European Food Safety Authority

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SCIENTIFIC OPINION

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

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the risks to human and animal health related to the presence of tropane alkaloids (TAs) in food and feed. TAs are secondary metabolites which occur in several plant families. Although more than 200 different TAs have been identified in various plants, respective data on toxicity and occurrence in food and feed are limited. Therefore, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) could only perform a risk assessment on (-)-hyoscyamine and (-)-scopolamine, the two TAs for which occurrence and toxicity data were available. Since the pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration, the CONTAM Panel concluded that it was appropriate to establish an Acute Reference Dose (ARfD) for these substances. Based on the results for decreased heart rate in a human volunteer study, the CONTAM Panel established a group ARfD of 0.016 µg/kg body weight (b.w.) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. Results on TAs in 124 food and 611 feed samples were collected in two Member States. Most of the food and feed samples were left-censored (below limit of detection/limit of quantification). A reliable exposure estimate was only possible for one food and one age class (toddlers). Based on the limited information, the CONTAM Panel concluded that the dietary exposure of toddlers could be up to seven times the group ARfD and could exceed the group ARfD on approximately 11 to 18 % of consumption days. TA toxicosis in livestock and companion animals is relatively rare.

KEY WORDS

tropane alkaloids (TAs), origin, chemistry, analysis, dietary exposure, risk assessment, health based guidance value

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the risks to human and animal health related to the presence of tropane alkaloids (TA) in food and feed.

TAs are secondary metabolites which naturally occur in plants of several families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae (including coca). The TAs are found in all parts of the plants and are responsible for the toxic effects of some of these plants. TAs contain an azabicyclo[3.2.1]octane ring structure. The common structural element is the tropane skeleton, (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octane. The group of TAs comprises more than 200 compounds and the wide range of compounds occurring especially in the Solanaceae family arises from the esterification of tropine with a variety of acids, such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid, and atropic acid.

Although more than 200 different TAs were so far identified in various plants, respective data on their occurrence in food and feed and on toxicity are limited. The most studied TAs are (-)-hyoscyamine and (-)-scopolamine, which in contrast to the (+)-enantiomers are formed naturally. The racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine is called atropine. Cocaine is also a prominent member of the group of TAs. However, since almost no data are available concerning the occurrence of cocaine in food and feed, it was not further considered in this opinion. Besides data on toxicity, some information on occurrence in feed and food were only available for (-)-hyoscyamine and (-)-scopolamine. Therefore, the CONTAM Panel could only perform a risk assessment on these compounds.

Plant extracts containing TAs have been used for centuries in human medicine and are still used, as are atropine, (-)-hyoscyamine and (-)-scopolamine. These uses include for example the treatment of wounds, gout and sleeplessness, and pre-anaesthesia. Furthermore, extracts from deadly nightshade (*Atropa belladonna*) were used to dilate pupils for cosmetic reasons and to facilitate ophthalmological examination. The genus *Datura* is long known for its content of TAs. In India, the root and leaves of *Datura stramonium*, commonly called thorn apple or Jimson weed, were burned and the smoke inhaled to treat asthma. This plant is widely distributed in temperate and tropical regions of the world. For this reason, seeds of this plant have been found as impurities in important agricultural crops such as linseed, soybean, millet, sunflower and buckwheat and products thereof. The consumption of a few berries from henbane (*Hyoscyamus niger*) or from *Atropa belladonna* has caused severe intoxication, including deaths in young children.

Atropine/(-)-hyoscyamine and (-)-scopolamine are readily absorbed from the gastrointestinal tract, quickly and extensively distributed into tissues, and excreted predominantly in the urine. *N*-demethylation and Phase II conjugation of atropine, (-)-hyoscyamine and (-)-scopolamine are known metabolic pathways in humans. The toxicological effects of (-)-hyoscyamine and (-)-scopolamine relate to their pharmacological effects, which are mediated by inhibition of muscarinic acetylcholine receptors in the central nervous system (CNS) and the autonomic nervous system (ANS). Inhibition of these receptors in the ANS results in decreased secretions from the salivary, bronchial and sweat glands, dilation of the pupils, paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion. (-)-Hyoscyamine and (-)-scopolamine differ in their ability to affect the CNS, with (-)-scopolamine having more prominent depressing central effects at therapeutic doses. The pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration, and therefore the CONTAM Panel concluded that it was appropriate to establish an Acute Reference Dose (ARfD) for these substances. Since they are not bioaccumulative, or genotoxic and do not exhibit chronic toxicity, the ARfD would also protect against effects of long-term exposure. Due to the common mode of action through receptor interaction, the CONTAM Panel considered it appropriate to establish a group ARfD for (-)-hyoscyamine and (-)-scopolamine assuming equivalent potency.

From the results of a study in which human volunteers were given a relevant mixture of (-)-hyoscyamine and (-)-scopolamine in food, the CONTAM Panel identified a no–observed-adverse-effect level (NOAEL) of 0.16 µg/kg body weight (b.w.), expressed as the sum of (-)-hyoscyamine and (-)-scopolamine as the basis for establishing a group ARfD. The Panel noted that the next higher dose in the human volunteer study of 0.48 µg/kg b.w. resulted in a transient statistically significant lowering of the heart rate, which is not adverse in healthy individuals but could be in more susceptible individuals, such as those with bradycardia. The Panel decided to apply an uncertainty factor of 10 for inter-individual differences to allow for the fact that this was a small study in young healthy male volunteers. The Panel divided the NOAEL of 0.16 µg/kg b.w. by the uncertainty factor of 10 and established a group ARfD of 0.016 µg/kg b.w. expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. The group ARfD is approximately two orders of magnitude lower than the lowest single doses of (-)-hyoscyamine and (-)-scopolamine used therapeutically.

Currently, only methods with mass spectrometric (MS) detection allow analysis of TAs at trace levels in food and feed. Basically, two MS based approaches are applied, either in combination with gas chromatography (GC) or predominantly with high performance liquid chromatography (HPLC).

Following a continuous call by EFSA in July 2010 for data in food and feed on a list of chemical contaminants, including plant toxins such as TAs, analytical data on TAs in 124 food samples and 611 feed samples were available in the EFSA database by the end of February 2013. The samples were collected in two Member States (the Netherlands and Germany) and all analysed and reported by the Netherlands. The data refer to atropine and (-)-scopolamine. When atropine was reported, the CONTAM Panel used these data as (-)-hyoscyamine. As the biosynthesis of TAs leads to (-)-hyoscyamine and (-)-scopolamine, any analytical results where no stereoselective separation is achieved are thus regarded in this opinion as 100 % (-)-hyoscyamine or (-)-scopolamine. Most of the food samples (83 %) were left-censored (below limit of detection/limit of quantification). Almost all food data with quantified TA concentrations belonged to the food category for infants and young children "Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids". The ingredients in these samples included wheat, maize, rye, oats and rice, indicating the possibility of contamination of different cereals. Risk characterisation was only possible for the toddlers' age class because a reliable exposure assessment was not possible for other age classes.

For comparison with the ARfD, it is necessary to consider estimates of acute exposure. The estimates of dietary exposure were based on the two available dietary surveys for toddlers reporting consumption of the selected food group, which are from Germany and Finland, and not necessarily representative of all European countries. Although the data represent only the food group for infants and young children 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids', these are the foods for toddlers most likely to contain TAs, and taking into account that other food samples did not contain detectable concentrations, the total exposure from all food sources is unlikely to be much higher.

The CONTAM Panel performed estimates of acute dietary exposure using both a deterministic and a probabilistic approach. Based on the limited available information, the dietary exposure of toddler consumers could be up to seven times the group ARfD (deterministic approach), and exceeded the ARfD in approximately 11 to 18 % of the consumption days (probabilistic approach).

Most feed data (91 %) were left-censored. More than half of the quantified samples were reported for compound feed. The highest levels of TAs were reported in samples of millet grains. Plants containing TAs are generally unpalatable, and will be avoided by most livestock unless other feed is unavailable. Therefore, animal exposure to the sum of (-)-hyoscyamine and (-)-scopolamine is primarily from consuming feed contaminated with TA-containing plant material. Except for rabbits with an estimated upper bound (UB) exposure of 2.5 µg/kg b.w., the estimated UB exposures for lactating and fattening ruminants, piglets, fattening pigs, sows, poultry, cats, dogs, horses and fish were all below 0.35 µg/kg b.w.

TA toxicosis in livestock and companion animals is relatively rare because TA-containing plant products appear to be unpalatable and animals try to avoid them where possible. Furthermore, compared to other livestock, poultry, rabbits and certain breeds of small ruminants are considerably less sensitive to TAs due to the expression of specific hydrolysing enzymes that inactivate the alkaloids. A NOAEL has been proposed for ruminants and a lowest-observed-adverse-effect level (LOAEL) for pigs, but these are significantly higher than estimated exposure.

The European Agency for the Evaluation of Medicinal Products (EMEA; now the European Medicines Agency (EMA)) and the European Food Safety Authority (EFSA) concluded in their evaluations in 1997 and 2008 respectively, that residues of TAs in edible tissues (milk, meat or eggs) were unlikely to constitute a risk for consumers following the legal use of *Atropa belladonna* and atropine as authorised veterinary medicines and no information has subsequently been published to alter these conclusions.

The CONTAM Panel recommended to better characterise TAs occurring in food and feed either naturally or as contaminants and that analytical data on the occurrence of TAs in cereals and oilseeds should be collected, including TAs not considered in this opinion occurring in food and feed commodities. Moreover, there is a need for: i) investigations into the agricultural conditions under which TAs occur in cereals and oilseeds; ii) defined performance criteria for the analysis of TAs in food and feed; iii) for certified reference materials containing TAs at levels of interest as well as for proficiency tests; iv) information on stability of TAs during food and feed processing and the identity and toxicity of potential degradation products; v) data on relative potency of (-)-hyoscyamine and (-)-scopolamine and for data on endogenous formation of (+)-hyoscyamine and its biological relevance; vi) toxicity data for TAs which are relevant in food and feed commodities other than those covered in this opinion.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The tropane alkaloids are commonly found in plants of three families, the Solanaceae, Erythroxylaceae, and Convolvulaceae families. The term tropane alkaloids refers to a group of more than 200 compounds best known for their occurrence in the family Solanaceae comprising over 100 genera and 3000 plant species. Many of the plants in the Solanaceae family contain tropane alkaloids, which are responsible for the toxic effects of the plants. The consumption of, for example, few berries from henbane (*Hyoscyamus niger*) or from deadly nightshade (*Atropa belladonna*) can cause death in young children.

The tropane alkaloids have in common a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbon atoms. The nitrogen atom at the end of the molecule, which characterizes the compounds as alkaloids, is in this group characteristically methylated. The most important tropane alkaloids are (-)-hyoscyamine, atropine ((±)-hyoscyamine) and (-)-scopolamine (also known as hyoscine). High concentrations of these alkaloids have been found particularly in *Datura stramonium* and *Datura ferox,* as well as in *Datura innoxia.* The pattern of tropane alkaloids differs significantly and in *Datura stramonium* (also known as thorn apple or Jimson weed) (-)-hyoscyamine prevails in most parts of the plant, whereas in *Datura ferox* (-)-scopolamine is the major alkaloid produced. *Datura* plants are toxic for animals if ingested in large amounts. Their seeds, which contain significant amounts of (-)-hyoscyamine and (-)-scopolamine, can be found as botanical impurities in certain seed products.

The Panel on Contaminants in the Food Chain issued on 9 April 2008 on a request from the Commission an opinion related to tropane alkaloids as undesirable substances in animal feed[4].

The Panel concluded that, while the presence of tropane alkaloids in feed constituted a risk for animal health in several animal species, it was unlikely that residues of tropane alkaloids in edible tissues, milk and eggs constituted a risk for consumers.

Occurrence

In the abovementioned opinion, it is mentioned that contamination of feed with *Datura* seeds is most likely to occur in oil-producing crops. Reference was made to a survey that was conducted between 1986 and 1988 in Germany and where several batches of linseed and soybean products were contaminated with parts of *Datura* seeds. Chemical analysis of contaminated samples showed mainly a contamination with (-)-scopolamine at levels between 0.1 and 33 mg/kg. More recent data were not reported, and the call for data launched by EFSA during preparation of that Opinion did not identify new data.

In the Rapid Alert System for Feed and Food (RASFF), 6 alert notifications are related to an unacceptable presence of tropane alkaloids and/or unacceptable presence of seeds containing tropane alkaloids in food or products intended to be used as an ingredient in food.

In buckwheat flour, atropine (at levels up to 110 µg/kg) and (-)-scopolamine (at levels up to 65 µg/kg) were found. Henbane seeds (*Hyoscyamus niger*) were found up to 0.42 % in poppy seeds.

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 tropane alkaloids in food and feed.

[4] Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Tropane alkaloids (from *Datura* sp.) as undesirable substances in animal feed. The EFSA Journal 2008, 691, 1-55. http://www.efsa.europa.eu/en/scdocs/doc/691.pdf

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

a) evaluation of the toxicity of tropane alkaloids for humans, considering all relevant toxicological endpoints and identification of the tropane alkaloids of toxicological relevance present in food;

b) exposure of the EU population to tropane alkaloids, including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc) and identify the relevant sources of exposure.

The scientific opinion as regards the presence of tropane alkaloids in animal feed should, *inter alia,* comprise an update, if necessary, of the Opinion of the Panel on Contaminants in the Food Chain on a request from the Commission related to tropane alkaloids as undesirable substances in animal feed, taking into account new data (toxicological, occurrence and other relevant information) which has become available since 2008.

ASSESSMENT

1. Introduction

Tropane alkaloids (TAs) are secondary metabolites which naturally occur in plants of several families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae (including coca). The TAs are responsible for the toxic effects of some of these plants and are found in all parts of the plants. The common structural element is the tropane skeleton. The group of TAs comprises more than 200 compounds and the wide range of compounds occurring especially in the Solanaceae family arises from the esterification of tropine (tropanol, see Appendix A) with a variety of acids, such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid, and atropic acid.

Datura stramonium, commonly called thorn apple or Jimson weed, belonging to the genus Datura, is long known for its content of TAs. The plant is widely distributed in temperate and tropical regions of the world. For this reason seeds of this plant have been found as impurities in important agricultural crops such as linseed, soybean, millet, sunflower and buckwheat and products thereof. The consumption of a few berries from henbane (*Hyoscyamus niger*) or from deadly nightshade (*Atropa belladonna*) has caused severe intoxication, including deaths in young children. The most studied TAs are (-)-hyoscyamine, (-)-scopolamine and cocaine. The racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine is called atropine.

Plant extracts containing TAs have been used for centuries in human medicine and are still used nowadays, as are atropine, (-)-hyoscyamine and (-)-scopolamine. These uses include for example the treatment of wounds, gout and sleeplessness, and pre-anaesthesia. Furthermore, *Atropa belladonna* extracts were used to dilate pupils for cosmetic reasons and to facilitate ophthalmological examination. In India, the root and leaves of the Jimson weed plant were burned and the smoke inhaled to treat asthma. From this observation, the introduction of TAs into Western medicine by British colonists in the early 1800s was derived. The first studies of the action of *Atropa belladonna* date from 1831 when Mein isolated the active constituent and named it atropine (Mein, 1831). The isolation of the same compound was independently described two years later by Geiger and Hesse (1833a, b).

Some TAs are well known as antagonists of acetylcholine (ACh) muscarinic receptors in mammals and can induce a variety of distinct toxic syndromes (anticholinergic poisoning). For the therapeutically used TAs, it is known that the naturally occurring (-)-L-enantiomers exhibit far stronger anticholinergic effects than the (+)-D-enantiomers.

Cocaine is also a prominent member of the group of TAs. Unlike other TAs, cocaine exerts its effects by acting mainly on the dopaminergic and serotonergic systems, and thus it cannot be considered to predict the toxicity of other members of the TA group. The German Federal Institute for Risk Assessment (BfR) has undertaken a health assessment of the cocaine content of a coca leaf extract-containing cola soft drink in which 0.4 µg cocaine/L had been detected. BfR came to the conclusion that no health risk is to be expected from consumption of this product because of its low cocaine content (BfR, 2009). Since no other data are available concerning the occurrence of cocaine in food and feed it was not further considered in this opinion.

Although more than 200 different TAs have been identified in various plants, data on their occurrence in food and feed and on toxicity are limited, which makes an exposure and risk assessment for the full range of these compounds impossible. Limited data on toxicity, and particularly on occurrence in food and feed were only available for atropine, (-)-hyoscyamine, scopolamine and (-)-scopolamine. Therefore, the CONTAM Panel could only perform a risk assessment for (-)-hyoscyamine and (-)-Scopolamine. Where analytical data in food and feed samples were reported as atropine or scopolamine, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) used and reported these data in this opinion as (-)-hyoscyamine and (-)-scopolamine, respectively. As the biosynthesis of

TAs leads to (-)-hyoscyamine and (-)-scopolamine, any analytical results where no stereoselective separation is achieved were thus regarded and reported as 100 % (-)-hyoscyamine or (-)-scopolamine.

1.1. Chemistry of tropane alkaloids

TAs contain an azabicyclo[3.2.1]octane ring structure. Thus, the common structural element is the tropane skeleton, (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octane, shown in Figure 1 (Lounasmaa and Tamminen, 1993). The group of TAs comprises more than 200 compounds and the wide range of compounds occurring especially in the Solanaceae family arises from the esterification of tropine with a variety of acids, such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid, and atropic acid.

The most studied natural TAs (-)-hyoscyamine and (-)-scopolamine (Figure 1) are esters of tropane-3α-ol (and the 6-7 epoxide of tropane-3α-ol) with tropic acid. The asymmetric α-carbon of tropic acid allows the formation of two stereoisomers. Atropine is the racemic mixture of (±)-hyoscyamine. Structures and chemical information on relevant TAs are given in Appendix A.

There is evidence that TAs co-occur with their corresponding TA *N*-oxides in plants. Studies conducted by Phillipson and Handa (1975, 1976) showed the presence of the equatorial and the axial isomers of (-)-hyoscyamine *N*-oxide (see Figure 1) in roots, stems, leaves, flowers, pericarps and seeds of *Atropa belladonna*, *Hyoscyamus niger* and *D. stramonium*. Of (-)-scopolamine *N*-oxide only the equatorial isomer was isolated from all parts of the latter two species and also from the leaves of *A. belladonna*. The roots, stems and leaves of *Scopolia lurida* and *S. carniolica* (henbane bell) were found to contain both *N*-oxides of (-)-hyoscyamine and one isomer of (-)-scopolamine *N*-oxide, while the former two *N*-oxides were found in the roots, stems, leaves, and fruits of *Mandragora officinarum* (mandrake). Analysis by thin layer chromatography (TLC) showed substantial amounts of *N*-oxides to be present in the leaves of *A. belladonna* (Phillipson and Handa, 1976). However, there is no confirmation of these findings by other analytical methods.

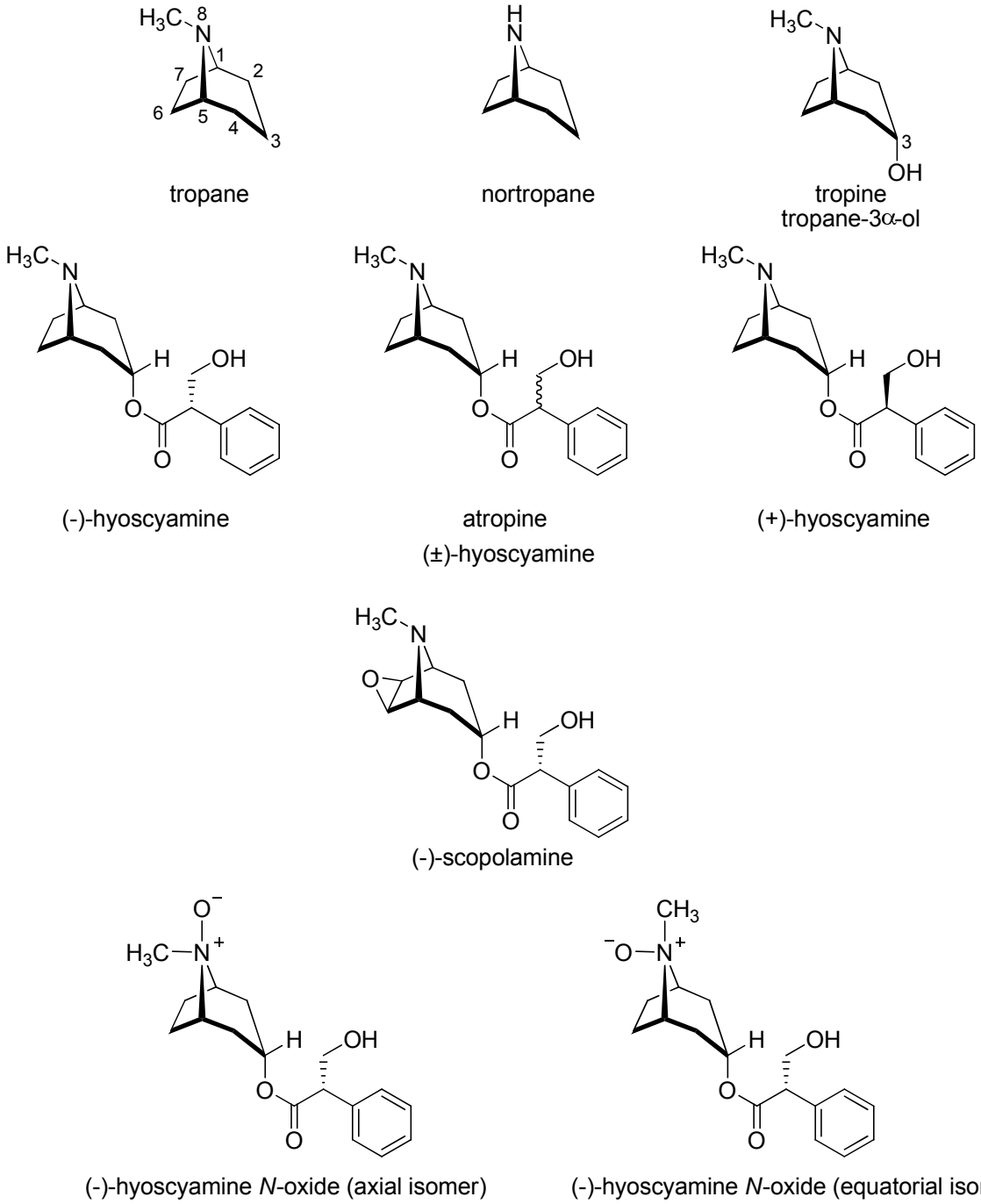


Figure 1: Tropane [(1R,5S)-8-methyl-8-azabicyclo[3.2.1]octane], nortropane and tropine skeletons of tropane alkaloids and structures of (+) / (-)-hyoscyamine, atropine and (-)-scopolamine. Both isomers of TA N-oxides are shown using (-)-hyoscyamine as an example.

