated rats. The absence of carcinomas and the regression indicate aetiology related to a non-genotoxic mode of action.

EAs act on a number of neurotransmitter receptors, particularly adrenergic, dopaminergic and serotonergic receptors. These receptor interactions could result in acute effects as well as longer term effects, therefore the CONTAM Panel considered it appropriate to establish both an acute reference dose (ARfD) and a tolerable daily intake (TDI) for EAs.

On repeated dosing of various EAs, these effects on receptors result *inter alia* in ischaemia, particularly in the extremities, such as the tails of rats, decreased body weight gain and changes in the levels of some hormones. Vasoconstriction was considered the critical effect and selected for the derivation of the HBGVs.

The $BMDL_{10}$ is 0.33 mg/kg b.w. per day for incidence of tail muscular atrophy from a 13-week rat feeding study of ergotamine. In considering an ARfD, the CONTAM Panel concluded that an uncertainty factor of 3 was required to take into account deficiencies in the database, such as incomplete information on reproductive toxicity. Together with the default uncertainty factor of 100 for intra and inter species differences, the CONTAM Panel applied an overall uncertainty factor of 300 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established an ARfD of 1 µg/kg b.w. (rounded to one significant figure).

In establishing a TDI, and in line with the recommendation of the EFSA Scientific Committee (EFSA, 2012), the CONTAM Panel concluded that an additional uncertainty factor of 2 should be applied for extrapolation from sub-chronic to chronic studies. The CONTAM Panel applied an overall uncertainty factor of 600 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established a TDI of 0.6 µg/kg b.w. per day (rounded to one significant figure).

Individual EAs can show a different profile of toxicity due to differences in receptor binding but, for those EAs for which data are available, there appear to be no marked quantitative difference in NOAEL/LOAEL and BMDL. There is a lack of information on the potency of most of the EAs found in food on the European market. Because of the potential for action via the same receptors and for conversion of -inine forms into the more active -ine forms, the CONTAM Panel concluded that the established HBGVs are a group ARfD of 1 µg/kg b.w. and a group TDI of 0.6 µg/kg b.w. per day for the sum of the EAs covered in this opinion assuming equal potency.

The established group ARfD is two fold below the lowest single dose of 2 µg/kg b.w. ergometrine used to induce uterine contractions. This dose has been used as a starting point used therapeutically and if ineffective, repeated and/or higher doses were administered. The CONTAM Panel concluded that 2 µg/kg b.w. is close to a NOEL and that the margin between this dose in a sensitive subpopulation and the group ARfD is adequate.

The lowest prescribed dose of ergotamine used in treatment of migraine (which has not shown convincing evidence of pharmacological activity) is approximately 10 to 20 times higher than the group ARfD and 20 to 40 times higher than the group TDI. Furthermore, the group TDI is 13 times lower than the maximum recommended dose for therapeutic use of ergotamine, which according to the medical literature should not exceed 8 µg/kg b.w. per day over a period of 30 days in order to avoid severe side effects.

The comparisons of the established HBGVs with doses used in human medicine provide additional support for the values of the group ARfD and the group TDI.

8. Risk characterisation

8.1. Human health risk characterisation

The mean and high acute and chronic dietary exposures to EAs were estimated based on occurrence data for the sum of at least six EAs, including ergotamine, ergocristine, ergocornine and ergosine in different foods. This scenario was considered optimal in that it did not result in an underestimation of the occurrence compared to the total of all 12 EAs analysed in a limited number of samples. Moreover, the impact of non-reported data on the exposure assessment was reduced.

The mean and high level estimated chronic dietary exposures to the sum of EAs for all age groups across European countries are below the group TDI of 0.6 µg/kg b.w. per day established by the CONTAM Panel. These estimates reflect the predominant EAs present in foods, and even allowing for a possible additional contribution from EAs that were not measured or reported, they do not indicate a health concern. This conclusion also applies to chronic dietary exposure of the specific subgroups with the potential for higher exposure than the general population, i.e. vegetarians and consumers of raw grains. Due to the limited available data, it was not possible to estimate exposure to EAs for subgroups following other specific diets, such as organic food.

The mean estimates of acute dietary exposure to the sum of EAs for all age groups across European dietary surveys are below the group ARfD of 1 µg/kg b.w., both for the general population and for the specific subgroup of raw grain consumers. In the case of high consumers' acute dietary exposure to the sum of EAs, maximum UB levels estimated for toddlers in the general population and 'other children' in the raw grain consumer subgroup were similar to the group ARfD. Taking into account the influence of left-censored data on the UB estimates, these exposures do not indicate a concern.

However, whilst the available data do not indicate a concern for any subgroup, the dietary exposure estimates relate to a limited number of food groups and a possible unknown contribution from other foods cannot be discounted.

8.2. Animal health risk characterisation

The risk characterization/conclusions reported by EFSA (2005) can only be updated with new information for pigs. Since individual alkaloids have not been investigated concerning their effects and potencies, only general conclusions regarding total alkaloid contents for *C. purpurea* and *C. Africana* can be made.

For *C. purpurea*, reduced body weight gains may be expected in the range 0.60 and 4.66 mg/kg of total alkaloids in feed when pigs are fed *ad libitum*. None of the adverse effects typically associated with ergot poisoning were observed in this study within this concentration range (Mainka et al., 2005a).

For *C. africana* sclerotia in which dihydroergosine accounted for approximately 90 % of the alkaloids, it was shown that diets for multiparous sows prior to farrowing should not exceed 1 mg alkaloid/kg, and should be limited to 0.1% for primiparous sows in order to prevent a significant reduction in plasma prolactin and first-lactation problems resulting from this. (Kopinski et al., 2007).

The risk of ergotism in livestock as a result of consuming contaminated cereal grains, or compound feeds manufactured from them, can be reduced where appropriate seed cleaning of grains is carried out. Certain forages present a risk in those areas where environmental conditions support the development of the sclerotia, but the adoption of husbandry measures, including crop rotation,[23] and grazing or topping of pastures prone to ergot infestation during the summer months (to reduce flower-head production) can reduce exposure.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to EAs has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO-IPCS, 2008). According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, model input (parameters) and other uncertainties.

9.1. Assessment objectives

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

9.2. Exposure scenario/Exposure model

In response to EFSA's request to submit occurrence data on EAs in food and feed, a total of 25 840 analytical results were received from 14 European countries. After a validation and cleaning step, 20 558 analytical results for EAs in 2 279 samples, of which 1 716 corresponded to food, 496 to feed and 67 to unprocessed grains of unknown end-use were considered in this opinion. These data on the occurrence of EAs include 803 samples of food and feed obtained through an Article 36 call. All samples were collected between 2004 and 2011.

Almost 60 % of the food samples available for the exposure calculations were sampled in just one country. Similarly, for feed around 50 % of the samples were also collected in only one Member State. Consequently, there is uncertainty over possible regional differences in EA contamination of food and feed, and the CONTAM Panel recognised that the data set is not representative of EA occurrence in food and feed on the EU market, especially as both data submissions were each received from only a few Member States.

Most of the reported food samples were non- or minimally processed foods (mainly grain milling products with 1 193 samples), with only approximately 250 samples of processed food available. Considering that most of the consumption data are reported as processed food there is an uncertainty in the exposure calculations due to this limited amount of occurrence data. On the other hand, the exposure calculations for few surveys also introduced some uncertainty due to the disaggregation of the reported food consumption data which hampered the matching with their respective analytical results and necessitated some modelling of recipes. The occurrence data on feed was also limited, with the main focus on rye and rye by-products (253 samples) and wheat and wheat by-products (161 samples). Only few samples of barley, despite the importance of this cereal as feeding material, were available

Among the food and feed samples, the number of EAs for which data were submitted ranged between 1 and 12 with different LODs and LOQs. The different number of EAs analysed in the food and feed

[23] Sclerotia do not usually survive in soil for more than one year, although under dry conditions they can survive for many years. See http://www.grainscanada.gc.ca/str-rst/ergot/em-mlce-eng.htm

samples introduced some uncertainty into the human and animal exposure assessments. Due to the substantial amount of left-censored data (60 % for food samples and more than 75 % for feed samples), the summation of the UB values of up to 12 EAs represents a conservative approach and an overestimation of exposure. This overestimation was minimised to some extent by using LOD and LOQ cut-offs of 10 µg/kg and 20 µg/kg, respectively.

Further sources of uncertainty include the limited data on the consumption pattern of vegetarians. There is also uncertainty about possible occasional high acute exposure of consumers of home made meals based on whole grains that may contain undistributed whole sclerotia in the portion used. The lack of appropriate occurrence and consumption data prevented the accurate assessment of the exposure to EAs in this group of the population. There are also insufficient data on consumption for infants, which adds uncertainty to the exposure calculations in this age group.

In addition to the limited amount of occurrence data in feed, the animal risk assessment is also hampered by limited representative feed consumption data across Europe.

Overall, there is considerable uncertainty regarding the total dietary exposure to EAs in the human and animal risk assessments.

9.3. Model input (parameters)

There are no prescribed fixed official methods or defined harmonised performance criteria for the analysis of EAs and laboratories can use any method of analysis, provided it can be demonstrated in a traceable manner that they fulfil the requirements according to ISO 17025. This may have added to the uncertainty in the analytical results. The limited number of defined reference compounds, isotope labelled internal standards and certified reference materials is a limitation when the method performance for the analytical procedures for analysis of EAs in food and feed is assessed. This adds to the overall uncertainty in the analytical results.

9.4. Other uncertainties

EAs other than those used therapeutically are not sufficiently characterised, with respect to their toxicological properties and relative potencies. For ergotamine and ergometrine, clinical data complying with current standards are missing, no-effect levels for pharmacological activity in humans are poorly defined, and reproductive toxicity data are lacking. There are also scarce data on adverse effects in livestock, fish and companion animals.

9.5. Summary of uncertainties

Table 17: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the exposure of EAs in food and feed.

Sources of uncertainty	Direction
Uncertainty in analytical results	+/-[(a)]
Impact of using upper-bounds for left-censored data on dietary exposure estimate	+
Low number of food samples available for exposure assessment	+/-
Extrapolation of occurrence data from only a limited number of European countries over a limited period of time to whole Europe	+/-
Lack of occurrence data on EAs from *C. purpurea* other than those considered in this opinion	-
Lack of occurrence data on EAs other than for *C. purpurea*	-
Limited data for population with specific consumption habits	+/-
Limited data on feed consumption and contamination across Europe	+/-
Limited data on toxicity of individual ergot alkaloids	+/-
Inclusion of the –inine forms in the group ARfD and group TDI	+
Limited data on the composition of EAs in feeding studies	+/-

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human and animal exposure to EAs through consumption of food and feed is considerable. The CONTAM Panel concluded that the risk assessment of human exposure to the sum of EAs considered in the opinion is more likely to overestimate than to underestimate the risk. For the risk assessment on animal exposure, given the level of uncertainty, it was not possible to conclude on possible overestimation or underestimation of the risk.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Ergot alkaloids (EAs) are naturally occurring tryptophan-derived mycotoxins produced by fungi, mainly members of the *Claviceps* spp. (external spore-producing fungi) and *Neotyphodium* spp. (internal endophytic fungi).