Compounds with a nortropane skeleton, without the methyl group in position 8, and without an ester group at position 3, have also been identified (Goldmann et al., 1990). These alkaloids, named calystegines, bear several hydroxyl groups in various positions of the nortropane backbone. Calystegines are very hydrophilic with calculated logP[5] values well below zero (e.g. -0.8 for the diols

[5] LogP is the decimal logarithm of the n-octanol-water partition coefficient. The n-octanol-water partition coefficient is the ratio of concentrations of a given substance in the n-octanol and aqueous phases at equilibrium.

down to -1.6 for the pentahydroxy alkaloids). Due to their hydrophilic properties they remain in the aqueous phase during common alkaloid extraction procedures (Dräger, 2004).

TAs with a tropic acid ester backbone, such as (-)-hyoscyamine and (-)-scopolamine, are chemically unstable in protic solvents. They degrade by two main routes: (i) inversion of the natural (*S*)-enantiomer to the (*R*)-form until a racemic mixture is reached and (ii) hydrolysis to tropic acid and the corresponding tropane backbone. In addition, minor dehydration to aposcopolamine/apoatropine occurs. According to a study by Blaschke et al. (1993) the hydrolysis and racemisation rates increase with increasing pH and temperature. At a pH of 3 apparently no racemisation and only slight hydrolysis occurs, while racemisation occurs and hydrolysis is more pronounced at pH values > 3. No data for pH values < 3 were reported in this study.

The physico-chemical data of TAs with a tropane skeleton were reviewed by Boit (1961), and of calystegines by Dräger (2004). The chemical and physical characteristics of atropine, (-)-hyoscyamine and (-)-scopolamine, have been summarised in an EFSA opinion on animal feed (EFSA, 2008).

Due to the lack of information on occurrence in food and feed and limited information on toxicity, the calystegines are not considered further in this opinion.

1.2. Plant species and biosynthesis

1.2.1. The families containing tropane alkaloids

TAs have been reported to occur in plants of seven Angiosperm (flowering plant) families. The families are Brassicaceae (syn. Cruciferae; the mustard family), Convolvulaceae (the bindweed or morning glory family), Erythroxylaceae (the coca family), Euphorbiaceae (the Spurge family), Proteaceae (no generally used common family name), Rhizophoraceae (the mangrove family) and Solanaceae (the nightshade or potato family) (Griffin and Lin, 2000; The Plant List, 2010). Among the seven families, especially Brassicaceae and Solanaceae are known for their many grown edible species, while the families Erythroxylaceae and Rhizophoraceae do not contain any important food species.

Within the Brassicaceae all edible species belong to the genus *Brassica*. Edible species include: *B. elongata* (elongated mustard), *B. fruticulosa* (Mediterranean cabbage), *B. juncea* (Indian mustard, brown and leaf mustards, Sarepta mustard), *B. napus* (rapeseed, canola), *B. narinosa* (broadbeaked mustard), *B. nigra* (black mustard), *B. oleracea* (kale, cabbage, broccoli, cauliflower, kai-lan, Brussels sprouts, kohlrabi), *B. perviridis* (tender green, mustard spinach), *B. rapa* (*syn B. campestris*) (Chinese cabbage, turnip, rapini, komatsuna), *B. rupestris* (brown mustard), *B. septiceps* (seventop turnip), and *B. tournefortii* (Asian mustard) (Rakow, 2004).

Convolvulaceae include the important food plant *Ipomoea batatas* (sweet potato) (Massal and Barrau, 1956) and furthermore *I. aquatica* (water spinach) (Austin, 2007), while an additional number of species have been reported as being used as 'famine foods' (Freedman, 2012).

There are no major food or feed plants within the Euphorbiaceae, although *Ricinodendron rautanenii*, has been reported as a wild food plant for all seasons in Zambia (Peters, 1987).

Proteaceae include tree nuts from the species of *Macadamia* (tetraphylla and integrifolia) and from Gevuina avellana (Halloy et al., 1996). In Australia a number of proteaceous species are also important sources of honey, such as *Grevillea robusta* (Orwa et al., 2009).

Edible *Solanaceae* spp. belong to the genera *Solanum*, *Capsicum*, *Physalis*, *Lycium*, *Lycianthes* and *Jaltomata*. The most intensively grown are *Solanum tuberosum* (potato), *S. lycopersicon* (tomato), *S. melongena* (brinjal eggplant) and *Capsicum annuum* (sweet and hot peppers). A number of other *Solanum* spp. are edible and grown or collected such as *S. macrocarpon* (the gboma eggplant) (Nee et al., 1999), *S. aethiopicum* (synonyms *S. gilo* and *S. incanum*; African eggplant) (Lester and Seck, 2004)

and others. Also a few additional species of capsicum are grown and eaten (e.g. *C. frutescens* (tabasco forms). Three species of physalis (e.g. *P. philadelphica* = tomatillo) are cultivated while four are regarded as being semicultivated. Two species of *Lycium*, namely *L. barbarum* and *L. chinense*, are grown in China. Also *Lycianthes asarifolia* is edible as are five species of *Jaltomata* (Nee et al., 1999).

1.2.2. Tropane alkaloids in plants used as food

Of the seven plant families that include TA-containing species, Brassicaceae, Convolvulaceae, Solanaceae and Proteaceae include plants used as food or feed. TAs have not been reported in the food plants from the Proteaceae family.

1.2.2.1. Brassicaceae

No Brassicaceae species of food relevance have been shown to contain (-)-hyoscyamine or (-)-scopolamine (Griffin and Lin, 2000). However, e.g. *Brassica oleraceae* has been shown to contain calystegines at concentrations up to 30 µg/g dry weight (d.w.) (Dräger, 2004; Brock et al, 2006).

1.2.2.2. Convolvulaceae

A number of genera within the Convolvulaceae (*Convolvulus, Evolvulus, Erycibe and Cochleare*) are well known for their content of various tropine esters with methoxy substituted benzoic acids, a group of compounds that is characteristic of TAs in this plant family. The compounds include convolvine (3α-veratroyloxynortropane), convolidine (3α-vanilloyloxynortropane) (Griffin and Lin, 2000). Tropine, pseudotropine and tropinone have been characterised in field bindweed (*Convolvulus arvensis)*, which is toxic to horses (Todd et al., 1995). So far, no reports have been published indicating any content of TAs in the food species *Ipomoea batatas* and *I. aquatica*.

1.2.2.3. Solanaceae

In their review of the chemotaxonomy and geographical distribution of TAs, Griffin and Lin (2000) used the classification of the Solanaceae into two sub-families Solanoideae and Cestroideae. This comprised a total of 12 tribes; seven within the sub-family of Solanoideae and five in the sub-family Cestroideae (Hunziker, 1979). Griffin and Lin (2000) in their review of the literature reported TAs to occur in seven of the 12 tribes, namely:

- Sub-family Solanoideae
    - Tribes
        - Datureae
        - Solandreae
        - Solaneae
        - Hyoscyameae
- Sub-family Cestroideae
    - Tribes
        - Anthocercideae
        - Nicandreae
        - Salpiglossidae

Of these, the tribe Datureae, comprising the two genera *Datura* and *Brugmansia*, contains the greatest range of TAs, but none of these genera include any food species.

Tribe Lycieae

Griffin and Lin (2000) did not consider the tribe Lycieae (which consists of the three genera: *Grabowskia*, *Lycium*, and *Phrodus* (Levin and Miller, 2005)) in their review on the distribution of TAs.

However, a very recent review on the phytochemistry, pharmacology and safety of Goji (*L. barbarum* and *L. chinense*) to a certain extent gives a slightly different picture (Potterat, 2010), as described below:

1. *L. barbarum.* There is one report on the presence of TAs in *L. barbarum* in which, based on TLC analysis, a content of 0.95 % of atropine and 0.29 % (-)-hyoscyamine on a d.w. basis was reported for the fruits of plants collected in India (Harsh, 1989). A similar content was reported for the shoots, and somewhat lower amounts were found in the roots. These findings, however, have not been confirmed by other studies, and are in obvious contradiction with the widespread consumption of the fruits and lacking reports of apparent toxicity (Potterat, 2010). In addition, more recent results obtained in a screening of *L. barbarum* berry varieties by high performance liquid chromatography-mass spectrometry (HPLC-MS), only trace amounts of (-)-hyoscyamine with maximum levels of 19 µg/kg (on d.w. basis) were detected (Adams et al., 2006) Drost-Karbowska et al. (1984) did not detect atropine and (-)-scopolamine in the roots of *L. barbarum*. No further reports on the occurrence of TAs in the leaves are known (Potterat, 2010).

2. *L. chinense*. No reports on investigations of the fruits or leaves for TAs were identified by Potterat (2010). Investigations of the roots showed the presence of calystegines and *N*-methylcalystegines, but not (-)-hyoscyamine or (-)-scopolamine (Asano et al., 1997; Potterat, 2010).

Tribe Solaneae

The rest of the edible plants belonging to the Solanaceae family is within this tribe, as represented by species of the genera *Capsicum, Jaltomata, Physalis*, *Solanum* and *Lycianthes*. From these, only *Physalis* and *Solanum* are reported to contain TAs.

1. *Physalis*. While the tomatillo (*P. philadelphica*; syn. *P. ixocarpa*) has not been reported to contain TAs, the Cape Gooseberry (*P. peruviana*) and the Chinese Lantern (bladder berry) named *P. alkekengi* have. *P. peruviana* has through a number of investigations been shown to contain several tropane and secotropane alkaloids all found in the roots and/or leaves, while no reports exist on any occurrence in the edible fruits (Griffin and Lin, 2000). *P. alkekengi* has twice been investigated concerning the content in the roots but not the fruits. Firstly, the roots were shown to contain tigloidine, 3α-tigloyloxytropane, cuscohygrine and phygrine; with a total alkaloid content varying from 0.02 to 0.025 % (0.084-0.104 % based on the dry weight of the root) (Basey and Woolley, 1973).

2. *Solanum*. According to the review of Griffin and Lin (2000) plants of the large genus *Solanum* do not contain (-)-hyoscyamine or (-)-scopolamine.

1.2.3. Tropane alkaloids in species that occur as contaminants (botanical impurities) in food and feed

1.2.3.1. Species of relevance for food and feed

Contamination of food or feed with parts (mostly seeds) of certain TA containing plant species can occur. A report from Adamse and van Egmond (2010) clearly points to contamination with seeds from *D. stramonium* (Jimson weed or thorn apple) as the most common problem. However, also seeds from other *Datura* spp. as well as berries of *Atropa belladonna* (deadly nightshade) (Adamse and van Egmond, 2010) and seeds of *Hyoscyamus niger* (henbane) have been reported as impurities in food (see Section 4.1). For feed materials, five sources of TA contamination were identified by van Kempen (1992), namely *D. stramonium*, *D. ferox*, *D. metel*, *D. wrightii* and *D. inoxia*, of which only the first two mentioned species were estimated to occur in relevant quantities in feed materials imported to Europe (Bucher and Meszaros, 1989). However, the CONTAM Panel noted that importers of oilseeds in the European Union (EU) have operated a large programme of microscopic examination of South American

soybean, and this was recently stopped because no contamination with *Datura* seeds had been found over nine years[6].

1.2.3.2. Occurrence in specific species

Datura species

The TA profile differs between species and between individual tissues/organs within each species. (-)-Hyoscyamine and (-)-scopolamine are the predominant TAs in all investigated species; although more than 65 different TAs have been reported to occur in the following species/varieties *D. ceratocaula*, *D. inoxia*, D. s*tramonium* var. *stramonium*, *D. stramonium* var. *tatula* and *D. stramonium* var. *godronii* (Berkov and Zayed, 2004; Berkov et al., 2006; Doncheva et al, 2006; Philipov et al., 2007). (-)-Hyoscyamine and (-)-scopolamine have also been detected in the flowers of *D. suaveolens* (syn *Brugmansia suaveolens* (Hall et al., 1977; Erhardt et al., 2008)). In Table 1, a compilation of available literature data on TA content for the major *Datura* spp. is presented. Only those studies are shown that have performed quantitative analysis using gas chromatography (GC) or high performance liquid chromatography (HPLC) based analytical methods and reported concentrations on a dry weight basis. For a comprehensive overview of available data see also EFSA (2008).

[6] Arnaud Bouxin, FEFAC, personal communication.

Table 1: Compilation of (-)-hyoscyamine and (-)-scopolamine concentrations in various plant tissues of the major *Datura* spp. Concentrations given as mg/kg dry weight.

Species (country of collection)	Plant part	(-)-Hyoscyamine	(-)-Scopolamine	Sum TAs	Reference
D. stramonium (incl var. tatula) (Hungary)	Stem Leaves Flower	360 - 5 910 430 - 4 710 1 690 - 3 970	20 - 3 320 130 - 1 790 1 360 - 2 740	380 - 8 830 560 - 6 430 3 050 - 6 710	Jakabová et al. (2012)
D. stramonium (Italy)	Roots Stem Leaves Flowers Seeds	n.d. - 121 1 - 915 134 - 831 270 - 299 170 - 387	n.d. - 14 n.d. - 129 16 - 73 66 - 106 12 - 89	n.d.-135 1 - 1 044 172 - 378 336 - 405 182 - 476	Miraldi et al. (2001)
D. stramonium (Italy)	Seeds	1 280	680	1 960	Caligiani et al. (2011)
D. stramonium (incl varieties) (Poland)	Leaves Seeds	425 - 1 655 710 - 1 380	230 - 715 520 - 1 275	1 000 - 1 885 1 235 - 2 655	Mroczek et al. (2006)
D. stramonium (USA)	Seeds	1 690 - 2 710	360 - 690	2 050 - 3 400	Friedman and Levin (1989)
D. ferox (Argentina)	Roots Stem Leaves Fruits Seeds	No data	36-900 29-200 40-3 200 130-210 1 500		Padula et al. (1976)
D. ferox (Argentina)	Seeds	n.d.	610-820	610 - 820	Vitale et al. (1995)
D. innoxia (Bulgaria)	Seeds	840	1 410	2 250	Berkov (2001)
D. innoxia (Hungary)	Leaves Stem Flower	20 - 60 400 30	940 - 4 530 1 950 3 940	960 - 4 550 2 350 3 970	Jakabová et al. (2012)
D. innoxia (Poland)	Leaves Seeds	325 2 860	840 675	1 165 3 535	Mroczek et al. (2006)
D. innoxia (Slovenia)	Nectar	3.4 - 37[(a)]	58 - 343[(a)]	61 - 380[(a)]	Boros et al. (2010)
D. metel (Hungary)	Leaves Stem Fruit+seeds	70 - 1 430 190 - 510 530	10 - 280 10 - 3 420 3 440	80 - 1 710 200 - 3 930 3 970	Jakabová et al. (2012)
D. metel (Poland)	Leaves Seeds	905 915 - 1 250	1 110 425 - 755	2 015 1 670	Mroczek et al. (2006)

n.d.: not detected; TAs: tropane alkaloids; USA: United States of America.
(a): in mg/L

Atropa belladonna

For leaves of *Atropa belladonna* the total alkaloid levels have been reported to vary considerably between different variants (breeding lines) and harvesting stages (Dhar and Bhat, 1982). Likewise, seed samples collected from 16 different locations within Europe were shown to vary greatly in their content of (-)-hyoscyamine and (-)-scopolamine, which are the two main alkaloids in this plant species, with (-)-hyoscyamine being more abundant (Simola et al., 1988). An overview of quantitative data available in the literature is given in Table 2.

Table 2: Compilation of (-)-hyoscyamine and (-)-scopolamine concentrations in various plant tissues of major *Atropa* spp. Concentrations given as mg/kg dry weight.

Species (country of collection)	Plant part	(-)-Hyoscyamine	(-)-Scopolamine	Sum TAs	Reference
A. belladonna (Europe)	Roots	500 - 3 700	n.d. - 900	500 - 4 000	Simola et al. (1988)
	Leaves	500 - 4 900	n.d. - 500	700 - 5 100	
	Seeds	1 200 - 6 900	n.d. - 500	1 300 - 7 300	
A. belladonna (Germany)	Roots	3 700	100		Sporer et al. (1993)
	Stem	1 800 - 3 900	70 - 130		
	Leaves	960 - 1 400	90 - 130		
	Fruit+seeds	2 800 - 9 200			
A. belladonna (Germany)	Roots	5 290	51		Wilms et al. (1977)
	Leaves	2 500 - 5 200	20 - 280		
A. belladonna (Iran)	Roots	570 - 880	17 - 31		Ashtiania and Sefidkonb (2011)
	Stem	740 - 770	28 - 180		
	Leaves	190 - 1 200	23 - 470		
A. acuminata (Iran)	Leaves	900 - 1 200	140 - 470		Ashtiania and Sefidkonb (2011)
A. baetica (Spain)	Roots	1 000 - 10 000	600		Zárate et al. (1997)
	Leaves	3 000	400		

n.d.: not detected; TAs: tropane alkaloids.

Hyoscyamus niger

The species *Hyoscyamus niger* also contains (-)-hyoscyamine and (-)-scopolamine as its main alkaloids. The (-)-scopolamine level is generally higher than that of (-)-hyoscyamine. *H. muticus* (Egyptian henbane) is particularly rich in TAs (Oksman-Caldentey et al., 1987; Mandal et al., 1991). An overview of data available in the literature is given in Table 3.

Table 3: Compilation of (-)-hyoscyamine and (-)-scopolamine concentrations in various plant tissues of major *Hyoscyamus* spp. Concentrations given as mg/kg dry weight.

Species (country of collection)	Plant part	(-)-Hyoscyamine	(-)-Scopolamine	Sum TAs	Reference
H. niger (Bulgaria)	Seeds	140	430	570	Berkov (2001)
H. niger (India)	Stem	200	320	520	Mandal et al. (1991)
	Leaves	100	350	450	
	Inflorescence	250	400	650	
H. niger (Iran)	Stem	170	530	700	Bahmanzadegan et al. (2009)
	Leaves	440	605	1 045	
	Flowers	945	1295	2 240	
	Seeds	1 100	1 890	2 980	
H. muticus (India)	Stem	3 410	n.d.	3 410	Mandal et al. (1991)
	Leaves	6 150	20	6 170	
	Inflorescence	7 200	430	7 630	
H. muticus (Egypt)	Whole plant	280-30 950	300-7 070		Oksman-Caldentey et al. (1987)
H. reticulatus (Turkey)	Roots	560±110[(a)]	trace		Kartal et al. (2003)
	leaves	360±40[(a)]	trace		
H. reticulatus (Iran)	Roots	189	97	286	Bahmanzadegan et al. (2009)
	Stem	520-1 080	385-690	905-1 770	
	Leaves	965-1 215	585-810	1550-2 025	
	Flowers	870	435	1 305	
	Seeds	1 170-1 915	755-1 645	1 925-3 560	

n.d.: not detected; TAs: tropane alkaloids.
(a): mean ± standard deviation

1.2.4. Biosynthesis

As disclosed from investigations using different solanaceous plants, TAs are to a great extent synthesized in young root cells and translocated to the aerial parts of the plant (Hashimoto et al., 1991). The synthesis of the tropine part starts from the amino acid ornithine via putrescine and *N*-methyl-putrescine which is transformed to 4-amino-butanal, catalyzed by diamine oxidase (DAO). This compound spontaneously ring-closes to form the 1-methyl-pyrrolinium cation which is transferred into tropinone and finally tropine (Zhang et al., 2007). Tropine is esterified with (*R*)-phenyllactate (stemming from phenylalanine) to (*R*)-littorine. At present at least two different routes leading from (*R*)-littorine to (-)-hyoscyamine have been described in literature (Li et al., 2006). (-)-Scopolamine is formed from (-)-hyoscyamine in a two step reaction involving the enzyme hyoscyamine 6β-hydroxylase (H6H) (Yun et al., 1992; Zhang et al., 2007). An overview of the biosynthesis is shown in Figure 2.

The calystegines are formed from pseudotropine which in turn originates from tropinone (Scholl et al., 2003). See also Figure 2.

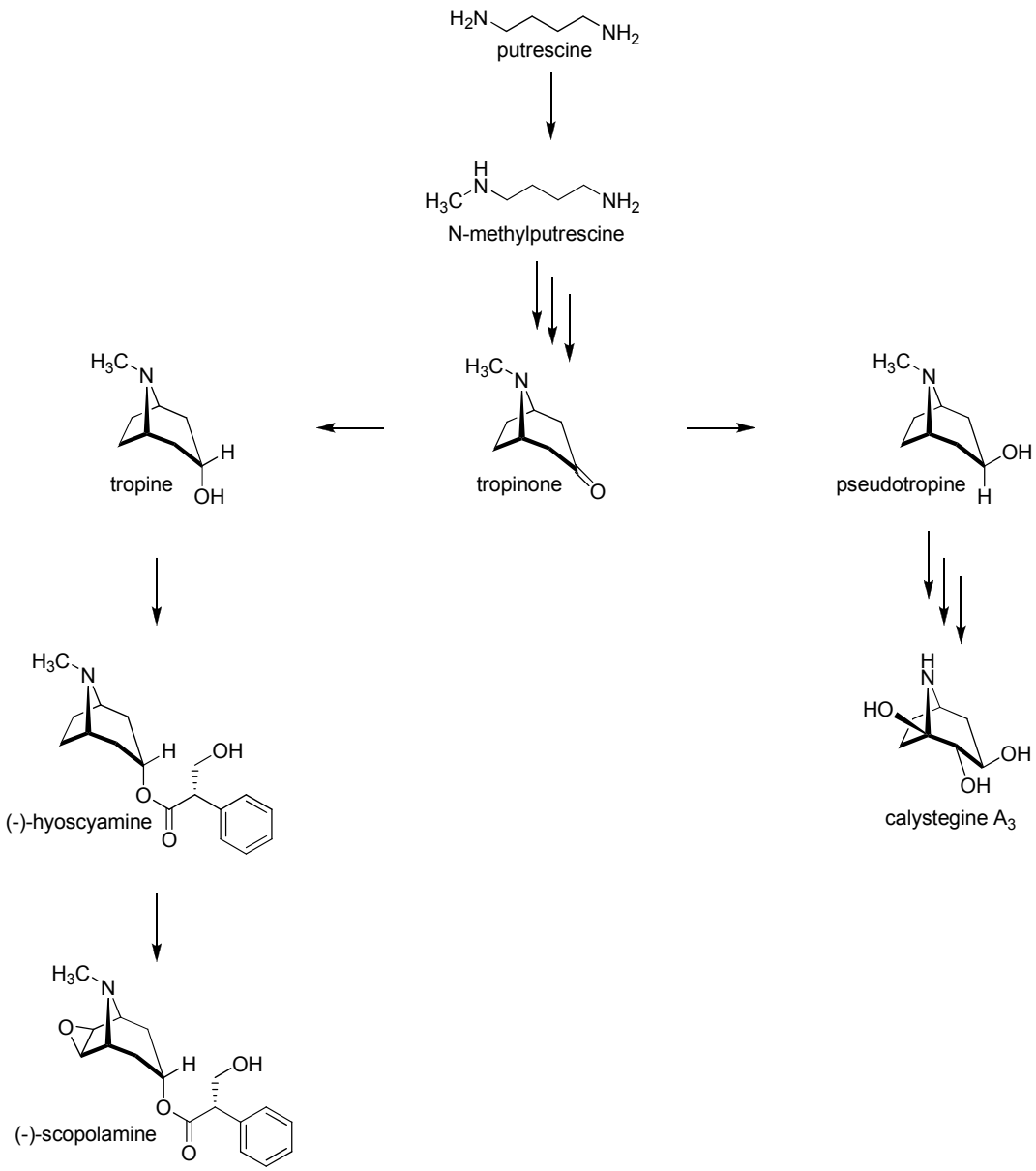


Figure 2: Overview of key steps in the biosynthesis of tropane alkaloids including calystegines. Modified according to Dräger[7].

1.3. Previous assessments

Human health assessments

WHO-IPCS (2002) assessed the use of atropine as an antagonist for poisoning by organophosphorus pesticides. The monograph reviewed the clinical and animal data relevant to the use of atropine, alone or in combination with oximes. The assessment concluded that in the case of unknown or mild poisoning, a dose of 1-2 mg atropine should be administered by intravenous (i.v.) injection in adults, and repeated every 5-10 minutes. Larger doses are required in case of moderate or severe poisoning. Under these clinical circumstances, adverse effects related to the use of atropine were reported to be infrequent and

[7] Biosynthesis of Calystegines (available at: http://ag-bioarznei.pharmazie.uni-halle.de/english/research/42162_42194/)

to include mydriasis, tachycardia and anticholinergic action in the central nervous system (CNS) leading to restlessness, hyperactivity and delirium.

The existing monographs in the present European Pharmacopoeia (7th edition) on TAs (atropine, (-)-scopolamine), their salts (e.g. (-)-hyoscyamine sulphate, atropine sulphate, (-)-scopolamine hydrobromide) and TA containing botanicals (e.g. Belladonna Leaf, Prepared Belladonna Leaf, Stramonium Leaf, Prepared Stramonium Leaf) reflect their diverse traditional therapeutic uses in human medicine (Ph. Eur.7, 2011) (see Section 7.5).

In 2008, l'Agence française de sécurité sanitaire des aliments (Afssa, now Anses (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail)) evaluated the relevance of setting an intervention threshold for TAs (atropine and scopolamine) in buckwheat flour. Based on a dose of 20 µg atropine/kg body weight (b.w.) (intramuscular (i.m.)) that caused reduced salivary secretion in five-year old children, an uncertainty factor of 30 (3 for the extrapolation to a dose without an effect and 10 for the inter-individual variability) and a maximum daily consumption of buckwheat flour of 100 g, an intervention threshold of 100 µg/kg buckwheat flour for the sum of atropine and scopolamine was derived (Afssa, 2008).

Animal health assessments

The Committee for Veterinary Medicinal Products of the European Agency for the Evaluation of Medicinal Products (EMEA; now the European Medicines Agency (EMA)) assessed the use of atropine as a medicine in farm animals (EMEA, 1998a). Atropine is used therapeutically in all food producing animals in doses varying between 0.02 and 0.2 mg/kg b.w. to treat drying secretions or gastrointestinal disorders, and up to 0.5 mg/kg b.w. as an antagonist for poisoning by organophosphorus pesticides. Since atropine is rapidly absorbed and eliminated, it is used for infrequent and non-regular treatment and animals are unlikely to be sent for slaughter immediately after treatment, no need to establish Maximum Residue Levels (MRLs) was identified by the Committee.

In a separate evaluation, the Committee for the evaluation of veterinary medicinal products assessed the use of *Atropa belladonna* as a herbal medicine in farm animals (EMEA, 1998b). According to the provisions of the German Homeopathic Pharmacopoeia, the ethanolic extract of the plant should have a maximum content in TAs of 0.1 % (calculated as (-)-hyoscyamine base) and should be diluted 1:100, leading thus to a final concentration of 0.01 mg/mL TAs in the diluted extract. Daily doses of 5-10 mL of the diluted extract are parenterally administered in farm animals (pig, sheep, goat, horse or cattle), corresponding to TA doses of 0.1 mg and 0.05 mg for large and smaller animals, respectively. In view of the low TA dose administered, the use of *A. belladonna* in a small number of individual animals for non-regular treatments, and the fact that animals are unlikely to be sent for slaughter immediately after treatment, no need to establish MRLs for the preparation *A. belladonna* was identified by the Committee.

In 2008, the CONTAM Panel assessed TAs from *Datura* spp. as undesirable substances in animal feed (EFSA, 2008). The CONTAM Panel concluded that TA intake through direct consumption of fresh *Datura* plants is unlikely to occur, but poisoning can occur via contamination of hay with *Datura* or of grain or oilseed products with *Datura* seeds. Due to insufficient information on the presence of TAs in feed materials, a conclusive exposure assessment was not performed. However, a worst case exposure estimate suggested that intake of seeds of *D. ferox* at the maximum level (ML) of 3000 mg/kg feed indicated by EU Directive 2002/32/EC[8] (see Section 2) may lead to adverse effects in pigs. The CONTAM Panel concluded that adverse effects of *Datura* poisoning in livestock, including dryness of the mucosa in the upper digestive and respiratory tract, constipation and colic in horses, pupil dilation, alterations in the heart rate and central nervous effects are caused by the anticholinergic action of TAs. Regarding species sensitivity, the opinion indicated pigs as the most sensitive species to *Datura*

[8] Directive 2002/32/EC of the Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10-22.

poisoning, whereas poultry and rabbits show a lower sensitivity presumably due to the more efficient metabolism of TAs in those species. No information was available on carry-over of TAs from feed into animal derived products under normal livestock conditions. However, the CONTAM Panel concluded that the kinetic data and the longstanding clinical use of atropine and butylscopolamine provide no evidence of an accumulation in animal tissues and that it was unlikely that the natural TAs (-)-hyoscyamine and (-)-scopolamine would reach concentrations in animal tissues that are pharmacologically active in consumers.

2. Legislation[9]

In order to protect public health, Article 2 of the Council Regulation (EEC) No 315/93[10] 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[11]. TAs in food are not regulated so far under this EU Regulation.

According to Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding MRLs in foodstuffs of animal origin, *Atropa belladonna* and atropine are classified as allowed substances for all food producing species. No marker residues or target tissues are stipulated and the Regulation states that no MRL is required for either substance. However, for *Atropa belladonna*, the Regulation specifies '*For use in homeopathic veterinary medicinal products prepared according to homeopathic pharmacopoeias, at concentrations in the products not exceeding one part per hundred only.*'

According to the Directive 2002/32/EC, products intended for animal feed must only be used if they are sound, genuine and of merchantable quality and therefore when correctly used do not represent any danger to human health, animal health or to the environment or could adversely affect livestock production. Annex 1, Section VI to Directive 2002/32/EC, contains a list of harmful botanical impurities that are undesirable in animal feed and their MLs in different feed commodities. The maximum content established in the EU for weed seeds and unground and uncrushed fruits containing alkaloids are presented in Table 4.

Table 4: EU legislation on weed seeds and unground and uncrushed fruits containing alkaloids in products intended for animal feed (Directive 2002/32/EC[8]).

Undesirable substance	Products intended for animal feed	Maximum content in mg/kg relative to a feedingstuff with a moisture content of 12 %
Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including	Feed materials and compound feed	3 000
- *Datura* spp.		1 000

[9] In this opinion, where reference is made to European legislation (Regulations, Directives, Decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.

[10] 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.

[11] 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.

3. Methods of analysis

3.1. Visual inspection

To meet current EU legislation (see Section 2) for animal feed, in general the content of weed seeds and unground and uncrushed fruits which contain alkaloids should be below 3 000 mg/kg; In particular for seeds of *Datura* spp. the content should be below 1 000 mg/kg. These criteria can be achieved by visual inspection of unprocessed raw feed material.

3.2. Extraction, sample clean-up and concentration

Like many other alkaloids, TAs can be extracted following the classical Stas-Otto procedure, which takes advantage of the basic nature of the alkaloids. Following the protocol, the alkaloids can be repeatedly distributed in two-phase systems between a polar aqueous (at low pH values) and a non-polar organic phase (at pH-values > 9) simply by changing the pH. This protocol allows sample clean-up and concentration of the TAs.

Applying those extraction conditions, it is necessary to consider racemisation, hydrolysis and dehydration side reactions of tropic ester TAs including especially (-)-hyoscyamine or (-)-scopolamine (see Section 1.1). Whenever those conditions are applied, the procedure should be performed either quickly or from moderately basified aqueous solution (sodium carbonate or ammonia) to avoid racemisation (Dräger, 2002). However, in most analytical studies on TA-occurrence and/or quantitation this fact is not addressed since most of the analytical procedures used do not apply enantiomeric separation of the TAs. Hence, most of the analytical results do not specify the stereochemistry and are given as (unspecified) concentrations of hyoscyamine/atropine or scopolamine. As the biosynthesis of TAs leads to (-)-hyoscyamine and (-)-scopolamine, any analytical results where no stereoselective separation is achieved are thus regarded in this opinion as 100 % (-)-hyoscyamine or (-)-scopolamine.

A number of different extraction procedures have been successfully applied, including pressurised solvent extractions, supercritical fluid extraction or microwave assisted extraction (Christen et al., 2008). Besides the Stas-Otto procedure of multistep liquid-liquid extraction (LLE), solid phase extraction (SPE) or solid phase supported LLE on diatomaceous earth is commonly used and mixed-mode SPE is applied frequently using reversed phase materials such as C18 (Papadoyannis et al., 1993), C8 or C4, together with cation exchange-material. As an alternative, a non-aqueous solid phase extraction method using strong cation exchange (SCX) SPE has been described by Long et al. (2012). A recent method by Mroczek et al. (2006) used pressurized liquid extraction in combination with mixed-mode reversed-phase cation-exchange (MCX) SPE for the analysis of (-)-hyoscyamine, (-)-scopolamine and (-)-scopolamine–*N*-oxide (see Section 1.1) from plant extracts achieving recoveries of 80 to 100 % (Mroczek et al., 2006)

3.3. Spectroscopic methods

Ultraviolet (UV) based methods are of limited value, since the TAs considered in this opinion show rather low absorption values at rather unspecific wavelengths (Bogusz and Erkens, 1994; Dräger, 2002; Kursinszki et al., 2005).

Recently, a fluorescent probe, based on an amphiphilic Schiff-base zinc (II) complex, was shown to be useful in the detection of various classes of alkaloids including TAs. It exhibited optical absorption changes and fluorescence enhancement upon formation of a 1:1 zinc (II) complex:alkaloid adduct in dichloromethane. The limit of quantification (LOQ) was 0.5 and 4.3 µg/mL for TAs and atropine, respectively (Oliveri and Di Bella, 2011).

3.4. Immunological methods

Immunological assays are a simple, cost effective and sensitive alternative for the quantification of biomolecules. Naturally, these detection systems depend on the availability of antibodies against the target molecule. Furthermore, the specificity of the antibodies is a crucial aspect of the application of

such test systems. In general, for multi-analyte detection and quantification e.g. for a class of compounds like TAs the specificity of the antibodies is a limiting factor in the validity of quantification of individual compounds in a complex mixture.

A sensitive radioimmunoassay (RIA) has been established for investigation of (-)-scopolamine production from cell and tissue cultures of *Datura* spp. (Savary and Dougal, 1990). A (-)-scopolamine-binding antiserum was generated. The assay used a commercially available labelled antigen, L-(*N*-methyl [^{3}H])-scopolamine methylchloride and was able to detect (-)-scopolamine in the range 0.15 to 3.0 ng.

A different approach was published by Fliniaux and Jacquin-Dubreuil (1987). Antibodies were obtained from the immunization of rabbits with racemic tropic acid conjugated to bovine serum albumin. This resulted in a broad specificity and enabled simultaneous analysis of a set of main TAs including (-)-hyoscyamine and (-)-scopolamine. The competitive enzyme-linked immunosorbent assay (ELISA) used for this analysis was found to be a sensitive method with good accuracy for the dosage of alkaloids in plant material: purified extracts, crude extracts, and dry powdered material. Atropine could be detected with a sensitivity of 0.1 ng. A racemisation step was necessary to detect (-)-hyoscyamine and (-)-scopolamine with the same sensitivity.

A lateral flow device based method was developed and inter-laboratory validated for the fast detection of some TAs. The antibody-based dipstick test detects (-)-hyoscyamine and (-)-scopolamine in animal feed at a target level of 800 µg/kg for the sum of both compounds (Van Egmond et al., 2013).

3.5. Gas chromatography and gas chromatography-mass spectrometry

GC is one of the predominant analytical techniques for screening, identification, and quantification of TAs in materials of plant origin as well as in biological fluids. The GC-analysis of TAs has been reviewed by various authors (Dräger, 2002; Christen et al., 2008; Aehle and Dräger, 2010). Many TAs are sufficiently volatile for direct GC-separation without derivatisation, allowing for analysis of TAs by GC-flame ionization detection (GC-FID) or gas chromatography-mass spectrometry (GC-MS). Identification of TAs in complex matrices is facilitated by the availability of electron-ionization mass spectrometry (EI-MS) databases of known TAs especially when used in combination with available retention index data (El-Shazly et al., 1997). Currently, GC-based methods, in particular in combination with MS-detection, are still routine methods for TA-analysis. Major applications for GC-MS are TA-identification and quantification in plant extracts and toxicological/forensic applications. Some recent representative GC-MS methods for TA-analysis are highlighted here.

El Bazaoui et al. (2009) reported a Stas-Otto-like LLE-extraction procedure for *D. stramonium* seeds. The extracts were analysed by GC-MS and the TAs were identified by comparing the obtained EI-MS spectra with data available from literature.

Recently, Caligiani et al. (2011) published a GC-MS based method for the detection of TAs as contaminants in buckwheat (*Fagopyron esculentum*) fruits, flours and commercial food products. The method allowed for a simultaneous detection of (-)-hyoscyamine and (-)-scopolamine by using GC-MS in selected ion monitoring mode (GC-SIM-MS). Special attention was given to extraction, sample clean-up and derivatisation to maximise recoveries and limits of detection (LOD). Nicotine was used as internal standard. The method validation covered response factor, recovery and assay precision. The reported LODs for (-)-hyoscyamine and (-)-scopolamine were 0.3 and 1 µg/kg, respectively, while the LOQs were 1 and 6 µg/kg, respectively.

3.6. High performance liquid chromatography and high performance liquid chromatography-(tandem) mass spectrometry

HPLC separation of TAs is usually performed on reversed phase (RP) columns. The UV chromophore of many TAs is weak and the UV spectrum shows low specificity, hence representing a major limitation for a sensitive detection in complex matrices. Nevertheless, there is a report on an HPLC-UV analysis of

some TAs (including (-)-hyoscyamine, (-)-scopolamine) in feedstuffs and biological samples (Papadoyannis et al., 1993). Bamifylline was added as an internal standard followed by a Stas-Otto sample extraction. The extracts were further purified and concentrated by SPE on C18 cartridges. LODs (S/N 2:1) of 13 and 12 ng on column were reported for (-)-hyoscyamine and (-)-scopolamine, respectively at a UV wavelength of 210 nm. LOQs (S/N between 5:1 to 10:1) were 38 ng on column for both compounds. Datura seeds, blood, urine and egg samples were tested. The method was validated (intra-day precision and accuracy, between-day precision and accuracy). The mean recovery rates of (-)-hyoscyamine and (-)-scopolamine were 95.5 % and 98.5 % in seeds, respectively. Mean recovery rates of (-)-hyoscyamine and (-)-scopolamine, in blood (and serum), urine and egg white at various concentrations ranged from 90 to 98 % for both compounds.

Plant biosynthesis results in only the (*S*)-enantiomers of hyoscyamine ((-)-hyoscyamine) and scopolamine ((-)-scopolamine). In terms of cholinergic receptor response, these are the main active forms of these compounds. Therefore, the separation of enantiomers is of some importance. Direct enantioseparation of (±)-hyoscyamine (atropine) and (±)-scopolamine by HPLC is possible and was demonstrated for various chiral stationary phases (CSP). Depending on solvent conditions used for the elution, UV or MS detection was applied. The enantioseparation of atropine and homatropine in Belladonna raw materials and tablets using a teicoplanin-coated CSP HPLC-UV method was reported by Cieri (2005). Another example is the separation of atropine by phases that contain α_1-acid glycoprotein (AGP) as chiral selector. Breton et al. (2005) used HPLC with an AGP CSP coupled to MS with an atmospheric pressure chemical ionization (APCI) interface operating in the positive mode and single ion monitoring for the chiral separation of atropine. Siluk et al. (2007) used a vancomycin-modified CSP to separate the enantiomers of atropine in plasma. Separation was achieved using gradient elution and detection was by APCI-MS. The reported LOQ was 0.5 ng/mL for both enantiomers. Developments in this area have been summarised by Dräger (2002) and Aehle and Dräger (2010).

TAs are nitrogen-containing polar analytes which are particularly amenable to analysis using high performance liquid chromatography-(tandem) mass spectrometry (HPLC-MS/(MS)). These techniques have become widespread and provide sensitive detection of TAs in positive ion electrospray ionization (ESI+). HPLC-MS/(MS) has been used for the identification and quantification of TA metabolites in biological matrices such as plasma, urine and faeces. Concentrations in plasma generally are much lower than in urine. A high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was developed and validated for the simultaneous detection of several TAs, including (-)-hyoscyamine, (-)-scopolamine, littorine and homatropine in plasma (John et al., 2010a). Plasma samples were extracted with acetonitrile and centrifuged before analysis. The LOQ was 0.05 ng/mL. John et al. (2010b) developed an elegant method to estimate the relative ratio of the atropine enantiomers in plasma using a non-chiral HPLC-MS/MS method. By adding atropineesterase to part of the sample, selective hydrolysis of (-)-hyoscyamine, but not (+)-hyoscyamine, takes place. By analysis of the samples with and without esterase treatment, the relative amounts of the hyoscyamine enantiomers could be calculated.

A simplified work-up protocol for the analysis of TAs in food and feed has been published by Adamse and van Egmond (2010). It is incorporated into a multi-analyte method together with ergot alkaloids. Quantification was achieved by means of multi-level standard addition. The LOD for (-)-hyoscyamine and (-)-scopolamine ranged from 3-5 µg/kg and the LOQ from 10-15 µg/kg.

Perharič et al. (2013b) used HPLC-MS/MS for the determination of (-)-hyoscyamine and (-)-scopolamine in buckwheat products. The samples were extracted with dichloromethane/methanol/ammonium hydroxide (70:25:5, v:v:v) and subsequently were concentrated. HPLC-MS/MS with positive ESI resulted in an LOD of 1 µg/kg and an LOQ of 3 µg/kg for both TAs.

There are not yet many reports in which TAs have been incorporated in multi-analyte HPLC-MS/MS methods. Recently, an HPLC-MS/MS method was published where the simultaneous determination of TAs (tropine, (-)-hyoscyamine, (-)-scopolamine, homatropine, anisodamine) and glycoalkaloids

(α-solanine, α-chaconine) in grains and seeds (wheat, rye, maize, soybean, linseed) was reported (Jandrić et al., 2011). Sample clean-up was achieved by a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach. Oily or fatty matrices such as soybean and linseed were cleaned by matrix solid phase dispersion (MSPD) on C18 material to remove co-extracted non-polar components. The analytes were separated isocratically on a vancomycin-modified CSP column. Chiral separation of atropine was attempted but was unsuccessful. (-)-Scopolamine-d_3 was used as internal standard and analytes were detected in ESI+ mode. The method performance showed good linearity, specificity, selectivity, accuracy, precision and ruggedness. The LODs ranged from 0.7 to 0.8 µg/kg and LOQs were in the range of 2.2-4.9 µg/kg (Jandrić et al., 2011).

The mass spectrometric analysis of TAs in plant tissues under ambient conditions using desorption electrospray ionization (DESI) has been described by Talaty et al. (2005). Fifteen different TAs could be identified from *D. stramonium* root without any sample preparation and requiring only a few seconds of analysis time. Direct analysis in real time (DART) MS has been used for the analysis of (-)-hyoscyamine and (-)-scopolamine in hairy root cultures of *Atropa acuminata* (Banerjee et al., 2008).

3.7. Capillary electrophoresis (CE)

Capillary electrophoresis (CE) and capillary zone electrophoresis (CZE) have been successfully applied in the analysis of TAs especially in plant tissues (Cataldi and Bianco, 2008; Aehle and Dräger, 2010). For example, (-)-scopolamine, (-)-hyoscyamine and anisodamine in *Flos daturae* could be separated, with detection by UV. The detection was linear in the range of 2.4-21.8 µg/mL for (-)-scopolamine, 4.0-36.0 µg/mL for (-)-hyoscyamine and 2.6-23.7 µg/mL for anisodamine. The developed method was applied for the analysis of herbal samples (Ye et al., 2001).

To improve sensitivity, electrochemiluminescence was introduced. With electrochemical detection in non-aqueous CE (NACE), LODs for (-)-scopolamine ranged from 0.055 µg/mL to 2.1 µg/mL in capillaries from 2 to 50 µm diameter. This was superior to CE with UV-detection using capillaries from 5 to 75 µm diameter. The LODs for (-)-scopolamine ranged from 1.2 µg/mL to 130.2 µg/mL (Blasco et al., 2009).

Analysis of TAs by CE coupled to mass spectrometric detection has also been reported. Mateus et al. (1999) used CE-MS with ESI+ to differentiate between (-)-hyoscyamine and its positional isomer littorine in *Datura* plant extracts. Recently, Posch et al. (2012) used NACE-MS for the analysis of TAs in crude *D. stramonium* extracts. The method was found to be very matrix tolerant. By combination of CE with time of flight (TOF) MS and with ion trap MS, Arráez-Román et al. (2008) were able to identify 7 different TAs in *Atropa belladonna* leaf extract.

CE-based techniques were also successfully used in the enantioseparation of TAs. For example, various TA-enantiomeric pairs, including (±)-hyoscyamine, (±)-homatropine and (±)-scopolamine were separated by the aid of cyclodextrin-modified microemulsion electrokinetic chromatography (MEEKC) and diode array detection (DAD) (Bitar and Holzgrabe, 2007). Developments in this area have been summarised by Dräger (2002) and Aehle and Dräger (2010).

3.8. Standards, reference materials, validation and proficiency tests

Reference compounds for (-)-scopolamine, (-)-hyoscyamine and atropine are readily available, while the availability of a number of other naturally occurring TAs (e.g. homatropine, aposcopolamine, anisodamine, anisodine) is limited. In many analytical approaches atropine is used as reference compound for the detection and quantification of (-)-hyoscyamine.