- In Europe *Claviceps purpurea* is the most widespread *Claviceps* species. It is known to infect more than 400 plant species, including some economically important cereal grains such as rye, triticale, wheat, barley, millet and oats.

- The biology of *C. purpurea* and the structures of their EAs have been intensively studied. In contrast, respective information for other EA-producing fungi, in particular *C. fusiformis*, relevant for pearl millet, and *C. africana,* relevant for sorghum, is limited.

- Endophyte infections of cool-season grasses such as tall fescue (*Lolium arundinaceum*) with *Neotyphodium* toxins are well known and characterised for their toxicity outside Europe, especially for ruminants and horses. However, as there are currently no indications of exposure of livestock to *Neotyphodium* toxins in Europe, the hazards of these toxins in forage crops have not been addressed in this opinion.

- The predominant EAs in sclerotia of *C. purpurea* are: ergometrine, ergotamine, ergosine, ergocristine, α- and β-ergocryptine, and ergocornine and their corresponding epimers (-inine

forms). The EA profiles and total amounts are highly variable and may depend on location, climate conditions, fungal genotype and host plant.

- EAs containing the lysergic acid backbone easily undergo spontaneous epimerisation of the carboxy substituent at the asymmetric centre in position C8. This epimerisation leads to mixtures of *D*-lysergic acid (-ine) and *D*-isolysergic acid (-inine) forms. Although the -inine forms are described to be biologically inactive, interconversion occurs under different conditions and thus both forms (-ine and -inine) of ergometrine, ergotamine, ergosine, ergocristine, α- and β-ergocryptine, and ergocornine were considered in this risk assessment.

- The mean total EA content of *C. purpurea* sclerotia reported in a limited number of European countries is approximately 800 µg/g. Values for individual sclerotia have been reported to vary between < 1 and 6 003 µg/g. In fresh sclerotia, as found in newly harvested grains, the ratio between -ine and -inine forms is approximately 3:1.

Methods of analysis

- Screening for ergot sclerotia content in grains to meet current European Union (EU) legislation (Regulation (EC) No 1272/2009[10] and Directive 2002/32/EC[6]) can be achieved by visual inspection, near infrared hyperspectral imaging or by determination of ricinoleic acid by gas chromatography – flame ionization detector.

- As the epimeric forms of EAs can interconvert, analytical methods should include the determination of both epimeric forms.

- At present, only high performance liquid chromatography with fluorescence detection (HPLC-FLD) and tandem mass-spectrometry (HPLC-MS/MS) allow the determination of individual EAs in food and feed commodities at relevant levels. Over the last few years, the focus in food and feed has been on the predominant EAs found in *C. purpurea* (ergocornine, ergocorninine, ergocristine, ergocristinine, α-ergocryptine, sum of α- and β-ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine) or subsets thereof.

- An HPLC-FLD method for the determination of the 12 EAs in grain and flour has been internationally validated.

- Availability of reference standards is limited. Isotopically labelled standards, useful for HPLC-MS/MS approaches are currently not available.

Occurrence and effect of processing

- The occurrence data on EAs in food and feed submitted to European Food Safety Authority (EFSA) indicated that ergotamine, ergocristine, ergosine and ergocornine are generally more abundant than α- and β-ergocryptine and ergometrine.

- A total of 20 558 analytical results from 13 different European countries were used to assess the exposure. This number of analytical results represents a total number of 2 279 samples, of which 1 716 corresponded to food, 496 to feed and 67 to unprocessed grains of unknown end-use.

- Most of the reported food data corresponded to non- or minimally processed foods, especially grain milling products. The highest concentrations of EAs were reported for rye grains, rye milling products and rye by-products.

- The amount of left-censored data reported was very high, with more than 60 % and 75 % of the results below limit of detection and/or limit of quantification for food and feed, respectively.

- In order to include in the assessments the maximum number of samples with a representative number of EAs, the occurrence data were selected based on the presence of the most abundant EAs, namely ergotamine, ergocristine, ergocornine and ergosine, and a minimum number of six reported EAs/sample.

- The scenario with at least six EAs provided an acceptable number of samples (more than 60 % of the total) and represented more than 80 % of the total analytical results available for food. This did not result in an underestimation of the occurrence compared to the total of all 12 EAs analysed in a limited number of samples.

- During processing, in particular baking, the total amount of EAs decreases and the ratio between the epimeric forms in general shifts towards the -inine forms.

- Milling processes result in redistribution of sclerotia particles in different milling fractions.

- For products consisting mainly of whole grains and consumed as such, distribution and diluting effects by milling and other steps of grain processing do not apply. In those cases whole sclerotia could get into a food serving and consequently lead to comparatively high EA-exposure for consumers of those products.

Human Exposure

- Estimation of human dietary exposure to EAs was highly influenced by the fact that most of the consumption data in the EFSA's Comprehensive European Food Consumption Database refer to processed food, for which a limited amount of occurrence data on these type of foods was available.

- The chronic dietary exposure in the adult population varied between 0.007 and 0.078 μg/kg b.w. per day for average consumers and 0.014 and 0.19 μg/kg b.w. per day for high consumers. The acute dietary exposure in the adult population ranged between 0.021 and 0.23 μg/kg b.w. per day for average consumers and 0.055 and 0.73 μg/kg b.w. per day for high consumers.

- The highest chronic exposure to EAs (considering minimum lower bound (LB) and maximum upper bound (UB) across European dietary surveys) was estimated in toddlers and 'other children'. For average consumers, the estimated chronic exposure in toddlers ranged between 0.03 and 0.17 μg/kg b.w. per day, and between 0.02 and 0.17 μg/kg b.w. per day in other children. For high consumers, estimated chronic exposure values in toddlers ranged between 0.07 and 0.34 μg/kg b.w. per day, and for 'other children' between 0.03 and 0.30 μg/kg b.w. per day

- Toddlers and 'other children' also showed the highest acute exposure to EAs (considering minimum LB and maximum UB across European dietary surveys). For average consumers, the estimated acute exposure in toddlers ranged between 0.08 and 0.42 μg/kg b.w. per day, and between 0.05 and 0.36 μg/kg b.w. per day in 'other children'. For high consumers, estimated acute exposure values in toddlers ranged between 0.21 and 1.03 μg/kg b.w. per day, and for 'other children' between 0.12 and 0.82 μg/kg b.w. per day.

- Those countries with relatively high consumption of rye bread and rolls showed the highest dietary exposure (both chronic and acute) across the different age groups.

- The assessment of the dietary exposure to EAs in specific groups of the population (vegetarians and consumers of unprocessed grains) was based on limited data. The results indicated that no significant differences were found between vegetarians and the general population, while consumers of unprocessed grains could have slightly higher dietary exposure to EAs than the general population.

Animal Exposure

- Exposure to EAs by livestock and domestic animals is most likely to occur as a result of consuming rations containing cereal grains and cereal by-products, and in particular rye, sorghum and millet and by-products derived from them. Within the EU rye, sorghum and millet are not widely used as livestock feeds, although where they are grown commercially these feeds may be more extensively used in livestock rations.

- Contaminated forages present a potential risk in those areas in which environmental conditions support the development of the sclerotia, but husbandry measures are available that reduce this risk. To date there have been very few reports of EA toxicosis in grazing livestock within Europe.

- Because the EA profiles and total amounts in feeds are very variable under field conditions, it is virtually impossible to relate specific reports of ergot toxicosis in livestock, as reported in the scientific literature, to exposure to individual alkaloids.

Hazard identification and characterisation

Toxicokinetics

- Data on toxicokinetics are sparse and are mainly limited to those EAs that are used as pharmaceuticals.

- The available literature suggests that EAs are absorbed from the gastrointestinal tract and subjected to oxidative biotransformation, primarily by cytochrome P450 3A4, and some EAs (e.g., ergometrine) can subsequently be conjugated with glucuronic acid. Biliary excretion represents the main elimination pathway except in ruminants.

Toxicity of ergot alkaloids

- Based on the LD_{50}, the EAs that have been tested exhibit moderate oral acute toxicity. Sublethal acute exposure induces signs of neurotoxicity, including restlessness, miosis or mydriasis, muscular weakness, tremor and rigidity.

- EAs act on a number of neurotransmitter receptors, particularly adrenergic, dopaminergic and serotonergic receptors.

- On repeated dosing of various EAs, these effects on receptors result *inter alia* in ischaemia, particularly in the extremities, such as the tails of rats, decreased body weight gain and changes in the levels of some hormones.

- Repeat dose studies in rats demonstrate no major quantitative difference in the toxicity of ergotamine, ergometrine and α-ergocryptine, with no-observed-adverse-effect-levels (NOAELs) in the region of 0.22 - 0.6 mg/kg b.w. per day.

- EAs have a number of effects on the reproductive process in rodents, including prevention of pregnancy by interfering with implantation, embryotoxicity, and inhibition of lactation. These

effects have generally been observed at higher doses than the lowest-observed-adverse-effect levels in the repeat dose studies.

- With the exception of ergotamine, only limited genotoxicity studies have been carried out on naturally occurring EAs. No mutagenic activity of ergotamine has been detected *in vitro*. Early studies showed it had some chromosome damaging effects *in vitro* and *in vivo* although the latter were weak and inconsistent.

- The Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that the available information on genotoxicity and carcinogenicity of EAs indicate that the observed tumours in rats fed crude ergot or ergotoxin were related to a non-genotoxic mode of action.

- The interaction of EAs with neurotransmitter receptors could result in acute as well as longer term effects. Therefore, the CONTAM Panel considered it appropriate to establish both an acute reference dose (ARfD) and a tolerable daily intake (TDI) for EAs.

- The CONTAM Panel concluded that the vasoconstrictive effect represented by tail muscular atrophy in rats was the critical effect for hazard characterisation and derivation of the health-based guidance values (HBGVs). A $BMDL_{10}$ of 0.33 mg/kg b.w. per day was calculated for the incidence of tail muscular atrophy in a 13-week rat feeding study of ergotamine.

- In establishing an ARfD, the CONTAM Panel concluded that an uncertainty factor of 3 was required to take into account deficiencies in the database, such as incomplete information on reproductive toxicity. Together with the default uncertainty factor of 100 for intra and inter species differences, the CONTAM Panel applied an overall uncertainty factor of 300 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established an ARfD of 1 µg/kg b.w. (rounded to one significant figure).

- In establishing a TDI, and in line with the recommendation of the EFSA Scientific Committee, the CONTAM Panel concluded that an additional uncertainty factor of 2 should be applied for extrapolation from sub-chronic to chronic studies. The CONTAM Panel applied an overall uncertainty factor of 600 to the $BMDL_{10}$ of 0.33 mg/kg b.w. per day and established a TDI of 0.6 µg/kg b.w. per day (rounded to one significant figure).