Isotopically labelled standards, useful for quantitative MS approaches, currently are sparingly available. The use of atropine-d_3 and (-)-scopolamine-d_3 as internal standards for isotope dilution has been described (Kintz et al., 2006; Jandrić et al., 2011).

So far, none of the methods for TAs in food or feed have been fully validated by inter-laboratory studies. In addition, no certified reference materials or proficiency studies are currently available for the determination of TAs in food or feed.

3.9. Conclusions

To meet current EU legislation (see Section 2) for animal feed, visual inspection of unground/uncrushed fruits for the presence of weed seeds is an accepted method. In the Directive, no TA-content is specified, but the content of *Datura* spp. seeds is regulated to be lower than 1 000 mg/kg feed.

Immunological approaches to screen food/feed for TAs are currently being developed but data or broad application experiences are not available, yet.

So far, most of the analytical methods available for TA-analysis in food and feed have focused on the occurrence of hyoscyamine/atropine and scopolamine without taking into account an enantioseparation of the individual TAs. In those cases, the reported data were considered as 100 % (-)-hyoscyamine and/or (-)-scopolamine, since the degree of racemisation cannot be estimated and because (-)-enantiomers are the forms that are biosynthesised.

For the routine analysis of (-)-hyoscyamine and (-)-scopolamine in biological matrices including food products, GC-MS or HPLC-MS/(MS) approaches can be applied. GC-MS was applied to the analysis of TAs in buckwheat fruits and food products containing buckwheat, with LODs for (-)-hyoscyamine and (-)-scopolamine of 0.3 and 1 µg/kg, respectively, and LOQs of 1 and 6 µg/kg, respectively. HPLC-MS/MS yielded similar results for the analysis of TAs in buckwheat products, with reported LODs of 1 µg/kg and LOQs of 3 µg/kg for (-)-hyoscyamine and (-)-scopolamine.

Reference standards for (-)-scopolamine, (-)-hyoscyamine and atropine are readily available, while the availability of a number of other naturally occurring TAs (e.g. homatropine, aposcopolamine, anisodamine, anisodine) and isotopically labelled standards is limited.

So far, none of the methods for TAs in food or feed have been fully validated by inter-laboratory studies. In addition, no certified reference materials or proficiency studies are currently available for the determination of TAs in food or feed.

4. Occurrence of tropane alkaloids in food and feed

4.1. Previously reported occurrence results

4.1.1. Food

Very few studies and surveys have been conducted in the past on the presence of TAs in food products and therefore only very limited data are available from the literature. Earlier chemical food contaminations were only reported when an intoxication occurred leading to hospitalisation. Screening the literature for reports on food-related intoxications led to eleven cases concerning TAs described within the period from 1978 to 2010, as overviewed in Adamse and van Egmond (2010). Five of these had to do with documented or suspected contamination of different types of herbal teas; i.e. burdock (*Arctium*) root tea (Bryson et al., 1978), nettle (*Urtica*) tea (Scholz and Zingerle, 1980), two incidents with comfrey (*Symphytum*) tea (Galizia, 1983; Routledge and Spriggs, 1989) and Paraguay (*Ilex paraguariensis*) tea (CDC, 1995). Other cases involved mallow (fruits of *Malva sylvestris*) contaminated with berries of *Atropa belladonna* (in Canada 1981 and 1984), canned green beans contaminated with flower buds of *D. stramonium* in France in 2010 (Department of Health and Sports, France, 2010), contamination of a stew made from self-picked material contaminated with Jimson weed (*D. stramonium*) material in the United States of America (USA) in 2008 (Russell et al., 2010) and wasp honey contaminated with TAs from *Datura* spp. in Venezuela in 1999 (Ramirez et al., 1999). It should be noted that in many of these cases no specific level of contamination was reported.

Perharič (2005) reported on buckwheat flour in Slovenia contaminated with up to 190 seeds/kg from *D. stramonium*. In Austria, a dish made from millet and carrot was contaminated with seeds from *D. stramonium*. Examination of the millet revealed *D. stramonium* seeds in a concentration of about 50 seeds/kg grain (Fretz et al., 2007).

Within the period covering 2006 to 2013, the Rapid Alert System for Food and Feed (RASFF) has reported a number of occurrences of thorn apple (*D. stramonium*) and henbane (*Hyoscyamus niger*) seeds in various food products. Thus, seeds of thorn apple were reported five times in millet samples; only one occurrence being quantitatively reported (130 seeds/kg organic millet from Austria and Hungary - year 2006). Furthermore, such seeds were reported four times in fruits and vegetables; a vegetable and bacon stir-fry mix from Spain (2007), two samples of canned green beans (2006/2007) and in a frozen vegetable-bean-seed mix (2013). Henbane seeds were reported to be present in poppy seeds twice in 2007 and once in 2008. The highest occurrence level was 0.42 %. Four reports were found on the occurrence of (-)-hyoscyamine and (-)-scopolamine in buckwheat flour, namely two in 2006, one in 2009 and one in 2012. The highest occurrence level was 110 µg/kg of (-)-hyoscyamine and 47 µg/kg of (-)-scopolamine in buckwheat flour from Hungary. In addition, there was one report on the presence of (-)-hyoscyamine in marshmallow root (*Athea officinalis*) in 2013.

In 2007, 26 samples of buckwheat (*Fagopyron esculentum*) grains and buckwheat flour and 2 samples of potato pancakes were analysed by HPLC-MS/MS in France. The sum of (-)-hyoscyamine and (-)-scopolamine was above 1000 µg/kg in ten samples (maximum 7 400 µg/kg) and the concentrations of (-)-hyoscyamine and (-)-scopolamine were below the LOD of 0.1 µg/kg in nine samples. In 2008, the study was repeated with 5 samples of buckwheat grains and 29 samples of buckwheat flour. That year, the sum of (-)-hyoscyamine and (-)-scopolamine was above 1000 µg/kg in 2 samples (maximum 1 340 µg/kg) and the concentrations of (-)-hyoscyamine and (-)-scopolamine were below the LOD of 0.1 µg/kg in 14 samples (Afssa, 2008). The country of production in most cases could not be identified.

Recently, Caligiani et al. (2011) used a GC-MS based method for the detection of (-)-hyoscyamine and (-)-scopolamine as contaminants in buckwheat fruits, flours and commercial food products from retail shops. The method was applied for the analysis of 2 commercial samples of buckwheat fruits, 1 hull and 6 flours and 7 food products made from buckwheat (3 pasta, 2 porridge, 1 cracker, and 1 flakes sample). In none of the samples were TAs detected above the LOD of 1 µg/kg.

Perharič et al. (2013b) reported on the analysis of 75 samples of buckwheat grain and buckwheat food products collected from millers and food shops throughout Slovenia. The survey was conducted following food poisoning incidents in 2003 which affected 73 consumers of buckwheat-based food products. The survey comprised 12 wholegrain samples, 13 samples of groats, 34 flour, 8 pasta, 4 bread and 4 žganci (semi-prepared buckwheat) samples. The buckwheat grain and groat samples were only visually inspected, but the 50 remaining products were analysed by GC-MS. The LOD of the GC-MS method used was 10 µg/kg, the LOQ 30 µg/kg. In 18 samples (-)-hyoscyamine and/or (-)-scopolamine was detected above the LOQ (11 flour, 4 pasta, 3 žganci samples), with a maximum of 26 000 µg/kg (-)-hyoscyamine and 12 000 µg/kg (-)-scopolamine in a sample of buckwheat flour originating from Hungary. Eleven of the 18 positive samples originated from Hungary, 4 from Czech Republic, 2 from China and 1 from Slovenia. The average content of the 18 positive samples was 1 922 µg/kg (-)-hyoscyamine and 1 034 µg/kg (-)-scopolamine and the median content was 245 and 100 µg/kg, respectively. The ratio of (-)-hyoscyamine /(-)-scopolamine was found to vary between 0.85 and 3.30, with a mean value of 1.71. The samples originating from Hungary appeared to be the most heavily contaminated (average contamination of the 11 samples was 4 729 µg/kg for the sum of (-)-hyoscyamine and (-)-scopolamine; the median contamination was 570 µg/kg). In contrast, the four positive samples from Czech Republic had only an average contamination of 37 µg/kg for the sum of TAs. Highest contamination levels were found in the buckwheat flour and in the buckwheat pasta samples.

4.1.2. Feed

Previous reported occurrence of TAs in feed materials was reviewed by EFSA (2008). The RASFF in 2012 reported occurrences of thorn apple (*D. stramonium*) seeds (1 862 mg seeds/kg) in sunflower seeds for bird feed from France. Red millet from Hungary intended for pet food had previously (in 2006) been found to contain 2 760 mg seeds/kg material.

4.2. Current occurrence results

4.2.1. Data collection summary

The Dietary and Chemical Monitoring Unit (DCM) launched in July 2010 a continuous call for data in food and feed on a list of chemical contaminants, including among them plant toxins such as TAs. European national food authorities and similar bodies, research institutions, academia, food and feed business operators and any other stakeholder were invited to submit analytical data on the presence of these contaminants. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a).

By the end of February 2013, analytical data on TAs in 124 food samples and 611 feed samples were available in the EFSA database. These data were reported as atropine and scopolamine for all of the samples while for 219 of the samples (46 food and 173 feed) additional data were also submitted on the total content of TAs (sum of atropine and scopolamine). As the biosynthesis of TAs leads to (-)-enantiomers, the reported analytical results were assumed to be (-)-hyoscyamine or (-)-scopolamine. In total, 1 689 analytical results corresponding to 735 samples, collected in the Netherlands and Germany and all reported by the Netherlands, were available. Figure 3 shows the distribution of these food and feed samples over the sampling years, with 2011 being the year when the highest number of samples was collected.

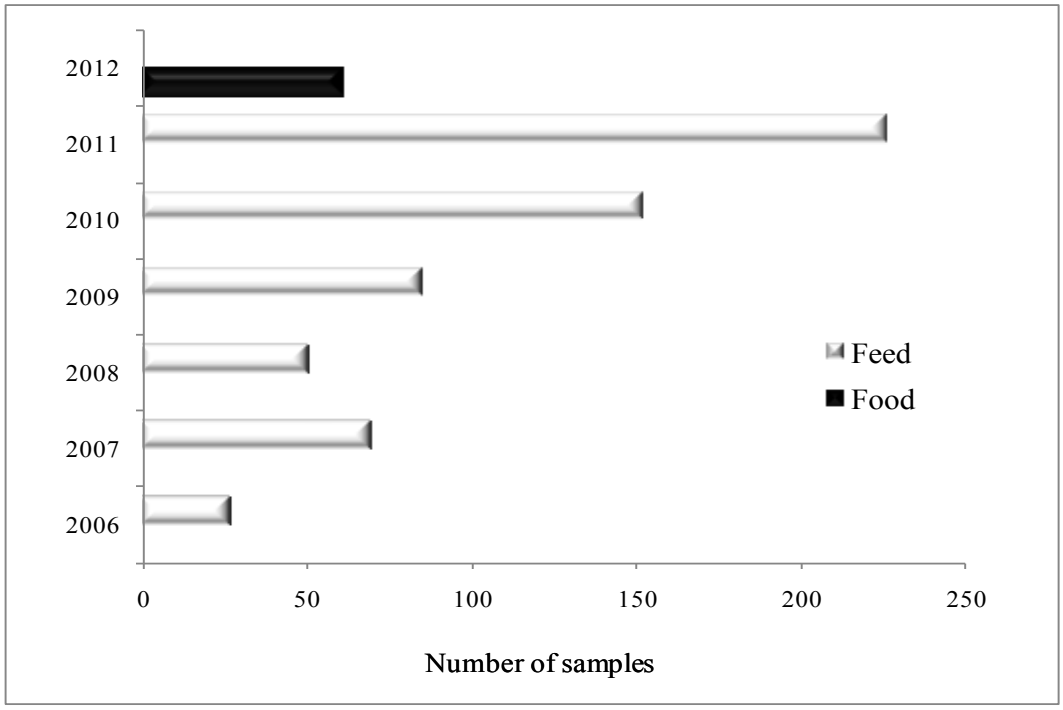


Figure 3: Distribution of food and feed samples over the sampling years.

To ensure the quality of data included in the assessment, several data cleaning and data validation steps were applied. Data were also checked for duplicates (same sample transmitted twice or repeated analysis of the same sample). All analytical results present in the database were initially considered suitable to carry out exposure assessment.

4.2.2. Data collection on food and feed commodities

4.2.2.1. Food samples

Sampling was mainly carried out in the Netherlands (112 samples) although a few samples were also collected in Germany (12 samples). All samples were collected between 2010 and 2012, with almost 50 % of the samples collected in the most recent year.

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.

Four different food groups were represented at FoodEx level 1 although the number of samples was not balanced among them. The food group with the highest representation was 'Food for infants and small children' with 93 samples. At FoodEx level 2 all 93 samples belonged to the food group 'Cereal-based food for infants and young children', with 56 samples corresponding to 'Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids', 10 samples to 'Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid' and 27 samples to 'Biscuits, rusks and cookies for children' (all at FoodEx level 3). The other food categories represented at FoodEx level 1 were 'Fruit and fruit products' with one sample of berries and small fruits, 'Grain milling products' with 7 samples of 'Breakfast cereals', 11 samples of 'Grain milling products' and 5 samples of 'Grains for human consumption', and 'Legumes, nuts and oilseeds' with 2 samples of 'Legumes, beans, dried' and 3 samples of 'Oilseeds' (see Figure 4).

It is important to mention that the food category 'Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids' refers to milled cereal products. In most of the cases the products were reported as containing a mix of different cereals (wheat, maize, rye, oats, rice, in different proportions). In one occasion rice was the only ingredient, and in some cases the cereals were accompanied by fruits. Half of the samples (28) were labelled as recommended for both age classes toddlers (1-3 years old) and infants (< 1 year). The remaining samples were only indicated for toddlers.

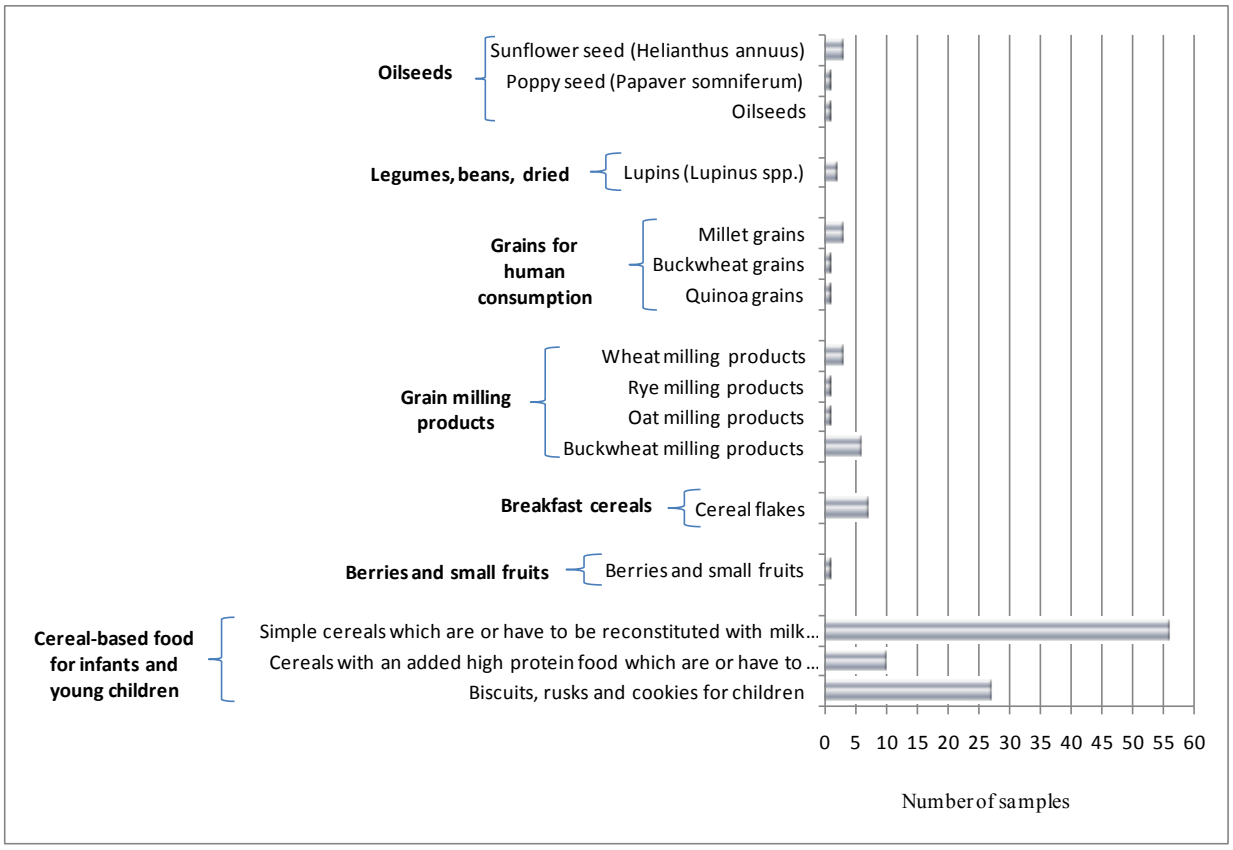


Figure 4: Distribution of the different food samples at FoodEx level 2 (in bold) and FoodEx level 3.

4.2.2.2. Feed samples

The sampling and reporting country for all feed samples was the Netherlands. Samples were collected between 2006 and 2011 (Figure 3).

A total of 611 feed samples were reported. Feed was classified according to the catalogue of feed materials specified in the Commission Regulation (EU) No 575/2011 (see Figure 5). Almost half of the samples belonged to the feed group 'Forages and roughage, and products derived thereof' (301 samples), although other feed categories were also represented: 'Cereal grains, their products and by-products' (122 samples), 'Oil seeds, oil fruits, and products derived thereof' (71 samples), 'Compound feed' (66 samples), "Other plants, algae and products derived thereof' (32 samples), 'Legume seeds and products derived thereof' (13 samples), 'Other seeds and fruits, and products derived thereof' (5 samples), and 'Tubers, roots, and products derived thereof'(1 sample). Compound feeds were grouped according to the species/production categories for which the feed is intended. Results were reported either as 88 % dry matter (438 samples) or as whole weight (173 samples). Considering the large number of left-censored data, conversion to a common moisture content was not performed.

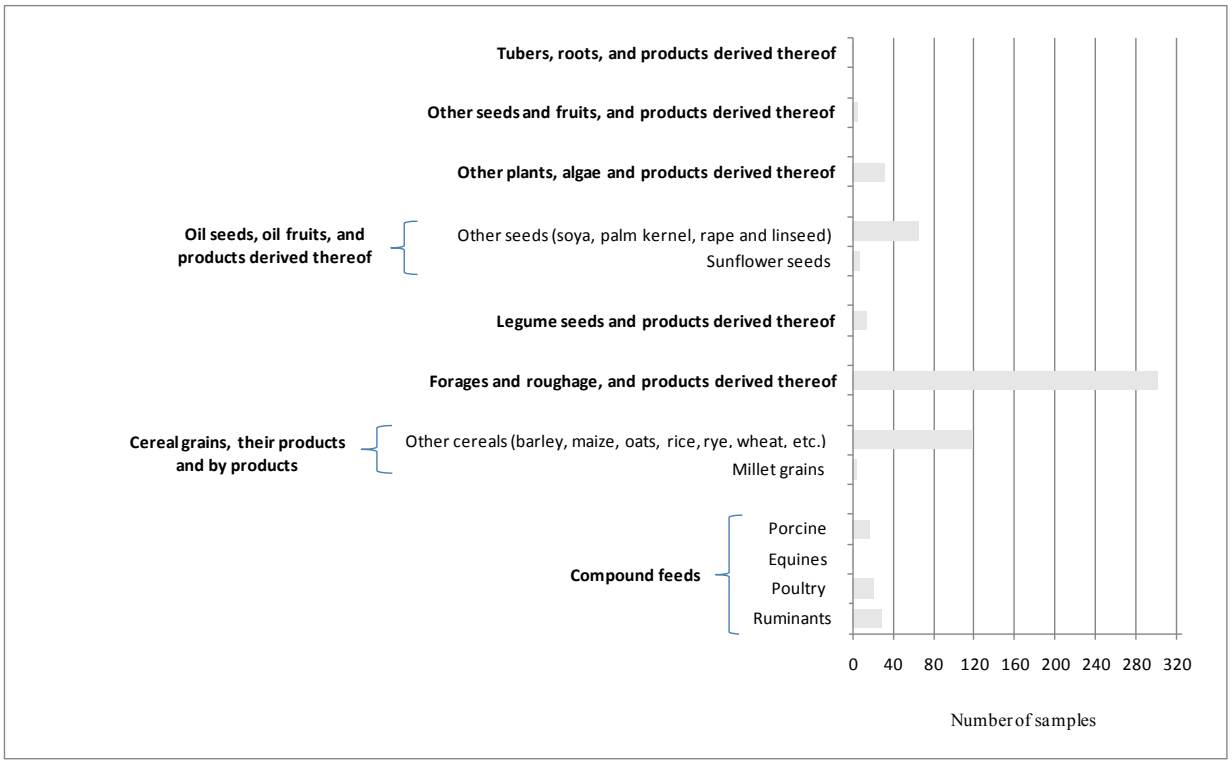


Figure 5: Available feed samples classified according to the catalogue of feed materials specified in the Commission Regulation (EU) No 575/2011.

4.2.3. Analytical methods used

4.2.3.1. Food samples

Data reported for food samples were all obtained using HPLC-MS/MS with an LOD of 0.3 µg/kg.

4.2.3.2. Feed samples

All data reported for feed samples were also obtained using HPLC-MS/MS. However, two methods with different sensitivity were applied. One HPLC-MS/MS method was reported with an LOD of 4.5 µg/kg, and a second HPLC-MS/MS method was reported with an LOD of 2 µg/kg.

4.2.4. Occurrence data by food and feed category

4.2.4.1. Occurrence data in food

The analytical results were reported corrected for recovery. 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). At the LB, results below the LOD were replaced by zero; at the UB the results below the LOD were replaced by the value reported as LOD.

Table 5 shows the available food samples with their concentrations (µg/kg) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine. Considering only the samples where the two compounds were quantified (11 samples) the levels of (-)-hyoscyamine were on average 3-fold higher than those of

(-)-scopolamine. (-)-Hyoscyamine was quantified in a total of 19 food samples while (-)-scopolamine was quantified in 13 food samples.

Most of the data were left-censored (103 out of 124 samples), where neither (-)-hyoscyamine nor (-)-scopolamine were quantified. Most of the quantified samples were reported for the food group 'Cereal-based food for infants and young children' at FoodEx Level 2. The other quantified samples were one sample of wheat bran (49.6 µg/kg, FoodEx Level 4) and one sample of mixed oilseeds (LB=0.32 µg/kg and UB=0.62 µg/kg, FoodEx Level 2)[12]. The CONTAM Panel decided not to consider these two samples for the exposure assessment. In the case of the sample of wheat bran, it was considered inappropriate to use the occurrence value of just one sample of such a specific food to derive the mean values of other foods included in upper levels of the food classification. For the sample of oilseeds, the existence of only one quantified sample together with its low TAs concentration and low consumption led to the exclusion of this sample from the exposure calculations.

When deciding at which FoodEx Level the occurrence data on 'Food for infants and small children' should be used, it was considered necessary to use them at FoodEx Level 3. Within FoodEx Level 2 (Cereal-based food for infants and young children), out of the four existing food groups, two of them did not report any quantified data and for a third one no data were reported. Therefore, the dietary exposure assessment to TAs was carried out using the fourth food group, 'Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids' at FoodEx Level 3. This food group reported mean concentration values of 4.5 µg/kg at the LB and 4.9 µg/kg at the UB. As can be seen in Table 5, (-)-hyoscyamine and/or (-)-scopolamine were quantified in a total of 19 samples of 'Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids'. Contamination with TA was mainly found in the cereal products that were indicated specifically for toddlers (a total of 28), with 50 % of the samples being contaminated. Among the other 28 samples that were indicated for both toddlers and infants, contamination with TAs was reported in five of them although, in general, lower levels than those found in the cereal products only for toddlers.

In several occasions the cereal products were reported as elaborated with a mix of different cereals, without further explanation. However, for some contaminated samples the mix of cereals was described on the label (wheat, maize, rye, oats, rice, in different proportions). One of the contaminated samples was described as made exclusively of rice.

[12] For the sample of mixed oilseeds the LB and UB are different as only one TA was quantified.

Table 5: Summary statistics of TA concentrations (µg/kg) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine in the different food samples. Left-censored data refer to samples where neither (-)-hyoscyamine nor (-)-scopolamine were reported. Concentration data were rounded to two significant figures. Only samples in bold were used for the exposure assessment (at FooodEx level 3).

FoodEx Level 1	FoodEx Level 2	FoodEx Level 3	N	NLC	Sum of (-)-hyoscyamine and (-)-scopolamine			
					Mean		Average last quartile	
					Lower bound	Upper bound	Lower bound	Upper bound
Fruiting vegetables	Berries and small fruits	Berries and small fruits	1	1	0.0	0.60	-	-
Food for infants and small children	Cereal-based food for infants and young children	Biscuits, rusks and cookies for children	27	27	0.0	0.60	-	-
		Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid	10	10	0.0	0.60	-	-
		Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids	**56**	**37**	**4.5**	**4.9**	**18**	**18**
Grains and grain-based products	Breakfast cereals	Cereal flakes (wheat, oat, mix)	7	7	0.0	0.60	-	-
	Grain milling products	Grain milling products	11	10	4.4	4.9	-	-
	Grains for human consumption	Buckwheat, millet, quinoa grains	5	5	0.0	0.60	-	-
Legumes, nuts and oilseeds	Legumes, beans, dried	Lupins (*Lupinus* spp.)	2	2	0.0	0.60	-	-
	Oilseeds (unspecified)	Oilseeds (unspecified)	5	4	0.060	0.60	-	-

N: number of samples; NLC: number of left-censored data.

4.2.4.2. Occurrence data in feed

As observed for the food samples, most of the reported data on feed were left-censored. From a total of 611 reported samples, in 557 neither (-)-hyoscyamine nor (-)-scopolamine were quantified. The left-censored data were treated by the substitution method as occurred for food samples. More than half of the left-censored data belong to the feed group 'Forages and roughage, and products derived thereof' (see Table 6). This feed group together with 'Legume seeds and products derived thereof' and 'Tubers, roots, and products derived thereof' were excluded from the exposure assessment as all the samples contained unquantified data (315 samples).

In Table 6 several sub-groups, within a specific group, are reported separately because of the very different occurrence values. Compound feeds were grouped according to the species/production categories for which the feed is intended (ruminants, poultry, equines and porcine). Among them, porcine compound feeds were those with the highest levels of contamination at the mean LB and UB as well as the subgroup where the maximum concentrations were reported (mean LB= 12 µg/kg, UB= 15 µg/kg). The lowest concentrations were reported for poultry compound feeds (approximately a 4 times lower concentration than in compound feeds for pigs). In five samples only levels of (-)-hyoscyamine were quantified, while in nine cases only (-)-scopolamine was reported. In those samples with both TAs quantified, (-)-hyoscyamine was always the predominant compound, except in one sample, with on average a 3-fold higher concentration than (-)-scopolamine.

A detailed analysis of the occurrence values in the group 'Cereal grains, their products and by-products' revealed the presence of three millet samples with high levels of (-)-hyoscyamine and (-)-scopolamine, particularly in one of the samples (1 600 µg/kg). These occurrence values, as well as published studies in the literature (Rwiza, 1991), indicate that millet seeds are prone to contamination with Datura seeds. Therefore, it was decided to consider separately millet seeds from the other cereals when carrying out dietary exposure assessment. As shown in Table 6, no contamination with TAs was reported in most of the other cereal samples (116 out of 118).

A similar analysis was carried out for the group 'Oil seeds, oil fruits, and products derived thereof'. The six samples of sunflower seeds (all quantified) are considered separately from the other samples based on their relatively high occurrence values (140 µg/kg as mean value for the sum of (-)-hyoscyamine and (-)-scopolamine)). After the millet seeds, the highest contamination was found in sunflower seeds. In the remaining feed groups, 'Other plants, algae and products derived thereof' and 'Other seeds and fruits, and products derived thereof' most of the samples did not contain quantified levels of TAs, although the few samples that contained quantified levels showed relatively high contamination.

Table 6: Summary statistics of TA concentrations (µg/kg) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine in the different feed samples. Left-censored data refer to samples where neither (-)-hyoscyamine nor (-)-scopolamine were reported. Only samples in bold were used for the exposure assessment. Concentration data were rounded to two significant figures.

N			N	NLC	Sum of (-)-hyoscyamine and (-)-scopolamine									
					Mean		Min		Max		Median		95th percentile	
					LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Compound feeds	**66**	**Ruminants**	**29**	**12**	**8.5**	**11**	**0.0**	**4.0**	**59**	**59**	**3.0**	**5.0**	**-**	**-**
		Poultry	**20**	**15**	**2.8**	**6.1**	**0.0**	**4.0**	**31**	**31**	**0.0**	**4.0**	**-**	**-**
		Equines	**1**	**0**	**4.0**	**6.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
		Porcine	**16**	**11**	**12**	**15**	**0.0**	**4.0**	**89**	**89**	**0.0**	**4.0**	**-**	**-**
Cereal grains, their products and by-products	**122**	**Millet grains**	**4**	**1**	**450**	**450**	**0.0**	**9.0**	**1600**	**1600**	**-**	**-**	**-**	**-**
		Other cereals (barley, maize, oats, rice, rye, sorghum, triticale, wheat)	**118**	**116**	**0.12**	**4.5**	**0.0**	**4.0**	**10**	**15**	**0.0**	**4.0**	**0.0**	**9.0**
Forages and roughage, and products derived thereof			301	301	0.0	9.0	-	-	-	-	-	-	-	-
Legume seeds and products derived thereof			13	13	0.0	9.0	-	-	-	-	-	-	-	-
Oil seeds, oil fruits, and products derived thereof	**71**	**Sunflower seeds**	**6**	**0**	**140**	**140**	**15**	**19**	**390**	**390**	**69**	**69**	**-**	**-**
		Other seeds (soya, palm kernel, rape and linseed)	**65**	**55**	**1.9**	**10**	**0.0**	**9**	**20**	**25**	**0.0**	**9.0**	**16**	**20**
Other plants, algae and products derived thereof			**32**	**28**	**20**	**28**	**0.0**	**9.0**	**360**	**360**	**0.0**	**9.0**	**-**	**-**
Other seeds and fruits, and products derived thereof			**5**	**4**	**24**	**31**	**0.0**	**9.0**	**120**	**120**	**-**	**-**	**-**	**-**
Tubers, roots, and products derived thereof			1	1	0.0	9.0	-	-	-	-	-	-	-	-

LB: lower bound; Min: minimum; Max: maximum; N: number of samples; NLC: number of left-censored data; UB: upper bound.

4.3. Food and feed processing

Crops grown for human or animal consumption do not contain (-)-hyoscyamine or (-)-scopolamine, and therefore toxicosis associated with food or feed is the result of contamination with foliage or seeds of the alkaloid-containing plants. A number of cases of toxicosis have been reported which were the result of cereal grains, oilseeds or maize (corn) contaminated with seeds of Jimson weed (*D. stramonium*). However, Jimson weed can now be controlled by a variety of pre- or post-emergence herbicides and appropriate seed cleaning, and where the weed is present it is the result of poor crop management.

Where seeds are harvested with the cultivated crop, they may be removed by mechanical separation. Compared to soybeans and cereal grains, *D. stramonium* seeds are small and are easily separated by mechanical screening (List and Spencer, 1976). This may explain the high level of TAs in samples of millet, which are smaller grains (see Table 6).

For most oilseeds, oil extraction involves the use of solvents, with the remaining meal being widely used as feed for livestock. List and Spencer (1976) confirmed that the majority (> 90 %) of TAs present in a 50:50 mixture of soybeans and Jimson weed seeds remained in the meal following oil extraction.

There is limited information on the stability and fate of TAs during storage and subsequent processing of food and feed, although it appears that TAs are relatively stable during drying and heat treatment of feed materials.

Reports of toxicosis in horses as a result of consuming hay contaminated with *D. stramonium* (Naudé et al., 2005) similarly suggest that TAs are stable under field drying conditions, and there are reports that livestock may be poisoned by *D. stramonium* where it is a contaminant of silage (Weaver and Warwick, 1984). There are no recent reports on the effect of ensiling on levels of TAs in contaminated silage. However, List and Spencer (1979) reported that livestock in the United States have perished as a result of consuming Jimson weed plants present in hay or silage, suggesting that the ensiling process does not destroy the TAs.

After baking, bread from wheat flour contaminated with Jimson weed seeds still contained 72-100 % of the TA content of the flour (Friedman and Levin, 1989). The breakdown of atropine and (-)-scopolamine in the crumb was 25 % and 13 %, respectively, while in the crust a respective reduction of 18 % and 28 % was found. The toxicosis that occurred in Turkey in 1949 as a result of consuming bread made from flour estimated to contain approximately 1 % of seeds from *D. stramonium* (Perharič, 2005) would also indicate that baking only partially reduces the TA content of the contaminated flour.

In a study on the effects of ingestion of low doses of atropine and (-)-scopolamine present as contaminants in food, Perharič et al. (2013b) prepared a traditional Slovenian dish, žganci. Atropine and (-)-scopolamine were added as standards in a 2:1 ratio to the buckwheat flour (atropine was added in the range of 0.25-25 mg/kg and (-)-scopolamine was added in the range of 0.125-12.5 mg/kg). Analysis by HPLC-MS/MS showed a reduction of the atropine content of nearly 60 % and of the (-)-scopolamine content of nearly 40 % after 12 minutes of cooking. The relatively high reduction during cooking (compared to bread baking) was attributed to the higher water content facilitating hydrolysis of the ester bond in atropine and (-)-scopolamine.

5. Food and feed consumption

5.1. Food consumption

5.1.1. EFSA's Comprehensive European Food Consumption Database

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built in 2010 based on information provided by EU Member States and the food consumption data for children obtained through an EFSA Article 36 project (Huybrechts et al., 2011). The Comprehensive Database version 1 contains results from a total of 32[13] different dietary surveys carried out in 22 different Member States covering more than 67 000 individuals (EFSA, 2011b). The Comprehensive Database includes individual food consumption data concerning infants (2 surveys from 2 countries), toddlers (8[13] surveys from 8 countries), children (16[13] 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).

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 methods were used between surveys to collect the data and thus direct country-to-country comparisons can be misleading. Similarly to what is described for the occurrence data, consumption records are also codified according to the FoodEx classification system. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011b).

The CONTAM Panel decided that acute dietary exposure to TAs should be evaluated because pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration (see Section 7.7). Therefore, all 32 dietary surveys (including those with only one day per subject) were potentially suitable for assessment of the dietary exposure (Appendix C, Table C1).

5.1.2. Food consumption data for different age and consumer groups

Only 423 eating occasions were found for consumption of 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' (FoodEx Level 3), the only food group in the Comprehensive Database for which the occurrence data were adequate for dietary exposure assessment (see Section 4.2.4.1). Traditionally, the different eating occasions reported for the same subject in one specific day are combined and the result, consumption day, is used for exposure calculation. This is due to the fact that for certain dietary surveys it is not clearly specified whether the eating occasions refer to one single meal or to the whole consumption in one specific day. Accordingly, the 423 eating occasions reported in the different surveys were initially grouped in a total of 266 consumption days. These consumption days mainly belonged to one age class, 'Toddlers' (243 consumption days), with few representatives for 'Other children' (16 consumption days), 'Infants' (6 consumption days) and 'Adults' (1 consumption day) (Table 7).

[13] When counting the total number of available dietary surveys and those for "Toddlers" and "Other children", the three Germans surveys named as Donald 2006, Donald 2007, and Donald 2008 are counted as only one survey since they were carried out using the same methodology (Dietary record). For more details on these surveys see Appendix C, Table C1.

Table 7: Consumption days across the different surveys that reported consumption of 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' at FoodEx Level3.

Dietary survey	Country	Number of consumption days[a]			
		Infants	Toddlers	Other children	Adults
AESAN-FIAB	Spain	-	-	-	1
DONALD (2006-2008)	Germany	-	197	13	-
DIPP	Finland	-	46	-	-
INRAN-SCAI 2005-06	Italy	6	-	2	-
STRIP	Finland	-	-	1	-
TOTAL		6	243	16	1

(a): When the number of consumptions days < 60, their use to calculate P95 exposure may not be statistically robust (EFSA, 2011b).

Considering the low number of consumption days in the other age classes, the CONTAM Panel decided to assess the dietary exposure assessment only in the age class 'Toddlers' (≥ 12 months to < 36 months old). Four surveys with consumption data on 'Toddlers' were available, three DONALD surveys (with 79, 56 and 62 consumption days) and the DIPP survey with 46 consumption days. In order to have a more statistically representative number of consumption days the three DONALD surveys were combined since they were conducted following the same methods (Table 7). Therefore, only those two surveys (DONALD (2006-2008) and DIPP) from two European countries were available for the exposure calculations.

Among the consumption data reported for toddlers, the 197 consumption days in the DONALD surveys were derived from 235 eating occasions while the 46 consumption days in the DIPP survey were derived from a total of 160 eating occasions. This indicates that in both surveys the consumption of the selected food occurred several times per day and individual, and this happened more frequently in the DIPP survey.

5.2. Feed consumption

As discussed above, TAs are found primarily in certain plants belonging to the *Solanaceae* family, including Jimson weed (*D. stramonum*), black henbane, (*Hyoscyamus niger*), mandrake (*Mandragora*), deadly nightshade (*Atropa belladonna*), *Brugmansia* spp., and *Solandra* spp. (Panter, 2005), none of which are cultivated for use as livestock feeds. However, it must be stressed that most reported cases of poisoning of animals have occurred when the seeds of TA-containing plants, and in particular seeds of *D. stramonium* or *D. ferox*, have been present as contaminants in cereals or oilseeds (van Kempen, 1992). TAs have also been reported in plants belonging to the *Erythroxylaceae*, and *Convolvulaceae* families. Some of these include fodder trees and shrubs, e.g. *Erythroxylum zambesiacum* and *lpomoea hardwickii,* which are important livestock feeds in dry tropical regions of Africa (Dicko and Sekena, 1992) but are not known to be used as feeds in Europe. Since none of these plants are commonly used as feeds for livestock, exposure to TAs therefore occurs as a result of accidental contamination of forages (in the case of grazing livestock) and/or contaminated grains.

For ruminant livestock (cattle, sheep and goats), forage crops represent their major - and often only - feed. These are predominantly grasses and legumes, and are generally highly palatable. In contrast, the leaves and stems of plants containing TAs have a pungent odour and taste making them unpalatable to most livestock, and therefore animals are likely to avoid these plants where they are present as weeds. Only where the amount of pasture available for grazing is severely restricted are livestock likely to consume these plants. Forages may also be conserved, either by drying or ensiling, for use when fresh forages are not available, and where TA-containing plants are present as weeds in the pasture they will be harvested along with the forage feed. Since the smell and taste associated with the TAs appears to be less noticeable in dried plants (EFSA, 2008), and livestock are unable to exercise the same selectivity as they do when grazing, the intake of TAs by ruminant livestock is likely to arise mainly where housed animals are fed contaminated forages.

Cereal grains and oilseeds are also widely used as livestock feeds. For ruminants, they are normally fed in conjunction with forages but for pigs and poultry they may account for 90 % or more of the total ration, with the actual amounts depending on the animal, its size and age and level of productivity. Appendix D gives example rations for different livestock used in assessing exposure.

6. Exposure assessment in humans and animals

6.1. Exposure assessment of tropane alkaloids in humans

6.1.1. Previously reported human exposure assessments

Only one dietary exposure assessment to atropine and scopolamine was found in the literature. As the biosynthesis of these TAs leads to (-)-hyoscyamine and (-)-scopolamine and no stereoselective separation is achieved in this work, analytical results are thus regarded as 100 % (-)-hyoscyamine or (-)-scopolamine. The exposure assessment was part of the risk assessment of buckwheat flour contaminated by thorn apple (*D. stramonium*) alkaloids carried out in Slovenia after a poisoning incident involving 73 consumers of buckwheat products (Perharič et al., 2013b). The exposure assessment included food samples involved in the outbreak, and it focused on those consumers affected by the poisoning and not on the general population. Most of the consumers (40 out of 46) reported the consumption of a typically Slovenian homemade dish (buckwheat žganci). Occurrence data were limited (84 samples). Most of the samples corresponded to buckwheat flour (43 samples) including nine samples of the buckwheat flour used by the poisoned consumers. Exposure assessment was mainly based on the concentration of TAs quantified in the samples of buckwheat flour and the amounts of this flour used as reported by the subjects. Dietary exposure was estimated for 12 individuals, with values that ranged between 0.7 and 137.6 µg /kg b.w. for (-)-hyoscyamine and 0.4 to 63.5 µg /kg b.w. for (-)-scopolamine (Perharič et al., 2013b).

6.1.2. Mean and high acute dietary exposure to tropane alkaloids

The acute dietary exposure to TAs was calculated on a per day basis for comparison with the acute reference dose (ARfD).

Deterministic approach

For the calculation of the acute dietary exposure, individual daily consumption data for 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' (FoodEx Level 3) were combined with the high level occurrence value of this food group. Since the number of samples in this food group was limited (< 60), the average of the last quartile was considered more statistically robust than the 95th percentile to represent the high level of contamination (see Table 5).

Acute dietary exposure was assessed for the age class 'Toddlers' (≥ 12 months to < 36 months old) for the two dietary surveys described in Section 5.1.2. Together with the acute exposure for average consumers, the 95th percentile exposure was calculated to represent the high consumers (Table 8). Acute dietary exposure for average consumers in the two surveys was 0.039 µg/kg b.w. per day and 0.107 µg/kg b.w. per day for the DONALD and DIPP surveys, respectively. Acute dietary exposure estimate for high consumers was 0.081 µg/kg b.w. per day in the DONALD surveys. All values used for the calculation of the average of the last quartile were quantified values. The exposure estimate for high consumers in the DIPP survey was not considered statistically robust due to the low number of consumption days (EFSA, 2011b).

Table 8: Acute dietary exposure to the sum of (-)-hyoscyamine and (-)-scopolamine (µg/kg b.w. per day) calculated for the age class 'Toddlers' in the two available surveys.

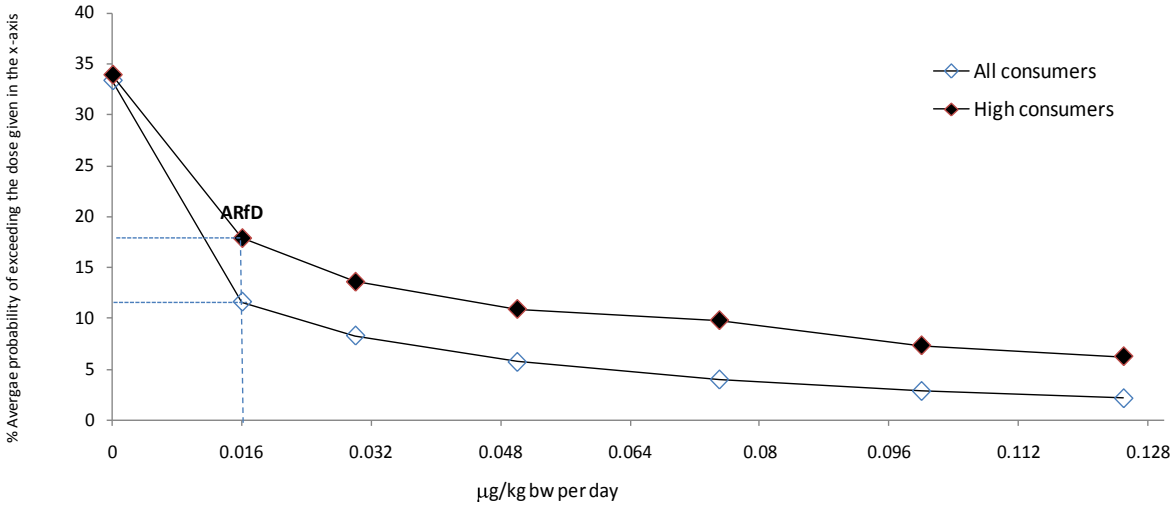
		DONALD (2006-2008) (N = 197)[a]	DIPP (N = 46)[a]
Toddlers	Average consumers	0.039	0.107
	High consumers	0.081	-[b]

(a): N refers to the number of consumption days available in each survey.
(b): The exposure value for high consumers is not reported since consumption days were lower than 60 (EFSA, 2011b).