- The available data do not allow determination of relative potencies of all EAs, but for those EAs for which comparable studies are available there appear to be no marked differences. The CONTAM Panel therefore concluded that the established HBGVs are a group ARfD of 1 µg/kg b.w. and a group TDI of 0.6 µg/kg b.w. per day for the sum of the EAs covered in this opinion, assuming equal potency.

- Knowledge on dose-effect relations in humans is mainly based on the therapeutic use of ergotamine and ergometrine salts.

- A lowest single dose of 2 µg/kg b.w. ergometrine has been used to induce uterine contractions. This dose has been used as a starting point therapeutically and if ineffective, repeated and/or higher doses were administered. The CONTAM Panel concluded that 2 µg/kg b.w. ergometrine is likely to be close to a no-observed-effect level and that the margin between this dose in a sensitive subpopulation and the group ARfD of 1 µg/kg b.w. is adequate.

- In the treatment of migraine the lowest prescribed dose of ergotamine (which has not shown convincing evidence of pharmacological activity) is 13 - 26 µg ergotamine/kg b.w., which is approximately 10 to 20 times higher than the group ARfD and 20 to 40 times higher than the group TDI. Furthermore, the maximum recommended oral therapeutic dose of ergotamine for adults is 8 µg/kg b.w. per day over a period of 30 days to avoid possible severe adverse effects

associated with overdosage such as peripheral vasoconstriction. This is 13 times higher than the group TDI. These comparisons with doses used in human medicine provide additional support for the value of the established group ARfD and group TDI.

Adverse effects in livestock, fish and companion animals

- Globally, toxicosis of livestock as a result of consuming ergot contaminated feed has been widely reported.
- From studies on pigs published since the last evaluation performed by EFSA (2005), a NOAEL of 0.15 mg EAs/kg feed for piglets has been identified.
- Poultry appear to be able to tolerate higher levels of ergot than other non-ruminant livestock. Studies published since 2005 would indicate a NOAEL of 1.4 mg EAs/kg feed.

Risk characterisation

- The mean and high level estimates of chronic dietary exposure to the sum of EAs, for all age groups across European dietary surveys, are all below the group TDI of 0.6 µg/kg b.w. per day established by the CONTAM Panel and do not indicate a health concern.
- The mean estimates of acute dietary exposure to the sum of EAs for all age groups across European dietary surveys are below the group ARfD of 1 µg/kg b.w. established by the CONTAM Panel and do not indicate a health concern.
- In the case of high consumers' acute dietary exposure to the sum of EAs, maximum UB levels estimated for toddlers in the general population and 'other children' in the raw grain consumer subgroup were similar to the group ARfD. Taking into account the influence of left-censored data on the UB estimates, these exposures do not indicate a concern.
- The exposure of vegetarians, with higher than average consumption of vegetables and grain products is likely to be within the range identified for the general population. Similarly, the estimated exposure of consumers of raw grain products is similar to that to the general population. Due to the limited available data, it was not possible to estimate exposure to EAs for subgroups following other specific diets.
- Whilst the available data do not indicate a health concern, the dietary exposure estimates relate to a limited number of food groups and a possible unknown contribution from other foods cannot be discounted.
- Estimates of exposure based on example diets and levels of EAs in cereal grains reported in Europe would suggest that under normal conditions the risk of toxicosis in livestock is low. Furthermore, the risk of ergotism in livestock as a result of consuming contaminated cereal grains, or compound feeds manufactured from them, is reduced where appropriate seed cleaning is carried out.

RECOMMENDATIONS

- Harmonised performance criteria for the analysis of EAs in feed and food should be established.
- There is a need for commercially available reference standards, in particular for isotope labelled internal standards and for certified reference materials.
- Collection of analytical data on occurrence of EAs in relevant food and feed commodities should continue. Special attention should be paid to processed foods and to speciality foods

consumed by specific groups. The EAs monitored should include at least the compounds identified in this opinion.

- As *C. africana* and *C. fusiformis* may be relevant for ethnic foods, special diets or imported feed into the EU, the occurrence of their predominant EAs, in particular dihydroergosine and agroclavine, respectively, should be monitored.

- More comprehensive consumption data for specific groups, such as vegetarians or raw grain consumers, are needed.

- The comparative target neurotransmitter receptor-binding activity of different EAs, including the -ine and -inine forms, should be studied. Better characterisation of the acute effects of the more potent EAs in appropriate animal models is needed.

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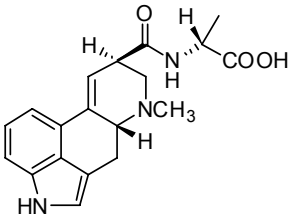
APPENDICES

A. CHEMICAL STRUCTURE OF THE MAIN ERGOT ALKALOIDS

Claviceps purpurea

_D_-lysergic acid derivatives



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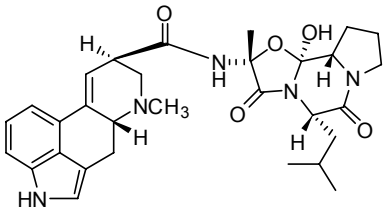

ergosine
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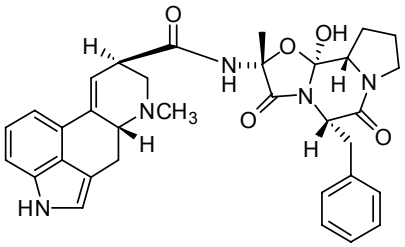
molecular weight: 547.6 g/mol



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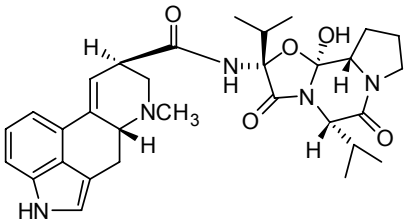
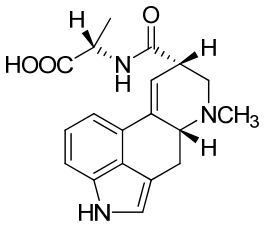
molecular weight: 581.7 g/mol



ergocornine, CAS: 564-36-3
molecular weight: 561.7 g/mol

isolysergic acid derivatives



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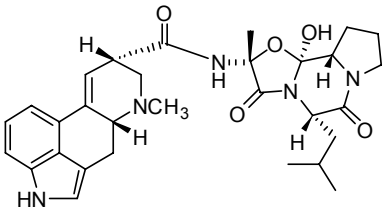

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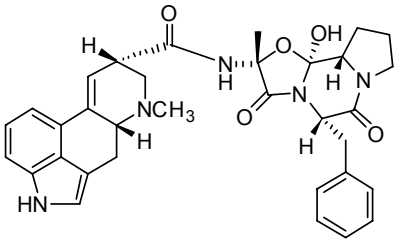

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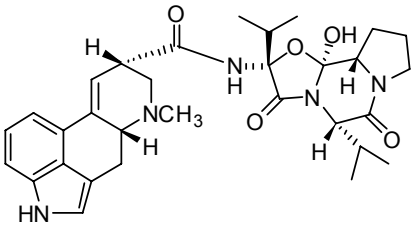

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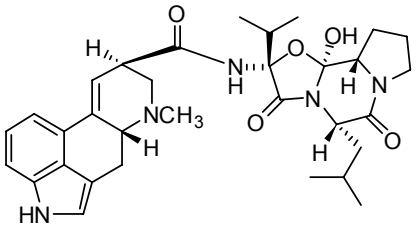
Claviceps purpurea (continued)

D-lysergic acid derivatives



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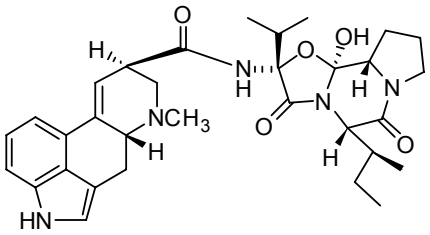
molecular weight: 575.7 g/mol



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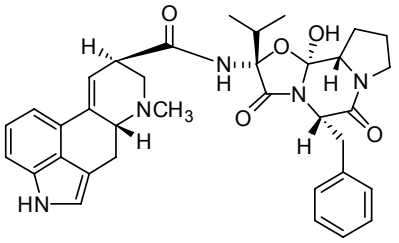

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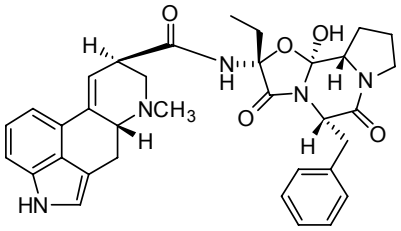
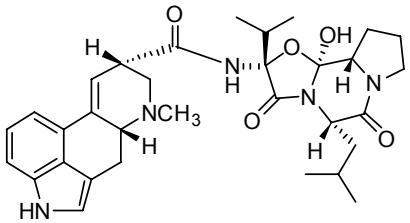


ergostine, CAS: 2854-38-8
molecular weight: 595.7 g/mol

isolysergic acid derivatives



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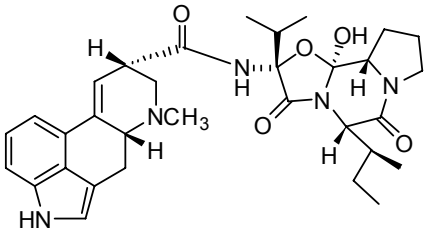
molecular weight: 575.7 g/mol



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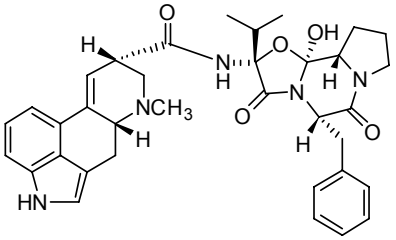

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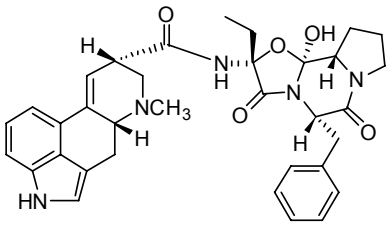
molecular weight: 609.7 g/mol



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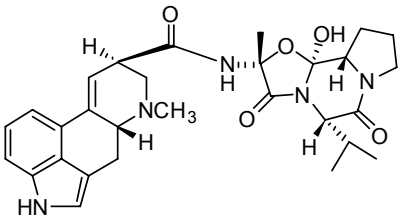
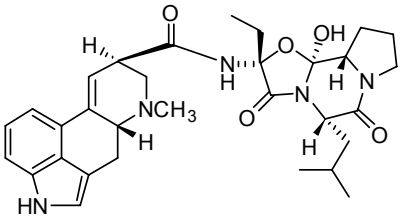
molecular weight: 595.7 g/mol

Claviceps purpurea (continued)