Probabilistic approach

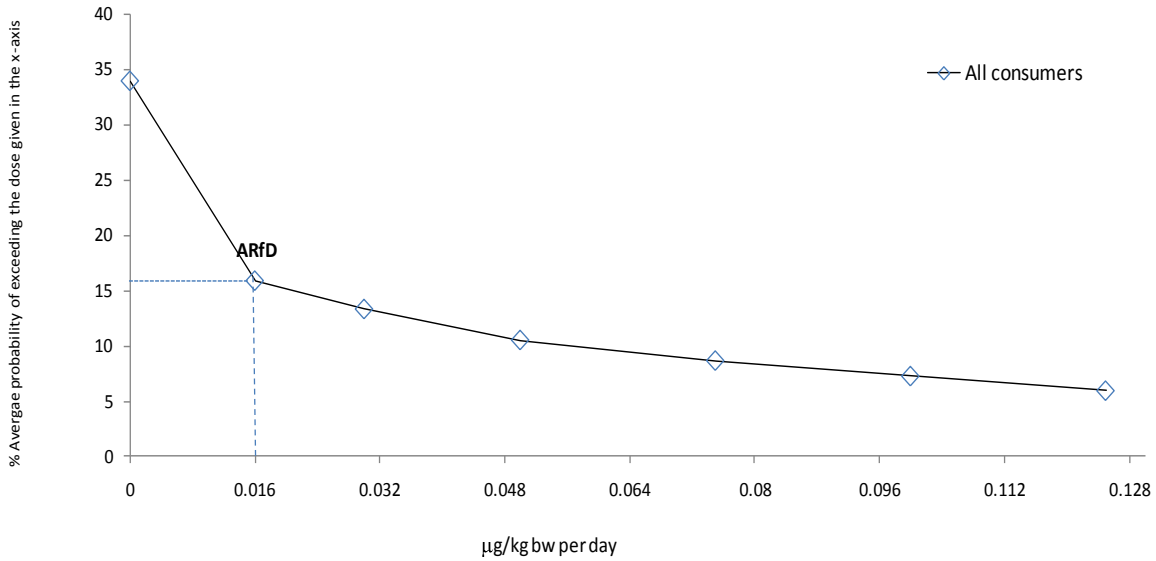
A refinement of the calculation of the acute dietary exposure was carried out by combining the toddler individual consumption data per day (consumption days) with the 56 occurrence values reported for 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' (FoodEx Level 3) at the LB. In each possible scenario of each consumption day, the estimated dietary exposure was compared to the group ARfD (0.016 µg/kg b.w., see Section 7.7.). An average probability was calculated by dividing the number of occasions the dietary exposure was above the group ARfD by the total number of occasions (56) in each consumption day. The average probability was estimated for all consumers and also specifically for high consumers (95th percentile consumption), the latter only for the merged DONALD surveys, as it possessed a statistically robust number of consumption days (EFSA, 2011b). Only days with consumption equal to or higher than the 95th percentile in the DONALD surveys (10 consumption days of the 197 available consumption days) were used for calculating exposure among high consumers. The average probability of being above the group ARfD was calculated as explained above.

No differences existed in the probability of being above the group ARfD using the LB or the UB approach. As shown in Figure 6 and 7, for all consumers the average probability was 11.6 % and 15.9 % in the DONALD surveys and DIPP survey, respectively. For high consumers the estimated average probability of being above the group ARfD was 17.9 %.



Legend: ARfD: acute reference dose; b.w.: body weight.

Figure 6: Distribution of the different average probabilities to exceed specific levels of exposure in the DONALD surveys for toddlers (all consumers and high consumers). Probabilities of being above the acute reference dose (ARfD=0.016 µg/kg b.w.; see Section 7.7.) are shown in the figure.



Legend: ARfD: acute reference dose; b.w.: body weight.

Figure 7: Distribution of the different average probabilities to exceed specific levels of exposure in the DIPP survey for all consumers. Probability of being above the acute reference dose (ARfD=0.016 µg/kg b.w.; see Section 7.7.) is shown in the figure.

As commented at the beginning of this Section, it is important to note that consumption days and not eating occasions were considered when the acute dietary exposure to TAs was assessed. This is especially important in the DIPP survey where 160 eating occasions were reported in only 46 consumption days. This fact implied that the consumption of food/day was much higher than in the DONALD surveys, where only in a few cases the consumption day contained more than one eating occasion/day of the same food (235 eating occasions/197 consumption days). Consequently, higher acute dietary exposure to TAs was observed in the DIPP survey compared to DONALD surveys.

6.1.3. Importance of non-dietary sources of human exposure

There is a potential for additional exposure to TAs from licensed medicines (see Section 7.5.).

6.2. Exposure assessment of tropane alkaloids in animals

A wide range of feeds and feeding systems are used for livestock and companion animals in Europe (Section 5.2). For many, feed is supplied in the form of commercially produced blends or compound feeds. While data on the TA content of compound feeds were provided by one Member State, the numbers of samples for any particular livestock species were generally small. For the majority of samples the product description was insufficient to identify the target species. As a result, it has not been possible to use data on the TA content of compound feeds to estimate exposure. Instead, the CONTAM Panel has identified example diets for a range of farm livestock and companion animals, details of which are given in Appendix D2, Table D4-D5.

In practice, livestock diets are formulated using a range of feed materials. As discussed above (Section 5.2) it is assumed that forages make no contribution to TA exposure by livestock. For most livestock, diets also normally contain a variety of non-forage feeds, particularly cereals, cereal by-products and oilseed meals. Insufficient data were provided on the TA concentrations for individual cereal types, and therefore LB and UB concentrations have been estimated for the general category "cereals and cereal by-products" (Table 6; Section 4.2.4.2). These, together with feed intakes (Appendix D1) have been used to estimate the exposure of livestock to TAs (Table 9).

As reported above (Section 4.2.4.2.), levels of TAs were particularly high in four samples of millet. Millet is an important food grain in many Asian and African countries, although both the growing crop and millet seeds can be used as feeds for livestock. However, production of millet in the EU is generally less than 80 000 tonnes per annum (according to the FAO database) and its most profitable market is in wild birdseed mixtures. Since millet is not widely used as a feed for livestock, exposure is based on levels of TA reported for all other cereal grains excluding millet (Table 9).

It must be stressed that the diet compositions used in estimating exposure are not 'average' diets, nor are they an attempt to describe 'worst case' scenarios. Rather, they are intended to provide an indication of likely exposure to TAs across a range of feeding systems in Europe.

6.2.1. Estimation of tropane alkaloids intake by farm livestock

6.2.1.1. Ruminants

Levels of TAs in grass and legume-based forages are low or non-existent (Section 4.2.4.2. and Appendix B, Tables B3 and B4), and therefore it has been assumed that they make no contribution to exposure. Table 9 provides estimated intakes by ruminants of TAs from non-forage feeds.

Based on levels of contamination in oilseed meals reported from Germany (Bucher and Meszaros, 1989), EFSA (2008) estimated a likely worst-case exposure to TAs by high yielding dairy cows. Assuming 12 kg compound feed per day, with an inclusion of 15 % of extracted soybean meal, they reported that this would result in an exposure of 20-80 µg/kg b.w., which is considerably higher than the estimate given in Table 9. It should be noted, however, that inclusion levels of soybean meal in dairy cow diets are normally significantly less than 15 %. Furthermore, it needs to be emphasized that these data date back to the late 1980s, and may not be typical of conditions today.

6.2.1.2. Pigs, poultry, rabbits, horses and fish

Based on feed intake data described in Section 5.2. and the mean LB and UB values for TAs in feeds (see Section 4.2.4.2.), estimates of the LB and UB exposures of TAs in diets and exposure by pigs, poultry, rabbits, horses and fish are given in Table 9.

As for ruminants, EFSA (2008) estimated likely exposure of pigs and poultry to TAs based on data from the German investigations (Bucher and Meszaros, 1989). For poultry consuming 120 g of extracted soybean meal, this would result in an exposure of 80-360 µg/kg b.w. at a body weight of 1.8 kg, and in pigs (40 kg body weight and 1600 g feed consumption) of 50-200 µg/kg b.w. Under the same assumptions the exposure from linseed products, which can be included at up to 40 % in diets for broilers and pigs, were calculated. In a worst-case scenario, in which animals obtain contaminated linseed expeller as sole linseed product at an inclusion rate of 40 %, exposure levels in broilers could reach 1 280 to 5 700 µg/kg b.w., and in pigs of 130-660 µg/kg b.w. Again, these estimates are considerably higher than those given in Table 9, which are based on levels in cereal grains reported above (Section 4.2.4.2.).

Diets of horses consist predominantly of forages, either fresh (grazed *in situ*) or conserved (e.g. as hay), supplemented with cereals where forages alone do not provide sufficient nutrients. The estimated exposure (Table 9) assumes 50 % of the diet consists of forages, with the remainder as cereals, cereal by-products, pulses feeds, minerals and vitamins.

The high estimated exposure for rabbits in Table 9 is due to the high proportion (20 %) of sunflower meal assumed to be in the diet. While this is the maximum likely to be used, in practice the amount will be influenced by the price and availability of other feed materials. As reported elsewhere (EFSA, 2008), rabbits have been identified to be comparatively resistant to TAs.

6.2.2. Estimation of tropane alkaloid intake by companion animals (cats and dogs)

Using the same approach, estimates of exposure to TAs by cats and dogs are given in Table 9.

Table 9: Dietary concentrations (µg/kg) and intake (µg/day and µg/kg b.w.) by farm livestock and companion animals for the sum of (-)-hyoscyamine and (-)-scopolamine, excluding data for millet. Separate estimates of exposure for (-)-hyoscyamine and (-)-scopolamine are given in Appendix E, Table E1.

		Diet concentration (µg/kg)	Intake (µg/day)	Intake (µg/kg b.w.)
Dairy: high yielding	LB	0.22	4.48	0.007
	UB	2.46	50.88	0.078
Beef: intensive cereal	LB	5.94	59.37	0.15
	UB	9.92	99.24	0.25
Beef: fattening	LB	0.07	0.65	0.002
	UB	0.87	8.39	0.021
Sheep: lactating	LB	3.57	10.00	0.17
	UB	6.38	17.87	0.30
Goats: lactating	LB	0.35	1.18	0.020
	UB	3.86	13.13	0.22
Goats: fattening	LB	0.19	0.28	0.007
	UB	2.13	3.20	0.080
Pig starter	LB	0.56	0.56	0.028
	UB	5.56	5.56	0.28
Pig finisher	LB	0.38	1.13	0.011
	UB	4.97	14.90	0.15
Lactating sow	LB	0.39	2.36	0.012
	UB	4.98	29.85	0.15
Chickens for fattening	LB	0.38	0.05	0.023
	UB	4.97	0.60	0.30
Laying hens	LB	0.50	0.06	0.030
	UB	5.49	0.66	0.33
Turkeys for fattening	LB	0.36	0.15	0.012
	UB	5.24	2.09	0.17
Ducks for fattening	LB	0.61	0.09	0.029
	UB	6.27	0.88	0.29
Horses	LB	0.05	0.44	0.001
	UB	2.30	20.66	0.046
Rabbits	LB	27.24	4.09	2.04
	UB	32.91	4.94	2.47
Salmonids	LB	0.26	0.011	0.005
	UB	2.34	0.09	0.047
Cats	LB	0.04	0.002	0.001
	UB	1.49	0.089	0.022
Dogs	LB	0.05	0.017	0.001
	UB	1.76	0.63	0.025

b.w.: body weight.

7. Hazard identification and characterisation

Most studies conducted to elucidate the toxicological or pharmacological properties of TAs use dosing and analytical strategies that do not differentiate between stereoisomers of hyoscyamine or its racemate, atropine, although there is evidence that (-)-scopolamine is not susceptible to racemisation in whole animal studies (Renner et al., 2005).

7.1. Toxicokinetics

7.1.1. Atropine

7.1.1.1. Absorption

Atropine is absorbed well from the human gastrointestinal tract. After a single oral administration of 2 mg ^{3}H-atropine in healthy volunteers, 90 % of the administered dose was estimated to be absorbed within one hour (Beerman et al., 1971). The absolute bioavailability of atropine has not been reported but 33-50 % of an oral dose is excreted into urine in a pharmacologically active form, suggesting substantial metabolism in the body (Kalser, 1971). Further evidence for rapid absorption was the reported maximal plasma atropine concentrations attained within 1 hour post-dosing (Beerman et al., 1971; Kalser, 1971; Ali-Melkkilaä et al., 1993).

7.1.1.2. Distribution

The distribution kinetics of atropine in humans is very rapid (half-life 1-2 min) following i.v. administration (Kanto and Klotz, 1988) and the magnitude of the apparent volume of distribution (210 L) is consistent with extensive tissue distribution (Hinderling et al., 1985). This rapid distribution of atropine to the tissues is consistent with the rapid onset of pharmacodynamic effects, which occur with the same time dependence as the plasma levels (i.e. stimulation of heart rate) (Kalser, 1971; Volz-Zang et al., 1995). Internal exposures to and effects of atropine appear to be greater in children and the elderly, albeit from different causes (i.e. a higher volume of distribution or decreased clearance, respectively (Virtanen et al., 1982)). Atropine readily crosses the human placenta based on similar concentrations occurring in maternal and fetal blood following i.m. administration to the mother (Kanto et al., 1981); however, under these conditions, penetration of the blood-brain barrier is more limited, based on lower concentrations occurring in maternal cerebrospinal fluid relative to blood.

7.1.1.3. Metabolism

Metabolism of atropine differs markedly between species with examples of glucuronidation and *N*-demethylation reactions reported (Kalser, 1971). A polymorphic serum carboxylesterase that cleaves atropine to tropic acid and tropine has been identified in rabbit serum, although such activity has not been observed in serum from humans, monkeys, goats, dogs, or guinea pigs (Harrison et al., 2006). In rodents, glucuronide conjugates of polar metabolites, but not of atropine itself, predominated in bile and urine; however, no tropic acid was present (Kalser, 1971). No evidence for formation of polar metabolites or tropic acid was reported in humans (Kalser, 1971). Glucuronidation of atropine was observed, albeit to a lesser extent than in rodents, by Kalser and McLain (1970) in early time points in urinary excretion profile in humans, but not in other studies (Gosselin et al., 1960; Kentala et al., 1990a). There is evidence for *N*-demethylation since exhalation of $^{14}CO_2$ was observed upon i.m. administration of 2 mg $^{14}CH_3$-N-atropine in human volunteers (1.4 – 3.0 % of total radioactivity measured in exhaled air in the post-treatment 3 hour period), whereas no radioactivity was detected in exhaled air when the same dose was administered with ^{14}C-labelling in the tropane ring. In both cases, total radioactivity was mainly excreted in urine over 24 hour post-dosing (ranging from 77 to 120 % in four volunteers) (Kalser, 1971). A pharmacokinetic study conducted by using GC-MS after i.v. administration of 1.35 – 2.15 mg atropine to human volunteers showed that tropine was a major metabolite (29 % of administered dose, Hinderling et al., 1985). The metabolism of $^{14}CH_3$-N-atropine was investigated in a single human volunteer by using HPLC analysis of urinary radioactivity and circular dichroism in comparison with profiles of authentic standards (Van der Meer et al., 1986). In this individual, 57 % of the administered radioactivity was

recovered in urine as (+)-hyoscyamine, 24 % as noratropine, 15 % as atropine-N-oxide, 3 % as tropic acid, and 2 % as tropine. The recovery of only (+)-hyoscyamine was interpreted as evidence of stereoselective metabolism of the active (-) isomer, primarily by *N*-demethylation and *N*-oxygenation with only a minor contribution from the hydrolysis pathway. Van der Meer et al. (1986) could not identify any atropine- or atropine metabolite -related conjugates (glucuronides, sulfates) after incubation of human urine samples with glucuronidase/sulfatase.

7.1.1.4. Excretion

In rodents and humans, more than 75 % of total radioactivity from radiolabelled atropine was excreted mainly in urine within 24 hour and 25-33 % of an oral dose to humans was excreted into urine in a pharmacologically active form as measured by a mouse eye bioassay, which was interpreted as unchanged atropine (Kalser, 1971). Fecal excretion appears to be a minor route (1.2-6.5 %, Beermann et al., 1971). Human excretion of parent compound in urine after single i.v. injection of 1.35 – 2.15 mg atropine was measured, using GC-MS, at 57 % along with 29 % as tropine (Hinderling et al., 1985); however, the remaining fraction was not identified. Glucuronide conjugates of polar atropine metabolites are excreted through the bile in rats and subjected to enterohepatic recirculation prior to excretion in urine (Kalser, 1971). The excretion of atropine into breast milk did not appear to be significant (O'Brien, 1974).

7.1.2. (-)-Scopolamine

7.1.2.1. Absorption

(-)-Scopolamine is absorbed rapidly from the gastrointestinal tract with maximal plasma concentrations observed within 0.5 hour (Renner et al., 2005). The absolute systemic bioavailability of orally administered (-)-scopolamine was measured at 13 %, based on areas under the curve (AUCs) for oral versus i.v. administration, suggesting substantial presystemic metabolism in the gastrointestinal tract and liver (Renner et al., 2005).

7.1.2.2. Distribution

Very limited information is available regarding the distribution of (-)-scopolamine but the magnitude of the apparent volume of distribution (141 L) is consistent with extensive tissue distribution (Renner et al., 2005). Based on similar concentrations in maternal and fetal blood following i.m. administration to the mother, it appears that (-)-scopolamine readily crosses the human placenta (Kanto et al., 1989); however, under these conditions, penetration of the blood-brain barrier appeared to be more limited, based on a lower concentration in maternal cerebrospinal fluid relative to blood.

7.1.2.3. Metabolism

Metabolism of (-)-scopolamine differs markedly between species with examples of O-glucuronidation, aryl and alkyl hydroxylation, tropic ester hydrolysis, dehydration of the tropic ester moiety, and *N*-demethylation reactions reported (Kentala et al., 1990b; Wada et al., 1991; Renner et al., 2005). The predominant metabolic pathway(s) were: in rats, aryl hydroxylation of the tropic acid moiety; in rabbits, hydrolysis of the tropic ester; in guinea pigs, tropic ester hydrolysis, dehydration, and *N*-demethylation; and in mice Phase II conjugation and *N*-demethylation (Wada et al., 1991). The information regarding human metabolism is incomplete. In humans, differently from what observed for atropine, glucuronidation/sulphation is a significant pathway, since approximately 22 % of i.m. administered (-)-scopolamine (5 mg/kg b.w.) was excreted in urine of pregnant women as Phase II metabolites (Kentala et al., 1990b). Significant pre-systemic metabolism in the gastrointestinal tract and liver was indicated by the 30 % increase in systemic bioavailability of orally administered (-)-scopolamine following pre-treatment of men and women with grapefruit juice, a well-recognized inhibitor of drug metabolism and transport (Ebert et al., 2000).

7.1.2.4. Excretion

Guinea pigs, mice, and some rabbits excreted into urine approximately 80-90 % of (-)-scopolamine injected subcutaneously (s.c.), and under the same conditions, rats excreted approximately 30 % (Wada et al., 1991). Following either i.v. or oral administration of 0.5 mg (-)-scopolamine to men and women, a urinary excretion of approximately 30 % as parent compound plus Phase II conjugates was measured within 24 hours after the administration (Ebert et al., 2000). Approximately 3 % of i.m. administered (-)-scopolamine (5 mg/kg b.w.) was excreted as parent compound in urine of pregnant women, while approximately 22 % was excreted as Phase II metabolites (Kentala et al., 1990b). The excretion of (-)-scopolamine into breast milk did not appear to be significant (O'Brien, 1974).

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

Acute toxicity was determined for atropine and (-)-scopolamine in several studies. Available LD_{50} values are summarised in Table 10 and Table 11.

Table 10: LD_{50} values determined in mice and rats for atropine.

Species	Route	LD_{50} (95 % confidence interval), mg/kg b.w.	Reference
Rat	i.v.	73[(a)]	Sax and Lewis (, 1992)
		89 (82-97)[(a)]	Wirth and Gösswald (1965)
		37 (32-44)[(b)]	Kalser et al. (1967)
		41 (40-43)[(b)]	Cunningham et al. (1949)[(c)]
		107 (98-116)[(b)]	Wirth and Gösswald (1965)
	i.m.	920[(a)]	Sax and Lewis (1992)
	i.p.	280 (225-350)[(b)]	Cahen and Tvede (1952)
		215 (203-227)[(b)]	Kalser et al. (1967)
	Oral	500 (442-565)[(a)]	Wirth and Gösswald (1965)
		600 (530-675)[(b)]	Wirth and Gösswald (1965)
		750 (620-900)[(b)]	Cahen and Tvede (1952)
Mouse	i.v.	30[(a)]	Sax and Lewis (1992)
		71 (63-81)[(a)]	Wirth and Gösswald (1965)
		31[(b)]	Sax and Lewis (1992)
		68 (60-77)[(b)]	Cunningham et al. (1949)[(c)]
		85 (75-97)[(b)]	Wirth and Gösswald (1965)
		87 (83-92)[(b)]	Lish et al. (1965)
		91[(b)]	Cazort (1950)[(c)]
	i.p.	320 (190-330)[(b)]	Cahen and Tvede (1952)
	Oral	1 050[(a)]	Frommel et al. (1961)
		75[(a)]	Sax and Lewis (1992)
		468 (376-581)[(b)]	Lish et al. (1965)
		400 (330-480)[(b)]	Cahen and Tvede (1952)

b.w.: body weight; i.v.: intravenous; i.m.: intramuscular; i.p.: intraperitoneal.
(a): Atropine (free base);
(b): Atropine sulphate;
(c): Reported by Wirth and Gösswald (1965).

Table 11: LD_{50} values determined in mice and rats for (-)-scopolamine.

Species	Route	LD_{50} (95 % confidence interval), mg/kg b.w.	Reference
Rat	Intraduodenal	670[(a)]	NTP (1997)
	Oral	1 270[(a)]	NTP (1997)
Mouse	i.v.	203[(a)]	NTP (1997)
		100[(b)] (32-316)	Atkinson et al. (1983)
	i.p.	650[(a)]	NTP (1997)
		400[(b)] (approximate LD_{50})	Morpurgo (1971)
	Oral	1 880[(a)]	NTP (1997)
		1 275[(b)]	Frommel et al. (1961)

b.w.: body weight; i.v.: intravenous; i.p.: intraperitoneal.
(a): (-)-Scopolamine hydrobromide trihydrate;
(b): (-)-Scopolamine (free base).

Buckett and Haining (1965) determined the acute toxicity of the two enantiomers of hyoscyamine and scopolamine in rats treated by i.v. injection (Table 12). The results indicated that stereoisomerism does not influence either lethality (LD_{50}) or the effective dose (ED_{50}) for induction of convulsions for hyoscyamine and scopolamine; however, this study also reported that several assays of peripheral and central anticholinergic effects (e.g. motility of the guinea pig ileum, midriasis, spontaneous motility) were preferentially activated by the (-)-enantiomers of hyoscyamine and scopolamine relative to the corresponding (+)-enantiomers.

Table 12: LD_{50} and ED_{50} values in mice exposed to the (+)- and (-)-enantiomers of hyoscyamine and scopolamine by intravenous (i.v.) injection as reported by Buckett and Haining (1965).