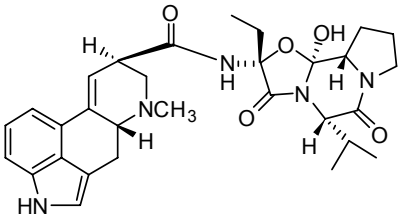
***D*-lysergic acid derivatives**



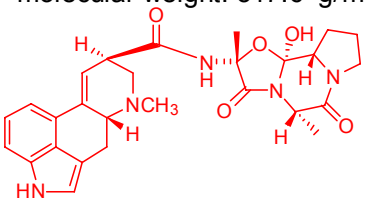
ergovaline, CAS: 2873-38-3
molecular weight: 533.6 g/mol



α–ergoptine, CAS: 29475-05-6
molecular weight: 561.7 g/mol



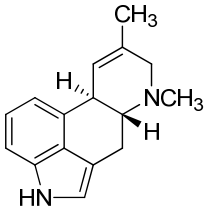
ergonine CAS:29537-61-9
molecular weight: 547.6 g/mol



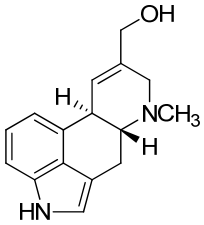
α–ergoalanine (8CI), CAS: 27010-13-5
molecular weight: 505.6 g/mol

(SEMI-SYNTHETIC)

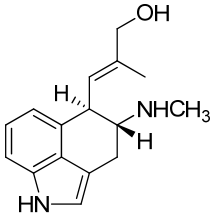
Claviceps fusiformis



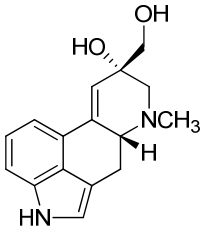
agroclavine, CAS: 548-42-5
molecular weight: 238.3 g/mol



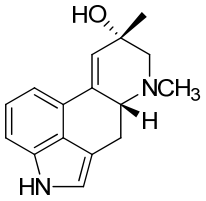
elymoclavine, CAS: 548-43-6
molecular weight: 254.3 g/mol



chanoclavine-I, CAS: 2390-99-0
molecular weight: 256.3 g/mol

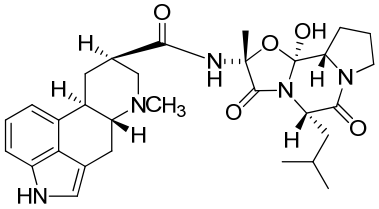


penniclavine, CAS: 519-13-1
molecular weight: 270.3 g/mol

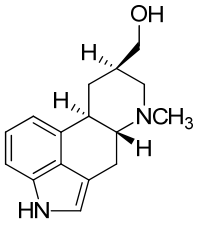


setoclavine, CAS: 519-12-0
molecular weight: 254.3 g/mol

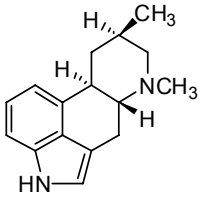
Claviceps africana



dihydroergosine, CAS: 7288-61-1
molecular weight: 549.7 g/mol

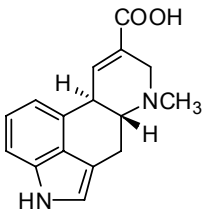


dihydroelymoclavine, CAS: 18051-16-6
molecular weight: 256.3 g/mol

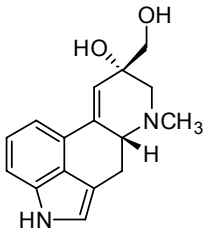


festuclavine, CAS: 569-26-6
molecular weight: 240.3 g/mol

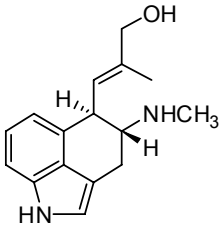
Claviceps paspali



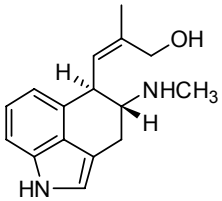
paspalic acid, CAS: 5516-88-1
molecular weight: 268.3 g/mol



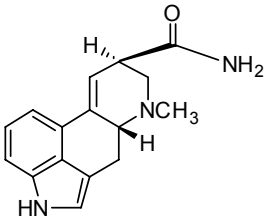
penniclavine, CAS: 519-13-1
molecular weight: 270.3 g/mol



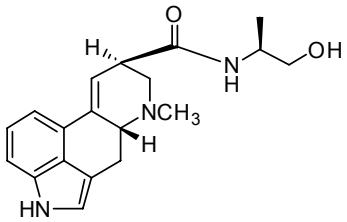
chanoclavine-I, CAS: 2390-99-0
molecular weight: 256.3 g/mol



isochanoclavine-I, CAS: 1150-44-3
molecular weight: 256.3 g/mol



ergine, CAS: 478-94-4
molecular weight: 267.3 g/mol



ergonovine, CAS: 60-79-7
molecular weight: 325.4 g/mol

B. ERGOT ALKALOID CONTENT IN SCLEROTIA

Table B1: Alkaloid content in ergot samples from the 2007 harvest (from Appelt and Ellner, 2009).

	Origin of the sample[a]		Cultivar	Weight of the sclerotia (mg)			Average total alkaloid content[b] (mg/kg)	Main alkaloid(s)	Alkaloids (mg/kg)[c]											
				Min	Max	Average in sample			EM	EMI	ES	EA	EC	EY	ET	ESI	EAI	ECI	EYI	ETI
Rye	Buchholz	ST	Fernando	16	483	99.3	608	Ergocornine	22.2	6.0	35.5	35.8	111.5	45.4	102.1	25.5	26.9	83.0	20.5	93.7
	Geslau	BY	Avanti	30	235	83.7	1 826	Ergocristine	138.7	24.8	180.2	307.9	309.9	117.8	345.1	64.2	115.5	92.5	33.7	96.0
	Neuenstadt a.K.	BW	Picasso	9	280	56.8	1 339	Ergocristine	79.8	17.8	126.6	301.1	78.5	66.7	416.2	35.2	72.1	28.3	20.1	96.7
	Natho	ST	Picasso	19	484	102.9	1 038	Ergocristine, ergotamine	29.7	8.4	9.4	247.1	35.2	92.0	298.8	37.4	100.5	16.5	29.2	101.1
	Pulspforde	ST	Picasso	20	774	90.6	621	Ergotamine	25.0	8.9	59.6	167.0	15.9	19.2	125.3	32.4	85.6	8.9	11.2	62.3
	Berlin	BE	Festus	18	436	88.2	280	Ergotamine	6.2	2.2	28.6	72.6	22.8	11.2	49.9	12.7	33.8	12.2	6.7	21.1
	Taucha	SN	Rasant	14	466	93.8	710	Ergotamine	20.0	4.4	56.7	200.7	49.7	67.3	1131.3	22.3	76.6	17.7	21.7	41.2
	Seyda	ST	Rasant	5	478	73.8	608	Ergotamine	14.6	4.0	30.5	122.5	58.4	67.8	90.1	27.4	64.9	38.0	30.2	59.5
	Flötz	ST	Ascari	11	486	90.3	640	Ergotamine	33.3	7.2	51.1	160.0	19.1	17.5	82.5	36.8	114.4	26.3	21.6	70.6
	Dessau	ST	Ascari	37	548	137.9	591	Ergocristine	24.5	9.1	62.4	84.3	39.0	40.9	130.5	34.7	53.4	23.6	23.2	65.0
	Kuhberge	ST	Rekrut	17	274	64.8	595	Ergocristine, ergotamine	36.0	7.6	66.0	110.6	24.5	36.7	114.3	41.3	58.4	14.8	16.3	68.5
	Horstdorf	ST	Visello	9	436	75.5	296	Ergotamine	10.4	3.2	39.1	54.6	20.0	20.1	51.3	20.9	30.0	11.5	10.0	24.6
	Berkau	ST	Amilo	18	1045	93.8	739	Ergocristine	34.6	19.0	86.3	35.8	47.7	49.0	266.8	61.8	23.4	46.1	42.2	26.7
Triticale	Bad Windsheim	BY	SW Talentro	19	362	86.2	2 076	Ergocristine	184.7	29.2	280.9	406.4	178.9	77.8	439.4	119.5	167.2	48.1	18.6	125.4
	Haar	BY	SW Talentro	10	291	71.5	1 694	Ergocristine, ergotamine	177.2	34.8	276.9	323.0	141.9	103.7	327.0	77.8	82.5	41.9	40.0	67.8
	Haar	BY	Versus Rubin	8	303	48.6	2 214	Ergocristine	159.0	49.6	243.4	256.3	252.3	127.4	615.1	77.7	111.7	105.7	49.7	165.9
	Dippoldiswalde	SN	Benetto	18	255	67.8	572	Ergocristine	30.6	5.5	118.4	67.7	41.5	29.9	97.5	54.2	34.8	28.8	14.0	49.1

(a): German federal states: ST: Saxony-Anhalt, BY: Bavaria, BW: Baden Württenberg, BE: Berlin, SN: Saxony.

(b): Total alkaloid content as sum of the 12 alkaloids analysed.

(c): EM ergometrine, EMI ergometrinine, ES ergosine, EA ergotamine, EC ergocomine, EY α-ergocryptine, ET ergocristine, ESI ergosinine, EAI ergotaminine, ECI ergocorninine, EYI α-ergocriptinine, ETI ergocristinine.

Table B2: Alkaloid content in ergot samples from the 2008 harvest (from Appelt and Ellner, 2009).

	Origin of the sample[a]		Cultivar	Weight of the sclerotia (mg)			Average total alkaloid content[b] (mg/kg)	Main alkaloid(s)	Alkaloids (mg/kg)[c]											
				Min	Max	Average in sample			EM	EMI	ES	EA	EC	EY	ET	ESI	EAI	ECI	EYI	ETI
Rye	Buchholz	ST	Fernando	3	378	55.2	3 336	Ergotamine	28.3	4.5	28.7	51.8	22.9	16.2	26.4	23.9	45.5	35.9	26.5	25.2
	Schleesen	ST	Fernando	23	448	120	549	Ergocristine	58.6	18.7	24.4	73.0	5.2	13.2	97.0	21.9	77.3	8.3	29.2	122.1
	Walsleben	ST	Visello	11	434	84.6	217	Ergotamine	13.3	3.4	14.3	68.0	4.5	5.1	7.4	13.9	57.6	8.8	9.6	10.7
	Naderkau	ST	Visello	43	781	168	245	Ergotamine	14.2	6.4	7.0	77.7	2.5	6.5	10.3	8.7	70.7	8.4	15.7	16.5
	Neuenstadt a.K	BW	Picasso	16	40	97.7	1 574	Ergocristine	168.1	34.3	106.9	154.1	130.5	74.5	418.0	77.4	95.2	96.9	52.4	166.0
	Aken	ST	Picasso	21	1 068	110.2	1 329	Ergotamine/ ergotaminine	109.4	18.6	64.9	328.2	12.7	20.4	147.6	73.9	314.4	25.9	35.4	177.8

(a): German federal states: ST: Saxony-Anhalt, BW: Baden Württenberg.
(b): Total alkaloid content as sum of the 12 alkaloids analysed.
(c): EM ergometrine, EMI ergometrinine, ES ergosine, EA ergotamine, EC ergocomine, EY α-ergocryptine, ET ergocristine, ESI ergosinine, EAI ergotaminine, ECI ergocorninine, EYI α-ergocriptinine, ETI ergocristinine.