Tested substance	LD_{50} (95 % confidence interval), mg/kg b.w.	ED_{50} for the production of convulsions (95 % confidence interval), mg/kg b.w.
(+)-hyoscyamine sulphate	81 (78-83)	102 (90-115)
(-)-hyoscyamine hydrobromide	95 (88-102)	110 (97-124)
(+)-scopolamine hydrobromide	154 (134-178)	54 (47-62)
(-)-scopolamine hydrobromide	163 (150-176)	54 (48-60)

However, in a study in guinea pigs treated by i.v. injection (-)-hyoscyamine sulphate was more acutely toxic by a factor of approximately two than the racemic mixture atropine (LD_{50} of 48 mg/kg and 88 mg/kg reported for (-)-hyoscyamine sulphate and atropine, respectively) (Lieber, 1957).

The CONTAM Panel noted that it is not possible to draw precise conclusions on the apparent inconsistency of the acute toxicity data for the (-)- and (+)-enantiomers of hyoscyamine and scopolamine.

7.2.2. Repeated dose toxicity

Studies have been conducted on the toxicity of a combination of **atropine** and **(-)-scopolamine** in ratios based on the relative proportions of these alkaloids measured in *D. stramonium* seeds. Following administration of 5.2 mg/kg b.w. per day of **atropine sulphate** plus 2.6 mg/kg b.w. per day of **(-)-scopolamine bromide** (4.5 and 2.1 mg/kg b.w. per day atropine and (-)-scopolamine base, respectively) for 4 weeks or of 4.2 mg/kg b.w. per day of **atropine sulphate** plus 1.6 mg/kg b.w. per day of **(-)-scopolamine bromide** (3.9 and 1.3 mg/kg b.w. per day atropine and (-)-scopolamine base, respectively) for 120 days by the i.p. route to male Wistar rats, the major findings were indications of liver damage, which were more severe with the longer term treatment (Bouzidi et al., 2011).

The toxicity of **(-)-scopolamine hydrobromide trihydrate** (89 % pure) has been studied by the US National Toxicology Program (NTP) in F344/N rats and B6C3F1 mice following administration by gavage for 16 days, 14 weeks or 2 years (NTP, 1997).

In the 16-day study, groups of 5 male and 5 female animals were dosed at 0, 150, 250, 450, 900 and 1 800 mg/kg b.w. per day (mice) or 0, 75, 150, 300, 600 and 1 200 mg/kg b.w. per day (rats). In the mice, one male and two females died at the highest dose, and one female died at the lowest dose. Body weight gains and the final mean body weights at all dose groups were similar to controls. Clinical findings included bilateral pupillary dilation and squinting in all treated animals. At the highest dose, the relative liver weights were statistically greater than those of the controls. Based on the mortality at 1 800 mg/kg b.w. per day in the 16-day study, a highest dose of 1 200 mg/kg b.w. per day was selected for the 14-week study in mice.

In the rats, there were no deaths. Body weight gain was statistically significantly decreased at doses ≥ 300 mg/kg b.w. per day, final body weight was about 90 % of control at 600 and 1 200 mg/kg b.w. per day. Clinical findings included bilateral pupillary dilation in all treated animals and red eyelids in both males and females at the highest dose. There were no biologically significant changes in organ weights or gross or microscopic lesions. The highest dose of 1 200 mg/kg b.w. per day was selected as the highest dose for the 14-week study (NTP, 1997).

In the 14-week study, groups of 10 male and 10 female rats and mice were dosed at 0, 15, 45, 135, 400 and 1 200 mg/kg b.w. per day. In the mice, one male died at 135 mg/kg b.w. per day, and 2 males and 1 female at 1 200 mg/kg b.w. per day. A cause of death was not reported. Body weight gains and the final mean body weights were statistically significantly lower than controls at all doses in the males and at ≥ 45 mg/kg b.w. per day in the females. Clinical observations included bilateral pupillary dilation, hyperactivity and hypoactivity. A minimal to mild mature neutrophilia, similar to that in the male rats, occurred in male mice at ≥ 45 mg/kg b.w. per day. The estrous cycle length was statistically significantly increased in the highest dose females; there were no changes in sperm parameters. Ten female and 15 male rats, divided amongst all except the lowest dose group, died from one week onwards. Of these, the deaths of 9 males and 5 females were considered to be due to tracheal and oesophageal obstructions, secondary to the inhibitory effects of (-)-scopolamine on salivary gland secretions and swallowing. Explanations were not provided for the other deaths. Body weight gains and the final mean body weights at all dose groups were statistically significantly lower than controls. Clinical findings included bilateral pupillary dilation in all treated rats. Small but statistically significant increases in haematocrit, haemoglobin concentration and/or erythrocyte count were seen in all except the lowest dose group, which was considered to be due to dehydration. A minimal to mild mature neutrophilia, presenting as higher segmented neutrophil numbers, occurred in all dosed male rats, which was not accompanied by microscopic evidence of neutrophilia. Absolute and relative liver weights were increased in the females at the highest dose, and absolute and relative thymus weights were decreased in most of the dose groups. There were no biologically significant changes in other organ weights or gross or microscopic lesions, and no changes in sperm morphology and vaginal cytology parameters (NTP, 1997).

In the mouse 2-year study, groups of 70 males and 70 females were dosed at 0, 1, 5 and 25 mg/kg b.w. per day. Ten males and 10 females of each dose group were evaluated at 15 months. Survival of treated animals was similar to that of controls. Mean body weights were similar to controls at 1 mg/kg b.w. per day, slightly lower than controls at 5 mg/kg b.w. per day and at 25 mg/kg b.w. per day were statistically significantly lower than in controls at both sexes from week 13 onwards. Clinical findings included bilateral pupillary dilation in all dose groups but there were no significant findings in ophthalmic examinations. Haematocrit, haemoglobin concentration and erythrocytes count were slightly lower in females at 25 mg/kg b.w. per day compared to controls, which was thought to be associated with anaemia due to lower body weights and decreased nutritional status. There were no treatment-related increases in non-neoplastic lesions, in contrast the incidence of some spontaneous lesions decreased compared to controls, which was considered related to the lower body weights. The neoplastic findings from this study are discussed in Section 7.2.5. (NTP, 1997).

In the rat 2-year study, groups of 60 male and 60 female rats were dosed at 0, 1, 5 and 25 mg/kg b.w. per day. Ten males and 10 females of each dose group except the 1 mg/kg b.w. per day females were evaluated at 15 months. The survival rates of female rats receiving 1 (mainly not (-)-scopolamine-related) and 25 mg/kg b.w. per day were statistically significantly lower than controls. Mean body weights at 1 and 5 mg/kg b.w. per day were similar to controls, whereas at 25 mg/kg b.w. per day the mean body weights were statistically significantly decreased in males from weeks 25-97, and from week 25 to the end of the study in females. Clinical findings included bilateral pupillary dilation in all dose groups but there were no significant findings in ophthalmic examinations. At 25 mg/kg b.w. per day, haematocrit was elevated in the males, and reticulocyte numbers were decreased in the females. Neurobehavioural changes were assessed on days 1, 90, 180, 270, 360 and 720, using tests for motor activity, grip strength, thermal sensitivity, startle responsiveness and passive avoidance. Decreased horizontal motor activity was observed in females on days 90, 180 and 360 at 25 mg/kg b.w. per day, startle response was lower on day 90 in the females at 5 and 25 mg/kg b.w. per day, passive avoidance was significantly lower on day 180 in the males at 25 mg/kg b.w. per day. The neoplastic findings from this study are discussed in Section 7.2.5. (NTP, 1997).

Overall, the CONTAM Panel concluded that, based on the bilateral pupillary dilation in rats and mice, the lowest dose of 1 mg/kg b.w. per day (-)-scopolamine hydrobromide trihydrate (corresponding to 0.7 mg/kg b.w. per day (-)-scopolamine) was a lowest-observed-effect level (LOEL) for pharmacological activity.

7.2.3. Developmental and reproductive toxicity

Malformations were reported in the fetuses of rabbits dosed with **(-)-scopolamine hydrobromide** in an article published in the Australian Journal of Biological Sciences (McBride et al., 1982). However, the journal later issued a notice that deliberate falsification occurred in the paper (Australian Journal of Biological Sciences, 1988). Therefore the CONTAM Panel considered that the results were invalid.

The NTP conducted teratology studies on **(-)-scopolamine hydrobromide trihydrate** at doses of 0, 10, 100, 450 and 900 mg/kg b.w. per day administered by gavage to CD-1 mice (23-32 dams per treatment group) and CD rats (21-28 dams per group) through gestation days 6-15. Caesarean sections were performed on gestation day 17 in mice and 20 in rats. The number and status of uterine implantation sites was recorded. Each live fetus was weighed, sexed, and examined for external, visceral, and skeletal malformations (NTP, 1987a,b).

In the mice, there were no adverse effects on prenatal viability, no evidence of teratogenesis, and only a marginal reduction in fetal body weight at doses of 450 and 900 mg/kg b.w. per day, which also caused marginal maternal toxicity (decreased body weight and weight gain). The no-observed-adverse-effect level (NOAEL) was 100 mg/kg b.w. per day (corresponding to 70 mg/kg b.w. per day (-)-scopolamine) (NTP, 1987a).

In the rats, at doses greater than or equal to 100 mg/kg b.w. per day, there was a marginal non-dose-related reduction in fetal body weight. At doses greater than or equal to 450 mg/kg b.w. per day, there was a significant increase in the incidence of short ribs. These effects were accompanied by significant dose-related maternal toxicity (reduced maternal body weight and weight gain). Marginal evidence of intrauterine growth retardation, and a non-dose-related trend toward an increase in the incidence of malformations was observed only at doses that caused significant maternal toxicity. The NOAEL was 10 mg/kg b.w. per day (corresponding to 7 mg/kg b.w. per day (-)-scopolamine) (NTP, 1987b).

7.2.4. Genotoxicity

Atropine

Early studies showed no mutagenic activity of atropine sulphate in the Ames assay, using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 in the presence of rat liver S9 (McCann et al., 1975).

(-)-Scopolamine

In an early study of Waskell (1978) no mutagenic effects were found for (-)-scopolamine in *Salmonella typhimurium* strains TA98 and TA100 both in the presence and absence of rat liver homogenate.

In an NTP assay (-)-scopolamine hydrobromide trihydrate (100 to 10 000 μg/plate) did not induce mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537, with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. No convincing induction of sister chromatid exchanges was found with (-)-scopolamine hydrobromide trihydrate in tests in cultured Chinese hamster ovary cells, at concentrations up to 500 μg/mL without S9, or 5 000 μg/mL with S9. (Some positive results were found but these were caused by alteration in pH produced by high concentrations of (-)-scopolamine hydrobromide trihydrate) (NTP, 1997).

No induction of chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with (-)-scopolamine hydrobromide trihydrate without S9. However, with S9, increases in the percentage of cells with aberrations were noted in each of two trials, at the highest concentration tested (5 000 μg/mL) even in the presence of HEPES buffer to maintain optimum pH (NTP, 1997).

No increase in the frequency of micronucleated normochromatic erythrocytes was found in peripheral blood samples obtained from male and female $B6C3F_1$ mice at the end of 14-week gavage studies of (-)-scopolamine hydrobromide trihydrate (0, 15, 45, 135, 400, 1 200 mg/kg b.w. per day) (NTP, 1997).

All together the available information, although limited, indicates no concern for genotoxicity by atropine and (-)-scopolamine.

7.2.5. Carcinogenicity

Atropine

Sprague-Dawley rats (30 males and 31 females) were administered atropine (i.p., 6 mg/kg b.w. per week) (Schmähl and Habs, 1976). The rats were kept under observation up to their natural death. Atropine had no influence on tumour frequency or mean life-span compared to a control group.

Cabello et al. (2001) carried out an investigation of organophosphorus pesticides on induction of mammary tumours in Sprague Dawley rats, which involved studying the effects of atropine (used in the treatment of poisoning with acetylcholinesterase inhibitors) on the effects produced by the pesticides. The effects of atropine were also studied separately from the pesticides. Virgin female rats (Sprague-Dawley, group of 70 animals) at 39 days of age were injected intraperitoneally with atropine (2.5 mg/kg b.w., twice a day for 5 days). The animals were housed three per cage for 28 months and were palpated weekly to detect formation of tumours. After a tumour was detected, the data were recorded and tumours were allowed to develop for one month. After that time the animals were sacrificed. None of the 70 animals treated with atropine had mammary tumours. The same result was found when combinations of atropine with parathion, malathion or eserine were used.

Tatsuka et al. (1989) investigated if atropine can promote experimental carcinogenesis in rat stomach caused by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Male Wistar rats (5 weeks old) were given drinking water containing 50 μg/mL MNNG for 25 weeks. At week 26 a group of 20 rats was given

0.5 mg/kg b.w. atropine per day in an olive oil suspension, up to week 52. Control animals received only olive oil after the MNNG treatment. Administration of atropine had no influence on the incidence of gastric cancer (controls 15/18 rats, atropine-treated 15/19 rats), but it significantly increased the number of gastric tumours per animal (control 1.2±0.2 gastric tumours/rat, atropine-treated 2.7 ± 0.4 gastric tumours/rat, $p < 0.001$). The CONTAM Panel noted that this study is inadequate to draw conclusions due to the design of the study, the limited number of animals and the high incidence of tumours in the non-atropine treated animals.

In summary, in two limited studies on the carcinogenic potential of atropine no carcinogenic effects were observed.

(-)-Scopolamine

Rats

Groups of 60 male and 60 female F344/N rats were administered 0, 1, 5, or 25 mg **(-)-scopolamine hydrobromide trihydrate**/kg b.w. per day in distilled water by gavage for 104 weeks. After 15 months, an interim evaluation of ten males and ten females from each dose group, excluding the 1 mg/kg b.w. per day female group, was carried out. The remaining animals were used for the carcinogenicity study. No carcinogenic activity was detected. The incidences of mononuclear cell leukaemia in males and females treated with 25 mg/kg b.w. per day were significantly lower than those of the control groups (NTP, 1997).

Mice

Groups of 70 male and 70 female $B6C3F_1$ mice were administered 0, 1, 5, or 25 mg **(-)-scopolamine hydrobromide trihydrate**/kg b.w. per day in distilled water by gavage for 104 to 105 weeks. After 15 months an interim evaluation of groups of control animals and animals from each dose level was carried out. The group size for this was 20 with the exception of the female control group and the 25 mg/kg b.w. per day female group where it was 19. The remaining animals were used for the carcinogenicity study. No carcinogenic activity was detected. The incidences of many spontaneously occurring non-neoplastic lesions were significantly lower in dosed mice than in the control groups and usually decreased with increasing dose. Dosed animals had lower body weights than controls and the decreased incidences of these spontaneous lesions were thought most likely to be a result of this (NTP, 1997).

In summary, under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity of (-)-scopolamine hydrobromide trihydrate in male or female F344/N rats or $B6C3F_1$ mice administered 1, 5, or 25 mg/kg b.w. per day.

7.3. Modes of action

The naturally occurring tertiary amines (-)-hyoscyamine and (-)-scopolamine are antimuscarinic agents which are antagonists of muscarinic ACh receptors. These receptors are primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves. Naturally occurring TAs appear to be relatively non-selective for the 5 different types of muscarinic receptors (M_1, to M_5) pharmacologically identifiable. At therapeutic doses these tertiary amine antimuscarinics have little effect on nicotinic ACh receptors.

The functions of the muscarinic receptor subtypes M_1 to M_5, which have distinct anatomical locations in the periphery and the CNS, are mediated by interactions with G proteins. M_1 receptors are found in the CNS (cerebral cortex, hippocampus and striatum), in autonomic ganglia and glands (gastric and salivary). M_2 receptors are widely expressed in the CNS, heart, smooth muscle and autonomic nerve terminals. M_3 receptors are also located in the CNS, and abundant in smooth muscle and heart. M_4 receptors are preferentially expressed in the forebrain. M_5 receptors are expressed at low levels in the CNS and the periphery. This subtype is predominant in dopamine neurons in the ventral tegmental area and the substantia nigra of the CNS (Brown and Taylor, 2006).

The predominant peripheral antimuscarinic effects of TAs are decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and paralysis of accommodation (cycloplegia), change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion.

(-)-Hyoscyamine and (-)-scopolamine differ in their antimuscarinic actions, particularly in their ability to affect the CNS. (-)-Hyoscyamine rarely has effects on the CNS at doses that are used clinically. In contrast, (-)-scopolamine has prominent depressing central effects at low therapeutic doses (see Section 7.5.2.1.) (Brown and Taylor, 2006). In neuropharmacology, (-)-scopolamine has been used as a reference compound to investigate the role of the cholinergic system in age- and dementia-related memory and cognitive deficits in experimental animals and humans (Flood and Cherkin, 1986; Ebert and Kirch, 1998). Regarding the role of the muscarinic receptors in the CNS, it has been hypothesized that ACh has a main involvement in attentional processes and plasticity of sensory neurons rather than learning and memory functions (Blokland, 1996; Everitt and Robbins, 1997; Rasmusson, 2000).

Klinkeberg and Blokland (2010) extensively reviewed the use of (-)-scopolamine to induce various neurobehavioural effects in different species. In particular, behavioural changes such as delayed visual stimulus discrimination, decreased visual or auditory attention were reported at systemic (-)-scopolamine doses as low as 0.005 mg/kg b.w. or 0.02 mg/kg b.w. in rats administered by i.p. injection, respectively; other neurobehavioural effects such as delayed auditory stimulus discrimination, increased locomotor activity and altered learning and memory functions were reported at higher doses. The overall picture is complicated by the high variability observed with different methods and in different animal models. Furthermore, the neurobehavioural effects at CNS level are often confounded or masked by the effects at peripheral level (e.g. pupillary dilation and visual stimulus discrimination) (Klinkeberg and Blokland, 2010). Finally, the CONTAM Panel noted that the studies reported in the Klinkeberg and Blokland review (2010) cannot be used for hazard characterisation, since no study by oral exposure is available and the studies were often not designed for establishing a dose-response relationship.

At toxic doses, (-)-hyoscyamine and (-)-scopolamine cause stimulation of the CNS with restlessness, disorientation, hallucinations, and delirium. As the dose increases, stimulation is followed by central depression leading to death from respiratory paralysis (Martindale, 2011a).

The anticholinergic activity of atropine has been historically attributed to its naturally-occurring form: (-)-hyoscyamine (Brown, 1990 as cited by Ghelardini et al., 1997). The affinity for the muscarinic receptor subtypes found in both functional and binding studies is always higher for (-)- than for (+)-hyoscyamine. The (-)-enantiomer was twice as potent as the atropine racemate in its antimuscarinic activity (Weiner, 1980). In functional experiments on guinea-pig ileum and rat atrium, Barlow et al. (1973, cited by Leffingwell, 2003) and Ghelardini et al. (1997) observed a 300 fold difference and 50 fold difference in affinities between (-)- and (+)-hyoscyamine, respectively. In genetically engineered Chinese hamster ovary cells, the affinity of the M_2 muscarinic receptors for (-)-hyoscyamine was 36 times higher than that for the (+)-enantiomer (Ghelardini et al., 1997). Ricny et al. (2002) confirmed that the action of hyoscyamine is stereospecific and receptor-mediated and observed an enantioselective action of (-)-hyoscyamine in the promotion of the synthesis of cyclic adenosine monophosphate (cAMP) in cardiac ventricular membranes, with the (+)-enantiomer having a 30-fold lower potency.

In other studies (Ghelardini et al., 1990, 1997), it has been reported that atropine in animal experiments is either a cholinomimetic or a cholinolytic drug, depending on the dose. Very low doses of atropine which were inadequate for blocking muscarinic receptors (1-100 µg/kg *in vivo*; 10^{-14}-10^{-12}M *in vitro*) increased cholinergic neurotransmission (antinociception of a cholinergic type in rodents and potentiation of the evoked contractions of guinea-pig ileum), while at higher doses (0.5-5 mg/kg *in vivo;* >10^{-9} M *in vitro)* the drug is endowed with the classic antimuscarinic property. A cholinomimetic paradoxical effect was also observed by Ferguson-Anderson (1952, cited by Ghelardini et al., 1997) and Brown (1990), who reported that in humans low doses of atropine caused an increase in gastric contraction frequency and amplitude, and decreased heart rate. Ghelardini et al. (1997) deduced that the dose-related behaviour of

atropine might be due to different activity of its two enantiomers. They found that (+)- and (-)-hyoscyamine have different stereochemical requirements on muscarinic presynaptic autoreceptors and muscarinic postsynaptic receptors. As a result, while (-)-hyoscyamine at low concentration level (5 µg/kg i.p.) did not change basal ACh release, (+)-hyoscyamine at the same dose induced a statistically significant increase in ACh release (+ 64 %) and thus being responsible for the indirect cholinomimetic activity of low doses of atropine (Ghelardini et al., 1997). Only (+)-hyoscyamine was able to induce antinociception in the mouse hot-plate test, while the (-)-enantiomer was completely inactive (Ghelardini et al., 1992, as cited by Bartolini et al., 2011). Similar results were obtained using the paw-pressure test in rats and the writhing test in mice (Ghelardini et al., 1997). *In vitro* experiments on electrically-evoked contractions of guinea pig ileum confirmed that the effectiveness of very low concentrations of atropine is due to the (+)-enantiomer. Its antinociceptive effect and its potentiating activity on ileum and diaphragm muscle contractions, obtained in the low dose-range, progressively disappear with increasing doses. In the antimuscarinic range of concentrations, (-)-hyoscyamine is more potent than (+)-hyoscyamine. The selective activity of low concentrations of (+)-hyoscyamine has been tentatively explained by the hypothesis that the (+)-enantiomer binds to presynaptic ACh receptors with negative feedback regulation. These presynaptic receptors could display constitutive activity in the absence of any ligand and (+)-hyoscyamine could act as an inverse agonist shifting the equilibrium of the presynaptic receptor towards the inactive form and thus triggering the ACh release in the synapsis (Romanelli et al., 1996; Dei et al., 1997). At higher doses of (+)-hyoscyamine the presynaptic effect on ACh release would be counterbalanced by the postsynaptic receptor blockade (Dei et al., 1997).

The CONTAM Panel concluded that the naturally occurring (-)-hyoscyamine and (-)-scopolamine are mainly responsible for the antimuscarinic activity. There is some evidence that the (+)-enantiomers could have cholinomimetic activity, however they are not naturally occurring in plants and there is insufficient information on potential for racemisation in animals or humans for a possible effect of the (+)-enantiomers to be taken into account in the risk assessment.

7.4. Adverse effects in livestock, fish and companion animals

There are considerable inter-species differences in the adverse effects of TAs (EMEA, 1998b). However, since *Datura* plants may contain a number of different alkaloids, it is difficult to identify the exact compound responsible for the intoxication when ingested via feed, as the observed biological effects might be attributable to several compounds.