C. DISTRIBUTION OF AVAILABLE FOOD SAMPLES

Table C1: Distribution of available food samples (n=1 716) across different food groups at FoodEx Level 1, FoodEx Level 2 and FoodEx Level 3 (after excluding non-qualifying data).

FOODEX LEVEL 1	FOODEX LEVEL 2	FOODEX LEVEL 3	Number of samples
Grains and grain-based products	Grains and grain-based products	Grains and grain-based products	3
	Grains for human consumption	Grains for human consumption	5
		Wheat grain	4
		Barley grain	3
		Rye grain	165
		Spelt grain	1
		Other grains	2
	Grain milling products	Grain milling products	14
		Wheat milling products	201
		Rye milling products	952
		Corn milling products	4
		Oat milling products	2
		Spelt milling products	2
		Other milling products	18
	Bread and rolls	Bread and rolls	119
		Wheat bread and rolls	29
		Rye bread and rolls	30
		Mixed wheat and rye bread and rolls	6
		Multigrain bread and rolls	18
		Unleavened bread, crisp bread and rusk	13
	Pasta (raw)	Pasta, wheat flour, with eggs	3
		Pasta, spelt flour	1
		Pasta, wheat wholemeal, with eggs	4
	Breakfast cereals	Cereal flakes	20
		Popped cereals	1
		Mixed breakfast cereals	2
		Grits	1
	Fine bakery wares	Fine bakery wares	3
		Biscuits (cookies)	40
Milk and dairy products	Milk and dairy products	Milk and dairy products	1
Herbs, spices and condiments	Seasoning extracts	Malt extract	2
Food for infants and small children	Cereal-based food for infants and your children	Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids	30
		Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid	5
		Biscuits, rusks and cookies for children	11
Snacks, desserts and other foods	Snack food	Tortilla chips	1

D. MAIN FOOD GROUPS SELECTED UNDER THE THREE EVALUATED SCENARIOS

Table D1: Main food groups selected under the three evaluated scenarios. Mean occurrence values and 95th percentile (P95) are expressed in µg/kg. Occurrence values are rounded up to the nearest whole number (0 decimal places).

	At least 6 EAs					At least 8 EAs					The 12 EAs				
	N	**Mean LB**	**Mean UB**	**P95[(a)] LB**	**P95[(a)] UB**	**N**	**Mean LB**	**Mean UB**	**P95[(a)] LB**	**P95[(a)] UB**	**N**	**Mean LB**	**Mean UB**	**P95[(a)] LB**	**P95[(a)] UB**
Rye grain	92	101	183	638	638	65	99	178	628	628	8	283	330		
Grains for human consumption (excluding rye grain)[(b)]	**14**	77	43	32	114	14	77	43			3	22	40		
Wheat milling products	191	30	39	141	141	191	30	39	141	141	167	34	41	185	185
Rye milling products	511	124	155	556	575	464	114	142	425	431	239	134	141	535	535
Grain milling products (excluding wheat and rye milling products)[(b)]	**28**	13	34	57	131	27	16	33			14	18	24		
Rye bread and rolls	24	30	45	94	178	24	30	45			21	31	43		
Wheat bread and rolls	29	3	15	16	20	29	3	15			21	4	13		
Multigrain bread and rolls	18	17	23	47	48	18	17	23			17	13	18		
Unleavened bread, crisp bread and rusks	13	3	16	10	20	13	3	16			8	4	13		
Bread and rolls[(b)]	**84**	14	25	63	92	84	14	25	63	92	67	15	24	63	69
Pasta (raw)[(b)]	**8**	0	20	0	20	8	0	20			-	-	-		
Breakfast cereals[(b)]	**22**	0	16	1	20	22	0	16			12	0	12		
Fine bakery wares[(b)]	40	3	15	14	20	40	3	15			30	4	13		
Cereals to be reconstituted with nutritious liquids/water or other protein free liquid	35	7	8	38	38	35	7	8			35	7	8		
Biscuits, rusks and cookies for children	11	19	20	79	79	11	19	20			11	19	20		
Cereal-based food for infants and young children[(b)]	**46**	10	11	61	61	46	10	11			46	10	11		

(a): The 95th percentile was only calculated for rye grain, wheat milling products, rye milling products and bread and rolls due to the low number of samples available for each category. For the rest of food categories the average value of the last quartile was calculated (only the values for the scenario used for exposure calculations are shown).
(b): Food categories at Foodex Level 2 grouping similar foods to cover incomplete consumption data when evaluating exposure assessment.

E. STATISTICS OF ERGOT ALKALOIDS REPORTED IN EACH FOOD GROUP

Table E1: Summary statistics of ergot alkaloids reported in each food group across FoodEx Level 2 and FoodEx Level 3 (in µg/kg). Food samples selected under scenario "at least six EAs" (n=1 049).

FOODEX LEVEL 2	FOODEX LEVEL 3	N	Variable	Mean	Min	Max	P50	P95
Grains and grain-based products	Grains and grain-based products	2	SUM LB	0	0	0		
			SUM UB	22	22	22		
Grains for human consumption	Grains for human consumption	5	SUM LB	19	0	87		
			SUM UB	84	39	168		
	Wheat grain	4	SUM LB	1	0	3		
			SUM UB	20	20	21		
	Barley grain	3	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Rye grain	92	SUM LB	101	0	1 022	0	638
			SUM UB	183	22	1 035	120	638
	Spelt grain	1	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Other grains	1	SUM LB	0	0	0		
			SUM UB	20	20	20		
Grain milling products	Wheat milling products	191	SUM LB	30	0	604	5	141
			SUM UB	39	12	604	15	141
	Rye milling products	511	SUM LB	124	0	5 645	23	556
			SUM UB	155	12	5 645	71	575
	Corn milling products	4	SUM LB	28	0	112		
			SUM UB	53	20	152		
	Oat milling products	2	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Spelt milling products	2	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Other milling products	20	SUM LB	14	0	57	8	
			SUM UB	23	12	62	17	
Bread and rolls	Bread and rolls	3	SUM LB	0	0	0		
			SUM UB	82	20	113		
	Wheat bread and rolls	29	SUM LB	3	0	17	0	
			SUM UB	15	12	20	13	
	Rye bread and rolls	24	SUM LB	30	0	256	8	
			SUM UB	45	12	281	16	
	Mixed wheat and rye bread and rolls	3	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Multigrain bread and rolls	18	SUM LB	17	3	88	9	
			SUM UB	23	13	92	15	
	Unleavened bread, crisp bread and rusk	13	SUM LB	3	0	14	0	
			SUM UB	16	12	20	14	
Pasta (raw)	Pasta, wheat flour, with eggs	3	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Pasta, spelt flour	1	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Pasta, wheat wholemeal, with eggs	4	SUM LB	0	0	0		
			SUM UB	20	20	20		
Breakfast cereals	Cereal flakes	20	SUM LB	8	0	155	0	
			SUM UB	22	12	157	12	
	Popped cereals	1	SUM LB	0	0	0		
			SUM UB	20	20	20		
	Mixed breakfast cereals	2	SUM LB	0	0	0		
			SUM UB	20	20	20		
Fine bakery wares	Biscuits (cookies)	40	SUM LB	3	0	23	0	
			SUM UB	15	12	25	13	
Milk and dairy products	Milk and dairy products	1	SUM LB	0	0	0		
			SUM UB	20	20	20		
Seasoning or extracts	Malt extract	2	SUM LB	0	0	0		
			SUM UB	20	20	20		
Cereal-based food for infants and young children	Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids	30	SUM LB	9	0	39	1	
			SUM UB	9	1	39	2	
	Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid	5	SUM LB	0	0	2		
			SUM UB	2	1	3		
	Biscuits, rusks and cookies for children	11	SUM LB	19	0	79	0	
			SUM UB	20	1	79	1	
Snack food	Tortilla chips	1	SUM LB	0	0	0		

F. CHRONIC AND ACUTE DIETARY EXPOSURE TO ERGOT ALKALOIDS

Table F1: Mean and 95th percentile (P95) chronic dietary exposure to ergot alkaloids (µg/kg b.w. per day) for total population in lower-bound (LB) and upper-bound (UB) scenario.

Code[(a)]	Range of dietary exposure (LB – UB) (µg/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BE/1							0.010-0.066	0.022-0.127	0.009-0.051	0.021-0.111	0.008-0.044	0.016-0.087	0.008-0.041	0.015-0.076
BE/2			0.052-0.173	-[(b)]	0.042-0.146	0.082-0.240								
BG/1	0.017-0.056	0.064-0.188	0.035-0.160	0.060-0.282	0.032-0.158	0.060-0.282								
CY							0.021-0.065	0.050-0.128						
CZ					0.043-0.127	0.095-0.248	0.038-0.104	0.080-0.198	0.028-0.065	0.063-0.127				
DE/1			0.068-0.148	0.165-0.335	0.055-0.134	0.117-0.262								
DE/2			0.069-0.146	0.162-0.301	0.055-0.136	0.116-0.229								
DE/3			0.069-0.140	0.166-0.318	0.058-0.140	0.131-0.238								
DE/4							0.029-0.072	0.075-0.150	0.028-0.061	0.066-0.125	0.034-0.066	0.072-0.127	0.034-0.066	0.079-0.140
DK					0.075-0.170	0.138-0.275	0.033-0.084	0.077-0.162	0.029-0.064	0.058-0.115	0.033-0.065	0.059-0.110	0.033-0.066	-[b]
EL					0.046-0.129	0.095-0.236								
ES/1									0.007-0.041	0.015-0.084				
ES/2							0.009-0.050	0.019-0.098	0.007-0.039	0.014-0.077				
ES/3					0.021-0.110	0.040-0.186	0.014-0.075	0.028-0.138						
ES/4			0.044-0.118	-[(b)]	0.022-0.109	0.050-0.202	0.016-0.079	0.036-0.159						
FI/1			0.075-0.171	0.148-0.323	0.045-0.155	0.094-0.287								
FI/2									0.026-0.052	0.060-0.108	0.026-0.053	0.059-0.108		
FI/3					0.034-0.105	0.073-0.187								
FR					0.017-0.111	0.032-0.192	0.011-0.069	0.023-0.131	0.009-0.047	0.018-0.088	0.008-0.044	0.016-0.087	0.008-0.041	0.015-0.076
HU									0.014-0.052	0.040-0.095	0.015-0.050	0.041-0.092	0.015-0.055	0.039-0.092
IE									0.008-0.043	0.014-0.080				
IT	0.013-0.049	-[(b)]	0.032-0.171	-[(b)]	0.022-0.149	0.051-0.283	0.013-0.089	0.026-0.164	0.009-0.058	0.017-0.102	0.008-0.053	0.015-0.088	0.008-0.057	0.015-0.100
LV					0.042-0.117	0.127-0.300	0.035-0.090	0.101-0.218	0.039-0.078	0.110-0.188				
NL/1									0.011-0.048	0.030-0.089				
NL/2			0.056-0.153	0.125-0.276	0.046-0.132	0.099-0.228								
SE/1									0.022-0.062	0.049-0.119				
SE/2					0.038-0.123	0.079-0.215	0.028-0.083	0.060-0.150						
UK									0.007-0.039	0.014-0.077				

(a): Details on the dietary surveys and the number of subjects are given in Table 4. (b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b).

Table F2: Mean and 95th percentile (P95) acute dietary exposure to ergot alkaloids (µg/kg b.w. per day) for total population in lower-bound (LB) and upper-bound (UB) scenario.

Code[(a)]	Range of dietary exposure (LB – UB) (µg/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
AT									0.021-0.055	0.055-0.141				
BE/1							0.035-0.077	0.084-0.179	0.029-0.060	0.071-0.144	0.028-0.049	0.060-0.115	0.028-0.049	0.058-0.109
BE/2			0.161-0.240	0.355-0.481	0.129-0.200	0.295-0.394								
BG/1	0.043-0.070	0.161-0.243	0.117-0.200	0.229-0.366	0.114-0.196	0.229-0.367								
BG/2							0.064-0.109	0.155-0.287	0.047-0.072	0.110-0156	0.053-0.076	0.100-0.154	0.054-0.078	0.123-0.181
CY							0.033-0.069	0.082-0.166						
CZ					0.088-0.152	0.185-0.297	0.073-0.123	0.163-0.264	0.047-0.075	0.103-0.161				
DE/1			0.140-0.282	0.523-0.971	0.111-0.230	0.373-0.738								
DE/2			0.131-0.263	0.511-0.964	0.104-0.219	0.350-0.709								
DE/3			0.140-0.265	0.567-1.030	0.115-0.241	0.431-0.820								
DE/4							0.054-0.109	0.198-0.375	0.050-0.098	0.175-0.339	0.063-0.120	0.204-0.390	0.063-0.121	0.197-0.383
DK					0.208-0.358	0.430-0.761	0.096-0.160	0.233-0.405	0.081-0.138	0.184-0.322	0.089-0.155	0.176-0.306	0.088-0.153	0.149-0.264
EL					0.047-0.129	0.115-0.290								
ES/1									0.026-0.049	0.062-0.112				
ES/2							0.033-0.060	0.082-0.148	0.026-0.047	0.062-0.105				
ES/3					0.062-0.126	0.121-0.238	0.044-0.086	0.093-0.173						
ES/4			0.075-0.132	-[(b)]	0.059-0.123	0.133-0.250	0.048-0.092	0.119-0.211						

Table F2: Continued.

Code[a]	Range of dietary exposure (LB – UB) (µg/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
EE									0.103-0.192	0.282-0.527				
FI/1			0.211-0.415	0.499-0.947	0.137-0.257	0.352-0.652								
FI/2									0.125-0.229	0.302-0.563	0.126-0.232	0.302-0.565		
FI/3					0.103-0.262	0.336-0.715								
FR					0.049-0.126	0.133-0.278	0.036-0.080	0.097-0.190	0.031-0.057	0.080-0.131	0.032-0.054	0.073-0.122	0.031-0.051	0.072-0.113
HU									0.051-0.075	0.110-0.160	0.048-0.073	0.109-0.152	0.052-0.079	0.102-0.140
IE									0.034-0.054	0.074-0.112				
IT	0.049-0.083	-[b]	0.130-0.245	0.374-0.546	0.087-0.188	0.248-0.380	0.055-0.111	0.136-0.223	0.039-0.074	0.093-0.149	0.034-0.065	0.078-0.125	0.037-0.069	0.080-0.136
LV					0.109-0.226	0.395-0.768	0.100-0.192	0.350-0.669	0.112-0.213	0.384-0.734				
NL/1									0.035-0.058	0.073-0.115				
NL/2			0.096-0.177	0.210-0.345	0.082-0.152	0.177-0.295								
PO			0.080-0.165	0.241-0.371	0.104-0.188	0.237-0.402	0.091-0.152	0.184-0.318	0.066-0.107	0.155-0.261	0.053-0.091	0.130-0.241	0.053-0.092	0.136-0.257
SE/1									0.053-0.114	0.164-0.334				
SE/2					0.058-0.148	0.176-0.375	0.042-0.099	0.120-0.252						
SK									0.060-0.115	0.228-0.429				
SL									0.034-0.070	0.082-0.170				
UK									0.027-0.051	0.066-0.114				

(a): Details on the dietary surveys and the number of subjects are given in Table 4.
(b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b).

G. BMDL CALCULATION - ERGOTAMINE (SPEIJERS ET AL., 1993)

Increased incidence of tail muscular atrophy was selected as the key effect. Ergotamine tartrate was administered (7 days/week for 13 weeks) at nominal concentrations of 0, 5, 20 and 80 mg/kg in the diet. The administered doses expressed as ergotamine were derived as 0, 0.41, 1.7 and 6.5 mg/kg b.w. per day, and 0, 0.36, 1.4 and 5.4 mg/kg b.w. per day for females and males, respectively.

BMDL was calculated by means of the software BMDS v2.1.2 (US EPA). All models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10 % (95 % confidence level) advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009). The models allowing for restrictions (e.g. Log Logistic or Multistage) were run both with and without the selected default restrictions.

G1. Incidence data

Males	Dose groups (mg/kg b.w. per day)	control	0.36	1.4	5.4
	Tested animals	10	10	10	10
	Incidence of muscular atrophy in tail	1	1	1	7
Females	Dose groups (mg/kg b.w. per day)	control	0.41	1.7	6.5
	Tested animals	10	10	10	10
	Incidence of muscular atrophy in tail	0	0	2	7

G2. BMR: 0.1 (extra risk)

G3. Model acceptability criteria: indicated in EFSA, 2009.

G4. Table of BMDL results:

Females

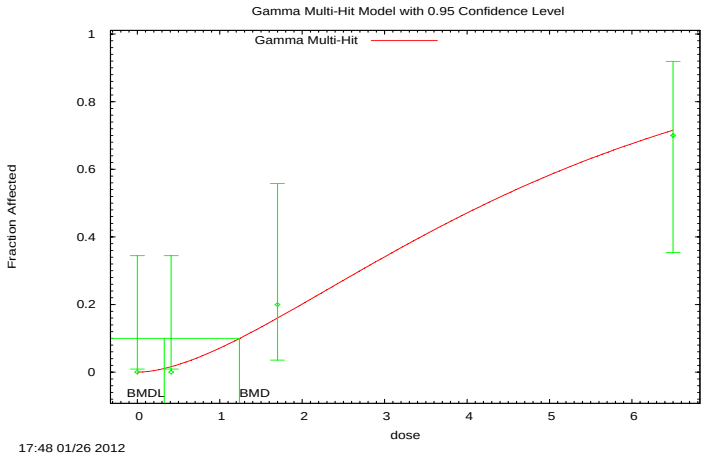
Models	Restriction	N. parameters	Log-likelihood	P-value	Accepted	BMD_{10} (mg/Kg b.w. per day)	$BMDL_{10}$ (mg/Kg b.w. per day)
Null model	–	1	-21.3266	–	–	–	–
Gamma	Power ≥1	2	-11.3374	0.7987	**Yes**	1.2381	0.4469
Weibull	Power ≥1	2	-11.3889	0.7586	**Yes**	1.2102	0.4414
LogLogistic	Slope ≥1	2	-11.2913	0.8364	**Yes**	1.2221	0.3959
LogProbit	Slope ≥1	2	-11.1953	0.9206	**Yes**	1.2240	0.6915
Multistage	Betas ≥1	2	-11.4923	0.6841	**Yes**	1.1977	0.4314
Logistic	–	2	-12.3906	0.2786	**Yes**	2.1885	1.3456
Multistage-Cancer	–	2	-11.4923	0.6841	**Yes**	1.1977	0.4314
Probit	–	2	-12.2098	0.3338	**Yes**	2.0006	1.2653
Quantal-Linear	–	1	-11.8714	0.6783	**Yes**	0.67667	0.4004
Gamma	No restr.	2	-11.3374	0.7978	**Yes**	1.2381	0.3273
Weibull	No restr.	2	-11.3889	0.7733	**Yes**	1.2102	0.4415
LogLogistic	No restr.	2	-11.2913	0.8364	**Yes**	1.2221	0.3954
LogProbit	No restr.	2	-11.1953	0.9206	**Yes**	1.2240	0.4390
Multistage	No restr.	2	-11.4923	0.6841	**Yes**	1.1977	0.3754
Full model	–	4	-11.1127	–	–	–	–

Comments on results

Female rats

All dichotomous models satisfied the log-likelihood and the goodness-of-fit acceptability criteria when compared to the null model and the full model.

The calculated $BMDL_{10}$ were in the range in the range 0.33 – 1.3 mg/kg b.w. per day. The lowest $BMDL_{10}$ of 0.33 mg/kg b.w. per day was calculated by the unrestricted Gamma model (plot shown below).



H. BMDL CALCULATIONS - ERGOMETRINE (PETERS-VOLLEBERG ET AL., 2000)

Decreased thyroxine (T4) levels in serum was selected as the key effect for modelling. Ergometrine maleate was administered (7 days/week for 4 weeks) at nominal concentrations of 0, 2, 10, 50 or 250 mg/kg in the diet. The administered doses expressed as ergometrine were derived as 0, 0.1, 0.7, 3.3 and 16.7 mg/kg b.w. per day.

BMDL was calculated by means of the software BMDS v2.1.2 (US EPA). Two continuous models, Hill and the Exponential family, as indicated by the EFSA guidance on the use of benchmark dose (EFSA, 2009) were applied. BMDLs were calculated for different BMRs (from 5 to 20 %, 95 % confidence level).

H1. Incidence data

Males	Dose groups (mg/kg b.w. per day)	control	0.1	0.6	2.9	15
	Tested animals	6	6	6	5	5
	T4 in serum (nM)	70 ± 10	62 ± 5	67 ± 8	57 ± 12	53 ± 7
Females	Dose groups (mg/kg b.w.per day)	control	0.1	0.7	3.0	15
	Tested animals	6	6	6	6	6
	T4 in serum (nM)	47 ± 6	50 ± 7	43 ± 6	49 ± 10	36 ± 10

H2. BMR: 0.05, 0.1 and 0.2 (Relative Deviation)

H3. Model acceptability criteria: indicated in EFSA, 2009.

H4. Table of BMDL results:

Males

Model		Restriction	N. parameters	Log-likelihood	Accepted	BMD (mg/kg per day)		BMDL (mg/kg per day)
Hill		Power >1	6	-78.770225	Yes*	**BMR**		
Null model (R)		Rho = 0	2	-84.466467		0.05	2.4	0.12
Full models	A1		6	-77.304917		0.1	2.7	0.35
	A2		10	-74.798865				
	A3		6	-77.303180				
Exponential, M2 (BMR 5 %)		Power >1 Rho = 0	3	-80.23206	Yes **	**BMR**		
Null model (R)			2	-84.46647		0.05	3.4	2.1
Full models	A1		6	-77.30492		0.1	6.9	4.3
	A2		10	-74.79886		0.2	15	9.1
	A3		6	-77.30492				

* full model A3 does not result in a significantly better fit. Tests of A1 versus A2 and A2 versus A3 indicate that homogeneous variance (Rho = 0) is appropriate.

** full model A3 does not result in a significantly better fit. M2 resulted the optimal model (M3 and M4 did not result in a significantly better fit than M2, data not shown).

Females

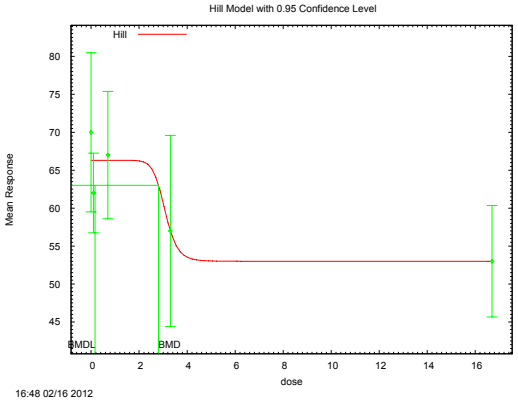
None of the models satisfied the acceptability criteria (data not shown).

Comments on results

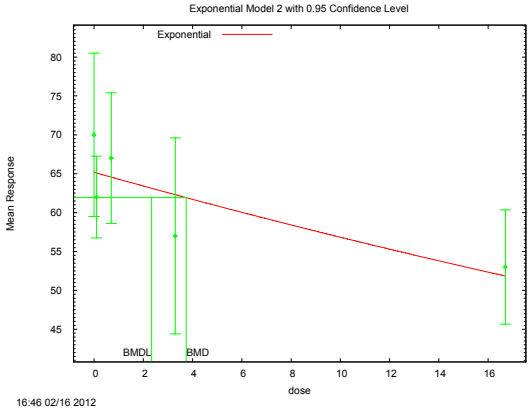
Male rats

The two continuous models satisfied the log-likelihood and the goodness-of-fit acceptability criteria when compared to the null model and the full model. BMDLs calculated at different BMR levels (from 5 % to 10 %) by the Hill Model ranged from 0.16 to 0.48 mg/kg b.w. per day (no BMDL could be calculated for higher BMR). BMDLs calculated at different BMR levels (from 5 % to 20 %) by the Exponential Model ranged from 2.1 to 9.1 mg/kg b.w. per day (no BMDL could be calculated for BMR 30 %). 2 plots of the Hill (a) and Exponential M2 (b) model fitting curves (BMR 5 %) are shown below. The CONTAM Panel noted that a large difference of about one order of magnitude exists between the BMD and BMDL values calculated by the Hill model. For this reason the results obtained by means of the Exponential model were considered more reliable.

(a)



(b)



I. BMDL CALCULATIONS - ERGOCRYPTINE (JANSSEN ET AL., 2000A, B)

The incidence of tail muscular degeneration was selected as the key effect for modelling. Ergocryptine was administered daily through the diet for 28-32 days at nominal concentrations of 0, 4, 20, 100 or and mg/kg, equivalent to 0, 0.34, 1.4, 6.6 and 44 mg/kg b.w. per day and 0, 0.36, 1.7, 8.9 and 60 mg/kg b.w. per day in males and females, respectively.

BMDL was calculated by means of the software BMDS v2.1.2 (US EPA). For the tail muscular degeneration all models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10 % (95 % confidence level) advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009). The models allowing for restrictions (e.g. Log Logistic or Multistage) were run both with and without the selected default restrictions.

Modelling of the incidence of tail muscular degeneration

I1. Incidence data

Males	Dose groups (mg/kg b.w.per day)	control	0.34	1.4	6.6	44
	Tested animals	6	6	6	6	6
	Incidence of muscular atrophy in tail	0	0	0	2	6
Females	Dose groups (mg/kg b.w. per day)	control	0.36	1.7	8.9	60
	Tested animals	6	6	6	6	6
	Incidence of muscular atrophy in tail	0	0	0	0	4

I2. BMR: 0.1 (extra risk)

I3. Model acceptability criteria: indicated in EFSA, 2009.

I4. Table of BMDL results:

Males

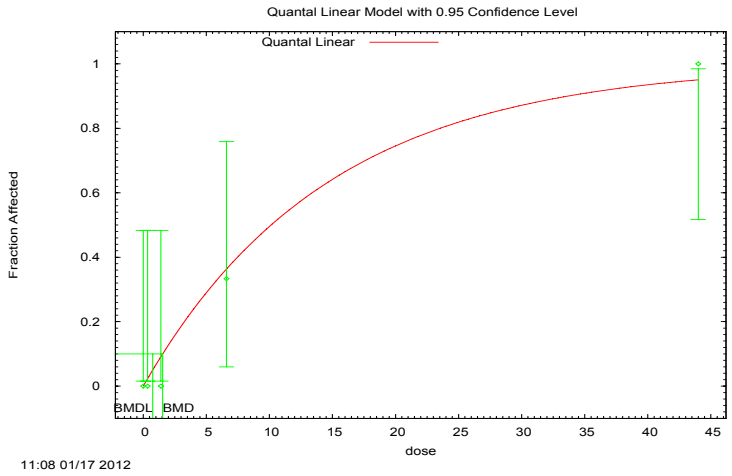
Models	Restriction	N. parameters	Log-likelihood	P-value	Accepted	BMD_{10} (mg/Kg b.w. per day)	$BMDL_{10}$ (mg/Kg b.w. per day)
Null model	–	1	-17.3975	–	–	–	–
Gamma	Power ≥1	2	-3.81909	1	**Yes**	5.3123	1.0768
Weibull	Power ≥1	2	-3.81917	1	**Yes**	5.3883	1.0768
LogLogistic	Slope ≥1	2	-3.81909	1	**Yes**	5.8001	1.4838
LogProbit	Slope ≥1	2	-3.81909	1	**Yes**	5.1297	1.5312
Multistage	Betas ≥1	1	-3.93173	0.9941	**Yes**	3.4618	1.0149
Logistic	–	2	-3.81909	1	**Yes**	6.1618	2.7519
Multistage-Cancer	–	2	-3.93173	0.9941	**Yes**	3.4618	1.0149
Probit	–	2	-3.81909	1	**Yes**	5.7670	2.4786
Quantal-Linear	–	1	-4.84838	0.725	**Yes**	1.5406	0.7488
Gamma	No restr.	2	-3.81909	1	**Yes**	5.3151	1.0594
Weibull	No restr.	2	-3.81918	1	**Yes**	5.3727	1.0691
LogLogistic	No restr.	2	-3.81909	1	**Yes**	5.8001	1.4838
LogProbit	No restr.	2	-3.81909	1	**Yes**	5.1462	1.4165
Multistage	No restr.	2	-3.93173	0.9941	**Yes**	3.4618	1.0149
Full model	–	4	-3.81909	–	–	–	–

Comments on results

Male rats

All dichotomous models satisfied the log-likelihood and the goodness-of-fit acceptability criteria when compared to the null model and the full model.

The calculated $BMDL_{10}$ were in the range in the range 0.75 – 2.75 mg/kg b.w. per day. The lowest $BMDL_{10}$ of 0.75 mg/kg b.w. per day was calculated by the Quantal-linear model (plot shown below).



Female rats

None of the models satisfied the acceptability criteria (data not shown).

J. BMDL CALCULATIONS - ERGOTAMINE (GRAUWILER AND SCHÖN, 1973)

The modelling was performed to assess the effects of ergotamine on fetal and maternal toxicity. The key effect for the maternal toxicity was the maternal b.w. gain (from the start of the exposure to the final day of the study), whereas the following parameters on fetal viability were modelled to account for the fetal toxicity: post implantation loss (versus number of implantations), the total prenatal mortality (versus number of corpora lutea) and the viable fetuses (versus number of implantations). Ergotamine was administered by gavage to pregnant rats from day 6 to day 15 of gestation at doses of 0, 1, 3, 10, 30 and 100 mg/kg b.w. per day.

BMDL were calculated by means of the software BMDS v2.1.2 (US EPA). For the fetal toxicity, all models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10 % (95 % confidence level) advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009). The models allowing for restrictions (e.g. Log Logistic or Multistage) were run both with and without the selected default restrictions. The CONTAM Panel acknowledged that the BMD analysis carried out using dichotomous models has limited validity, as it does not take into account litter specific data. However, due to the absence of litter specific data in the original publication, it was not possible to apply the nested dichotomous models.

The modelling of the maternal b.w. gain was performed by considering two continuous models, Hill and the Exponential family, as indicated by EFSA (2009). A default BMR of 5 % is indicated by EFSA (2009) for continuous data. However, in view of the low toxicological relevance of a 5 % decrease in b.w. gain and of the large standard deviations of the calculated average b.w. gain in the control and treated groups, a BMR of 10 % (95 % confidence level) was considered appropriate.

Modelling of the maternal b.w. gain

J1. Incidence data

	Dose groups (mg/kg b.w. per day)	control	1	3	10	30	100
Females	Tested animals	28	23	26	23	21	12
	b.w. at start of treatment (g)	246 ± 18	246 ± 20	237 ± 14	237 ± 18	252 ± 11	252 ± 15
	b.w. at sacrifice (g)	342 ± 37	335 ± 37	326 ± 22	292 ± 38	310 ± 32	262 ± 16
	Calculated b.w. gain (g)	96 ± 41	89 ± 36	89 ± 26	55 ± 42	58 ± 34	10 ± 22

J2. BMR: 0.1 (Relative Deviation)

J3. Model acceptability criteria: indicated in EFSA, 2009.

J4. Table of BMDL results:

Model		Restriction	N. parameters	Log-likelihood	Accepted	BMD (mg/kg per day)	BMDL (mg/kg per day)
Hill		Power >1 Rho ≠ 0	5	-539.4660	**Yes***	4.8	2.5
Null model (R)			2	-566.5939			
Full models	A1		7	-537.4219			
	A2		12	-531.7014			
	A3		8	-535.8292			
Exponential M2		Power >1 Rho ≠ 0	4	-539.7343	**Yes****	5.1	3.7
Null model (R)			2	-566.5939			
Full models	A1		7	-537.4219			
	A2		12	-531.7014			
	A3		8	-535.8292			

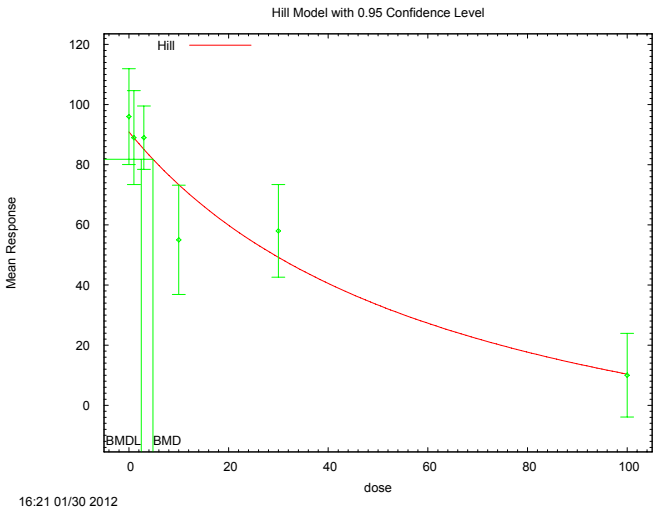
* full model A3 does not result in a significantly better fit. Tests of A1 versus A2 and A2 versus A3 indicate that non homogeneous variance (Rho ≠ 0) is appropriate.

** full model A3 does not result in a significantly better fit. M2 resulted the optimal model (M3 and M4 did not result in a significantly better fit than M2)

Comments on results

The two continuous models satisfied the log-likelihood and the goodness-of-fit acceptability criteria when compared to the null model and the full model.

The calculated $BMDL_{10}$ were in the range in the range 2.5 - 3.7 mg/kg b.w.per day. The lowest $BMDL_{10}$ of 2.5 mg/kg b.w.per day was calculated by the Hill model (plot shown below).



Modelling of the fetal toxicity parameters

J5. Incidence data

Dose groups (mg/kg b.w. per day)	control	1	3	10	30	100
Number of corporea lutea	451	359	348	332	314	168
Number of implantations	382	293	290	263	259	148
Viable fetuses	363	278	268	166	130	24
Pre implantation loss[1] (%)	15.3	18.4	16.7	20.8	17.5	11.9
Post implantation loss[2] (%)	5.0	5.1	7.6	36.9	49.8	83.8
Total prenatal mortality[1] (%)	19.5	22.6	23.0	50.0	58.6	85.7

(1) % of corpora lutea.
(2) [2]% of implantation sites.

J6. BMR: 0.1 (extra risk)

J7. Model acceptability criteria: indicated in EFSA, 2009.

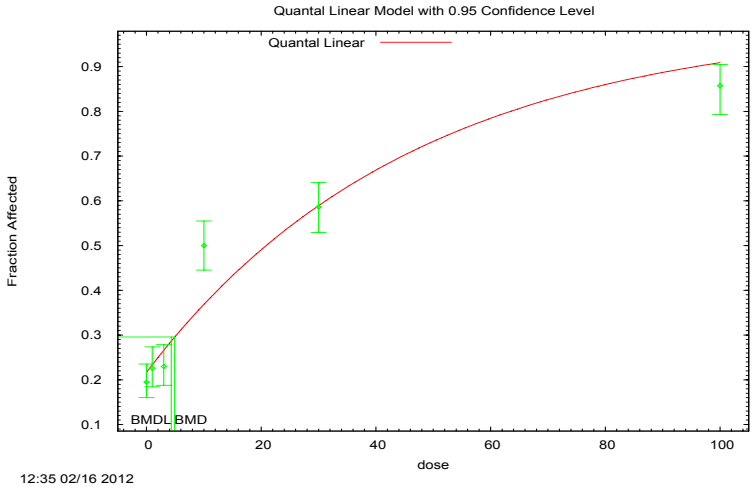
J8. Table of BMDL results:

(example for modelling of total fetal mortality versus number of corpora lutea)

Models	Restriction	N. parameters	Log-likelihood	P-value	Accepted	BMD_{10} (mg/Kg b.w. per day)	$BMDL_{10}$ (mg/Kg b.w. per day)
Null model	–	1	-1306.43	–	–	–	–
Gamma	Power ≥1	2	-1130.25	<0.001	**No**	–	–
Weibull	Power ≥1	2	-1130.25	<0.001	**No**	–	–
LogLogistic	Slope ≥1	2	-1121.88	<0.001	**No**	–	–
LogProbit	Slope ≥1	2	-1137.8	<0.001	**No**	–	–
Multistage	Betas ≥1	1	-1130.5	<0.001	**No**	–	–
Logistic	–	2	-1151.21	<0.001	**No**	–	–
Multistage-Cancer	–	2	-1130.5	<0.001	**No**	–	–
Probit	–	2	-1153.06	<0.001	**No**	–	–
Quantal-Linear	–	1	-1130.25	<0.001	**No**	–	–
Gamma	No restr.	3	-1123.48	<0.001	**No**	–	–
Weibull	No restr.	3	-1122.74	<0.001	**No**	–	–
LogLogistic	No restr.	3	-1121.86	<0.001	**No**	–	–
LogProbit	No restr.	3	-1121.18	<0.001	**No**	–	–
Multistage	No restr.	3	-1123.34	<0.001	**No**	–	–
Full model	–	6	-1114.08	<0.001	–	–	–

Comments on results

For all the fetal toxicity effects, the fitting of the dichotomous models (with and without restrictions) resulted as statistically signficantly different from the full model and thus no acceptable BMD/BMDL could be calculated (see example of fitting results given above and example of plot given below – Quantal-Linear model fitting for total fetal mortality versus number of corpora lutea). This result is likely attributable to the application of dichotomous models instead of the nested dichotomous models using litter specific data. However, as an indication, it may be worthwhile noting that in most of the cases the $BMDL_{10}$ calculated for the fetal toxicity parameters ranged between 2 and 6 mg/kg b.w. per day.



ABBREVIATIONS

17-KS	17-ketosteroids
17-OHCS	17-hydroxycortiosteroids
γ-GT	γ-glutamyltransferase
AA	Amino acids
AESAN	Spain Dietary Survey, code ES/1
AESAN-FIAB	Spain Dietary Survey, code ES/2
AFSSA	French Food Safety Agency
ALT	Alanine aminotransferase
ARfD	Acute reference dose
ASNS	Austria Dietary Survey
AT	Austria
BE	Berlin (German federal state)/ Belgium
BG	Bulgaria
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limits
BMR	Benchmark response
b.w.	Body weight
BW	Baden Wurttenberg (German federal state)
BY	Bavaria (German federal state)
CAS	Chemical Abstracts Service
Childhealth	Cyprus Dietary Survey, code CY
CID	Collision-induced dissociation
CNS	Central nervous system
CONTAM Panel	EFSA's Scientific Panel on Contaminants in the Food Chain
CRP_2008	Slovenia Dietary Survey, code SI
CYP3A4	Cytochrome P450 3A4 isozyme
CY	Cyprus
CZ	Czech Republic
Danish Dietary Survey	Denmark Dietary Survey, code DK
DCM	EFSA's Dietary and Chemical Monitoring Unit
DE	Germany
Diet National 2004	Belgium Dietary Survey, code BE/1
DIPP	Finland Dietary Survey, code FI/1
DK	Denmark
DM	Dry matter
DNFCS 2003	The Netherlands Dietary Survey, code NL/1
DONALD 2006	Germany Dietary Survey, code DE/1
DONALD 2007	Germany Dietary Survey, code DE/2
DONALD 2008	Germany Dietary Survey, code DE/3
EA	Ergot alkaloid / Ergotamine
EAI	Ergotaminine
EC	Ergocornine
ECI	Ergocorninine
EE	Estonia
EFSA	European Food Safety Authority
EFSA_TEST	Latvia Dietary Survey, code LV
EL	Greece
ELISAs	Enzyme linked immunosorbent assays
EM	Ergometrine
EMAN	European Mycotoxin Awareness Network
EMEA	European Agency for the Evaluation of Medicinal Products
EMI	Ergometrinine

enKid	Spain Dietary Survey, code ES/4
ES	Ergosine/ Spain
ESI	Electrospray ionization/Ergosinine
ET	Ergocristine
ETI	Ergocristinine
EU	European Union
EXPOCHI	Article 36 project 'Individual food consumption data and exposure assessment studies for children'
EY	α-ergocryptine
EYI	α-ergocriptinine
FDA	US Food and Drug Administration
FI	Finland
FINDIET 2007	Finland Dietary Survey, code FI/2
FLD	Fluorescence detection
FR	France
GC	Gas chromatrography
GC-MS	Gas chromatography-mass spectrometry
GDs	Gestational days
GLDH	Glutamate dehydrogenase
GLP	Good Laboratory Practice
HBGV	Health-based guidance value
HCCSCA	Hungarian Case–Control Surveillance of Congenital Abnormalities
HPLC	High performance liquid chromatography
HU	Hungary
IE	Ireland
INCA2	France Dietary Survey, code FR
INRAN-SCAI 2005-06	Italy Dietary Survey, code IT
IR	Infrared
IT	Italy
i.v.	intravenous
IZZ_FAO_2000	Poland Dietary Survey, code PO
LB	Lower bound
LFGB	German Food and Feed Law
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOQ	Limit of quantification
LSD	Lysergic acid diethylamide
LV	Latvia
MLs	Maximum levels
MRL	Maximum Residue Limits
MRM	Multi reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
National Nutrition Survey	Germany Dietary Survey, code DE/4
National Repr Surv	Hungary Dietary Survey, code HU
NDNS	The United Kingdom Dietary Survey, code UK
NDS 1997	Estonia Dietary Survey, code EE
NFAn	Sweden Dietary Survey, code SE/2
NIE	Near infrared spectrometry
NL	The Netherlands
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NP-HPLC	Normal phase high performance liquid chromatography
NRI	Near infrared spectroscopy

NSFC	Ireland Dietary Survey, code IE
NUT INK05	Spain Dietary Survey, code ES/3
NUTRICHILD	Bulgaria Dietary Survey, code BG/1
NSFIN	Bulgaria Dietary Survey, code BG/2
p	Probability
PBCEC	Porcine brain capillary endothelial cells
PO	Polonia
PSA	Primary and secondary amine
Regional Crete	Greece Dietary Survey, code EL
Regional Flanders	Belgium Dietary Survey, code BE/2
RIAs	Radioimmunoassays
RIKSMATEN 1997-98	Sweden Dietary Survey, code SE/1
RP	Reversed phase
s.c.	Subcutaneous
SCE	Sister chromatid exchange
SD	Standard deviation
SE	Sweden
SI	Slovenia
SISP04	Czech Repulic Dietary Survey, code CZ
SK	Slovakia
SK_MON_2008	Slovakia Dietary Survey, code SK
SN	Saxony (German federal state)
SPE	Solid phase extraction
ST	Saxony-Anhalt (German federal state)
STRIP	Finland Dietary Survey, code FI/3
T4	Thyroxine
TDI	Tolerable daily intake
TLC	Thin layer chromatography
UB	Upper bound
UHPLC-MS/MS	Ultra high performance liquid chromatography – tandem mass spectrometry
UK	The United Kingdom
UV	Ultraviolet
VCP kids	The Netherlands Dietary Survey, code NL/