Plants containing TAs are unpalatable to most livestock, and therefore toxicity in grazing livestock is relatively uncommon. Although not frequent, *Datura* intoxications, sometimes fatal, have been described in the veterinary literature, particularly in horses and cattle following the consumption of contaminated hay. Other cases of incidental intoxications in buffaloes, sheep, goats, swine, mules, and ostriches have been reported (Cooper and Johnson, 1984). Rabbits and poultry species are believed to be more resistant to TA, presumably due to the (varying) expression of specific hydrolytic enzymes (atropine-hydrolases) that can cleave and inactivate the majority of TAs (Werner and Brehmer, 1967). Henbane (*Hyoscyamus niger*) is also widely acknowledged as being poisonous, but there are few reports of toxicosis as a result of consuming the plant (Cooper and Johnson, 1984), probably due to the disagreeable odour and sticky texture of the plant that makes it unattractive to livestock.

The predominant symptoms of intoxications in livestock are consistent with the well-known anticholinergic effects of TAs, which may include hyposalivation, tachycardia, hyperventilation, pupil dilation, restlessness, nervousness, muscle tremor, convulsions, delirium and death from asphyxia. In small ruminants such as goats and sheep, typical symptoms also include drowsiness and reduced ability to stand (Piva and Piva, 1995).

There is some uncertainty regarding the susceptibility of different livestock species to TA poisoning. Piva and Piva (1995) suggested that pigs were the most susceptible farm livestock species, followed by cattle, horses and chickens. However, Naidoo (2012) proposed that the order should be horses > pigs > cattle > poultry, based on susceptibility and chance of exposure.

The adverse effects in livestock and companion animals were reviewed by EFSA (2008); since little new information has been published subsequently, their conclusions are briefly summarised below.

7.4.1. Ruminants

7.4.1.1. Cattle

Ruminants generally avoid eating Jimson weed unless other vegetation is unavailable, but may be poisoned by ingesting it as a contaminant of hay or silage (Weaver and Warwick, 1984). One of the first effects following ingestion of Jimson weed seeds is rumen stasis. In a study reported by Nelson et al. (1982), cattle fed approximately 107 *Datura* seeds per kilogram body weight developed anorexia and rumen stasis; they did not succumb to atropine intoxication because they stopped eating the seeds (Nelson et al, 1982). When 11 one year-old heifers were given a feed containing different amounts of *D. stramonium* seeds (0, 8.8, 881, or 4 408 seeds per kg diet) for 14 days, the animals given the highest dose failed to consume their daily ration (Nelson et al., 1982). They developed anorexia from the first day; subsequent symptoms included bloat, dry mucosal surfaces followed by mieosis and constipation. Toxicity in cattle as a result of consuming feed contaminated with *D. stramonium* therefore appears to be a self-limiting problem, with rumen atony and anorexia preventing further intoxication until the blood levels of alkaloids are reduced to allow normal intestinal function to resume. Unless a feed that is highly contaminated with Jimson weed seed is ingested (greater than 0.09 % of body weight) or force-fed, death should be a rare consequence of Jimson weed seed contamination (Nelson et al, 1982).

Based on the limited available data, EFSA (2008) concluded that cattle are sensitive to Datura alkaloids, and that although signs of toxicity are likely to occur at levels exceeding 500 µg (-)-hyoscyamine plus 100 µg (-)-scopolamine per kg b.w., levels of up to 300 µg/kg b.w. (total alkaloids) may be safe. Since no data have been published since 2008 to alter this conclusion, the CONTAM Panel propose this as a NOAEL for cattle.

7.4.1.2. Small ruminants (sheep and goats)

Sheep and goats appear to be able to ingest large amounts of dried belladonna plant material (*Atropa belladonna*) without adverse effects due to their ability to express an enzyme (atropinesterase) in plasma, which hydrolyses the alkaloid (Piva and Piva, 1995; EMEA, 1998a; Harrison et al., 2006). However, the level of atropinesterase in goats varies with season (the concentration of the enzyme is lower in winter) and between breeds (Liebenberg and Linn, 1980; El Dirdiri et al., 1981). In a study reported by El Dirdiri et al. (1981), desert sheep given 10 g/kg b.w. per day of fruits or leaves of *D. stramonium* died within 38 days; however when similar diets were fed to Nubian goats death only occurred after 136 days. The symptoms observed for both sheep and goats included reduced water consumption, anorexia, intermittent hyperesthesia, tremors, drowsiness and recumbence.

7.4.2. Pigs

Pigs are amongst the most sensitive of the farm livestock species to TAs (Piva and Piva, 1995; Naidoo, 2012). The adverse effects of progressive atropine poisoning in pigs include a reduction in feed intake and growth rate, gastrointestinal motility and secretary activity, increased respiration and cardiac rate and pupil dilation. In a study in which pure (-)-hyoscyamine and (-)-scopolamine (ratio 2:98) was fed to fattening pigs, Piva (1993) identified a provisional NOAEL of 1500 µg TA/kg feed. In a review of available data, Piva and Piva (1995) also concluded that the threshold limits for toxicosis of pigs (20-60 kg b.w.) consuming feed with Datura alkaloids is approximately 1500 µg TA/kg feed, which corresponds to approximately 60 µg/kg b.w. for a 60 kg pig. In a subsequent study, Piva et al. (1997) observed that the intake of alkaloids added to a commercial feed as a synthetic mixture (in the ratio 0.98 (-)-scopolamine:0.02 (-)-hyoscyamine, corresponding to the ratio found in *D. ferox*) at 1500 µg TA/kg feed resulted in growth inhibition. They concluded that 1500 µg TA/kg feed which is much lower than the estimated EU limit of 4.68 mg/kg - is not acceptable for long-term exposure, and a lower limit for *D. ferox* should be considered. However, they concluded that further studies are needed to establish

whether the toxicity of synthetic alkaloids in pigs differs from that of naturally occurring alkaloids in feedingstuffs.

However, as with other livestock, pigs find the seed unpalatable and are likely to reject the contaminated feed before becoming seriously intoxicated (Worthington et al., 1981; Janssens and de Wilde, 1989).

Conflicting reports exist as to the effects of *Datura* seed consumption on the developing foetus. Piglets were reported to have developed bony deformities (arthrogryposis) after sows had consumed Jimson weed (*D. stramonium*) in early gestation (Leipold et al., 1973). The pregnant sows had farrowed in a pen surrounded by a dense stand of *D. stramonium*, and there were indications that the sows had foraged in this area, although there were no estimates of intake. The sows had shown typical signs of *D. stramonium* toxicity during the second and third months of pregnancy. However, this teratogenic effect could not be reproduced experimentally (Keeler, 1981).

7.4.3. Rabbits

In common with sheep and goats, certain breeds of rabbit may be unaffected following the intake of TAs because of their ability to produce the enzyme atropinesterase, which causes the rapid hydrolysis of TAs (Harrison et al., 2006). However, not all breeds appear to express this enzyme, and production of the enzyme appears to be seasonal (Liebenberg and Linn, 1980).

7.4.4. Poultry

Poultry appear to possess an atropine hydroxylase-like enzyme that inactivates TAs, and are therefore believed to be more resistant than other farm animals to the effects of exposure to TAs (Werner and Brehmer, 1967). A number of studies involving both laying hens and broiler chickens were reviewed by EFSA (2008). These involved feeding poultry with diets supplemented with *D. stramonium* or *D. ferox* seeds, or mixtures of purified (-)-hyoscyamine and (-)-scopolamine in ratios typical for *D. ferox*. Initial responses following ingestion of TAs included mild diarrhoea, reduction in feed intake and reduced body weight gain or egg production, particularly at higher doses. However, these effects tended to be transient, and the duration of the studies did not affect the health or productivity of the birds. EFSA (2008) concluded that, on the basis of the available data, levels up to 3 % *D. stramonium* seeds (containing predominantly (-)-hyoscyamine) in the diet of broiler chicks and hens would have no influence on performance during short-term exposure. Furthermore, *D. ferox* (containing (-)-scopolamine as the major alkaloid) contamination at an inclusion rate of 150 mg alkaloid/kg feed showed no adverse effects in poultry. In laying hens, doses of >150 mg TAs (98 % (-)-scopolamine, 2 % (-)-hyoscyamine) per kg feed produced significant increases in the cardiac rate of hens after five weeks, although breeding frequency was not affected.

7.4.5. Horses

Horses are sensitive to atropine (EMEA, 1998a), and there are several reports of toxicosis following ingestion of Jimson weed seeds (Barney and Wilson, 1963; Williams and Scott, 1984; Schulman and Bolton, 1998), teff hay[14] contaminated with young *Datura* plants (*D. stramonium* and *D. ferox*) (Naudé et al., 2005; Gerber et al., 2006) or freshly harvested maize, contaminated with young *D. stramonium* plants (Binev et al., 2006). In the latter case, clinical symptoms included hyperthermia, tachycardia, polypnoea, with dyspnoea, acute gastric dilation, secondary intestinal gas accumulation and complete refusal of feed. Patho-morphological studies showed a toxic liver dystrophy, and extensive dystrophic and necrotic changes in the kidneys and myocardium (Binev et al., 2006). In the study of Gerber et al., (2006), toxicity was confirmed by the presence of hyoscyamine and scopolamine (18.5 and 1553 µg/g, respectively) in the urine. Naudé et al. (2005) concluded that the toxic oral dose of TAs in the horse is approximately 100 µg/kg b.w. of (-)-hyoscyamine (corresponding to 200 µg/kg b.w. atropine). EMEA (1998a) reported that a subcutaneous dose of > 10 µg atropine/kg b.w. can give adverse effects in horses. As a result of its paralytic effect on the gastrointestinal tract, severe colic can be induced.

[14] Produced from highly digestible, low sugar grasses, making it particularly suitable for horses.

7.4.6. Fish

There are no reports of adverse effects of TAs on fish.

7.4.7. Companion animals (cats and dogs)

EFSA (2008) noted that cases of toxicosis have been reported in dogs and cats as a result of accidental ingestion of ornamental house plants. Based on studies with (-)-butylscopolamine (the derivative of (-)-scopolamine that is used in veterinary therapy) the oral no-effect level in dogs is 1-3 mg/kg b.w. for gastrointestinal tract mobility and tachycardia (EMEA, 1997). In common with poultry, domesticated and wild birds appear to be more resistant against TAs (EFSA, 2008).

7.4.8. Carryover

As reported by EFSA (2008), traces of (-)-scopolamine have been detected in eggs laid by hens fed a standard diet supplemented with 150 mg/kg (-)-hyoscyamine and (-)-scopolamine in the ratio 2:98 (the characteristic ratio of (-)-hyoscyamine and (-)-scopolamine in *D. ferox*) (Kovatsis et al., 1993), but no (-)-scopolamine was detected in eggs of hens receiving 1.5 or 15 mg/kg feed.

There are reports that both atropine and (-)-hyoscyamine cross the placenta and can be found in small quantities in human breast milk[15]. However, TAs have not been identified in the milk from cows fed *Datura*-contaminated feed, although an off-flavour of milk has been reported (Forsyth, 1979). Binev et al. (2006) reported that suckling foals exhibited no signs of intoxication, even though their mothers had consumed feed contaminated with *D. stramonium* and showed the typical signs of intoxication.

Pharmacokinetic studies with cattle, horses and pigs following oral administration of (-)-scopolamine have shown that the highest concentrations are found in the liver and kidneys. However, in all species (-)-scopolamine was excreted rapidly (EMEA, 1997).

As reported by EMEA (1998a), atropine is used therapeutically for the treatment of gastrointestinal disorders and intoxication by cholinesterase inhibitors in nearly all food-producing animals. Doses vary according to the method of administration (oral, s.c., i.v. or ocular). Although no residue depletion studies were available, EMEA (1998a) concluded that these were not necessary since pharmacokinetic data indicated rapid absorption and elimination of atropine.

Overall, no new information has been published to alter the conclusion of EMEA (1997) and EFSA (2008) that it is unlikely that residues of TAs in animal food products (milk, meat or eggs) represent a risk for consumers as a result of the legal use of *Atropa belladonna* and atropine as an authorised veterinary medicine or from consumption in feed.

Outside the EU, i.e. in China, studies were recently performed to evaluate the effect on goat meat quality of atropine sulphate injected pre-slaughter. The available information in English does not include the dose level, however, analysis of liver tissue and meat (muscle) did reveal atropine residue levels of 1.23 and 0.82 mg/kg, respectively (Li, 2010). Injection with atropine sulphate improved meat tenderness, increased pH and water capacity and reduced drip loss. The CONTAM Panel noted that such use of atropine is illegal in the EU but has the potential to become an emerging risk from imports from outside the EU.

7.5. Human pharmacological and toxicological data

Data on dose related pharmacological or toxicological effects available due to the medical use of (-)-hyoscyamine, atropine and (-)-scopolamine may be used as a basis for the risk assessment of food contaminated with these TAs and are presented below.

[15] International Medication Systems (UK) Ltd. Summary of Product Characteristics: Atropine Injection BP Minijet. Available online: http://www.medicines.org.uk/EMC/medicine/4963/SPC/Atropine+Injection+BP+Minijet++%28International+Medi cation+Systems%29/#PREGNANCY

According to literature, the naturally occurring enantiomers, (-)-hyoscyamine and (-)-scopolamine, exhibit far stronger pharmacological and toxicological effects than the (+)-enantiomers (Forth et al., 2009). Thus (-)-hyoscyamine has about twice the antimuscarinic activity of atropine (Martindale, 2012). (+)-Hyoscyamine is reported to have a 10-40 fold weaker pharmacological potency compared to (-)-hyoscyamine (Bracher et al., 2006; Fachinformation, 2006).

7.5.1. (-)-Hyoscyamine and atropine

7.5.1.1. Pharmacodynamics

Atropine and (-)-hyoscyamine are tertiary amine antimuscarinic alkaloids with both central and peripheral actions. (-)-Hyoscyamine and atropine (as a racemic mixture of hyoscyamine) induce qualitatively the same pharmacological and toxicological effects. (-)-Hyoscyamine and atropine have antispasmodic actions on smooth muscle and reduce secretions, especially salivary and bronchial secretions. They also reduce perspiration, depress the vagus and influence the heart rate. When given orally they reduce smooth-muscle tone and diminish gastric and intestinal motility. Concerning the eye, they cause dilation of the pupils (mydriasis) and paralysis of accommodation (cycloplegia). (-)-Hyoscyamine and atropine at increasing doses stimulate and then depress the CNS (Bracher et al., 2006, 2011; Martindale, 2011a, 2012).

For mode of action see Section 7.3.

The medical use of (-)-hyoscyamine is less common than that of atropine (Bracher et al., 2006).

7.5.1.2. Therapeutic applications and dosage

(-)-Hyoscyamine

(-)-Hyoscyamine is used mainly in the relief of conditions associated with visceral spasm. (-)-Hyoscyamine as a base is typically given in oral doses of 0.15 to 0.3 mg up to four times daily (1.2 mg per day), but it is more usually employed as the sulphate dihydrate. Its hydrobromide is also used. The suggested single doses of (-)-hyoscyamine sulphate dihydrate are 0.125 to 0.25 mg orally or sublingually every four hours as needed, up to a maximum of 1.5 mg in 24 hours (Martindale, 2012). For (-)-hyoscyamine sulphate dihydrate Bracher et al. (2011) recommend oral single doses of 0.15 to 0.3 mg and avoidance of single doses exceeding 0.5 mg or daily doses exceeding 1.5 mg.

Atropine

Atropine is commonly used for medicinal purposes as atropine sulphate monohydrate (Bracher et al, 2011; Martindale, 2011a).

Because of the effect on heart rate, atropine is used in the treatment of bradycardia and asystole of various causes, including in acute cardiopulmonary resuscitation procedures. It is used as a premedicant in anaesthetic practice and to counteract the muscarinic effects of anticholinesterase agents such as neostigmine and other parasympathomimetics. Atropine is used as an antispasmodic and for its antisecretory effects in gastrointestinal disorders, as an adjunct to opioid analgesics for the symptomatic relief of biliary or renal colic, to treat or prevent bronchospasm and in the treatment of poisoning with mushrooms that contain muscarine and in organophosphorus pesticide poisoning. Atropine is used topically in ophthalmology as a mydriatic and cycloplegic (e.g. Martindale, 2011a).

For most medical indications atropine is administered by s.c., i.m. or i.v. injection (e.g. Martindale, 2011a). Oral administration of tablets is described for the treatment of gastrointestinal, biliary or renal colics and in order to reduce gastric or pancreatic secretion in single oral doses of 0.5-1 mg atropine sulphate monohydrate which may be repeated twice up to a daily dose of 1.5-3 mg for adults. For children 2-5 years of age the single oral dose amounts to 0.25 mg and the maximum daily dose to 0.75 mg atropine sulphate monohydrate (Fachinformation, 2006; Bracher et al., 2011).

7.5.1.3. Adverse reactions, intoxication and clinical studies

Reports on adverse reactions and intoxications of atropine are generally indicated to be also relevant for (-)-hyoscyamine taking into account that (-)-hyoscyamine has about twice the antimuscarinic activity of atropine (Bracher et al., 2006, 2011; Martindale, 2012).

Dose dependency of adverse and toxic effects of orally administered atropine

As summarised in several monographs, following an oral dose of 0.5 mg atropine (at the low end of therapeutic dose), reported side effects are slight cardiac slowing, some dryness of the mouth and inhibition of sweating. An oral dose of 1 mg atropine is associated with definite dryness of the mouth, thirst, acceleration of the heart, sometimes preceded by slowing, and mild dilation of pupils. Following an oral dose of 2 mg atropine, reported effects are rapid heart rate, palpitation, marked dryness of the mouth, dilated pupils and some blurring of near vision. At a dose of 5 mg atropine the before mentioned symptoms are more pronounced. Furthermore difficulties in speaking and swallowing, restlessness and fatigue, headache, dry and hot skin, difficulties in micturition and reduced peristalsis are observed. At oral doses of 10 mg atropine and more, additional manifestations occur, such as rapid and weak pulse, practically obliterated iris, very blurred vision, flushed, hot, dry and scarlet skin, ataxia, excitement, hallucinations, delirium, and coma (Bracher et al., 2006, 2011; Brunton et al., 2006).

Adverse reactions of atropine associated with therapeutic doses

Dryness of the mouth, reduced sweating, dry, hot and reddened skin, tachycardia and impaired vision (due to mydriasis and cycloplegia) are very frequently (> 1/10) observed adverse effects of therapeutic oral atropine administration (Fachinformation, 2006).

Other peripheral adverse effects mentioned, which may accompany oral therapy with atropine, are difficulty in swallowing and talking, arrhythmias, and difficulty in micturition, as well as reduction in the tone and motility of the gastrointestinal tract leading to constipation. Some of the central effects of atropine seen at toxic doses (see below) are said to possibly also occur at therapeutic oral doses, such as restlessness, confusion, hallucinations, excitement, delirium, occasionally seizures, comatose conditions (Fachinformation, 2006).

Hypersensitivity to atropine is not uncommon and may occur as conjunctivitis or a rash (Fachinformation, 2006; Martindale, 2011a).

The therapeutic oral use of atropine in recommended dose ranges may cause blurred vision, dizziness, and other effects that may impair a patient's ability to perform skilled tasks such as driving (Fachinformation, 2006; Martindale, 2011a).

Clinical studies

In an older study with 129 apparently healthy children of different age the minimal effective dose (MED) of atropine was determined by regarding the suppression of salivation as an onset of effect (Unna et al., 1950). The variations in individual susceptibility to atropine were large in all age groups. The average oral MED/kg b.w. was somewhat smaller in infants of 1 to 12 months (16 µg/kg b.w.) and from 12 to 36 months (14 µg/kg b.w.) than in older children in the age group of 3 to 6 years (22 µg/kg b.w.) and 6 to 12 years (20 µg/kg b.w.). Fever was observed when the MED was exceeded 2 - 3 fold.

Nine healthy adult volunteers (three females and six males, age ranging between 27 and 40) were treated orally with atropine sulphate (Murrin, 1973). All the nine subjects were dosed with 14 µg/kg b.w. atropine[16], and three and six subjects (same volunteers) were also dosed at 7 and 28 µg/kg b.w., respectively, with a minimum period of three days between two treatments in the same subject. The

[16] No information is available in the publication whether the doses were calculated on the considering the molecular mass of the salt or of the active substance only.

subjects were monitored for heart rate, blood pressure, salivary secretion and pupillary dilation as from 30 minutes before treatment until five hours post treatment. The heart rate was statistically significantly lower when subjects were treated with 7 μg/kg b.w. (starting from 30 minutes post treatment and persisting for one hour), and statistically significantly higher at 28 μg/kg b.w. A dose related reduction in salivary secretion was observed at all the three doses, with maximum effects reached 1-2 hours post treatment. No significant changes were observed for blood pressure or pupillary dilation.

Volunteer subjects (5 males and 1 female, age ranging between 29 and 40 years) were orally exposed to atropine or (-)-scopolamine[17] (Mirakhur, 1978). For atropine, doses of 0.5, 1.0 and 2.0 mg were administered. Those corresponds to 12, 23 and 47 μg/kg b.w. atropine for the only female subject (based on the actual body weight reported in the publication), and to 7.6, 15 and 30 μg/kg b.w. atropine for the male subjects (calculated on the mean of individual body weight reported in the publication). All subjects were exposed to all treatment doses with at least one week of recovery between two consecutive treatments. Heart rate, arterial pressure, oral temperature, pupillary measurements, sweat gland activity and salivary secretion were recorded. Control observations were carried out under resting conditions before the treatment, and measurements were performed 30 minutes and 60 minutes after the administration and repeated each hour up to 6 hours post treatment. A biphasic dose response was observed for heart rate changes in subjects treated with atropine, consisting of slight, non-statistically significant decreases at 0.5 and 1.0 mg atropine, and a slight, non-statistically significant increase at 2.0 mg atropine. Salivary secretion decreased in subjects treated with 1.0 mg and 2.0 mg atropine, and a slight reduction was observed also at 0.5 mg atropine. A decreasing trend in sweat gland activity was recorded at all doses tested with the effect being more pronounced with atropine in comparison to (-)-scopolamine. Finally only 2.0 mg atropine induced appreciable pupillary dilation, with the other two atropine doses having only negligible effects.

Seven healthy, non-smoking male volunteers (age ranging between 21 and 56 years, average body weight of 87.9 kg) were orally administered 30 μg/kg b.w. atropine[17] (Volz-Zang et al., 1995). Heart rate, blood pressure and salivary secretion were monitored under resting conditions starting from 30 minutes before the treatment up to 3 hours after the treatment. On a different day, separated by at least five days, the volunteers were monitored without treatment. Heart rate was slightly increased with a maximum achieved 135 minutes after the treatment with atropine (not statistically relevant) in comparison to controls. A statistically relevant decrease in salivary secretion was observed from 70 minutes post treatment until the end of the observation period in comparison with the controls (maximum decrease of 84.3 %).

Intoxications and adverse effects of (-)-hyoscyamine and atropine associated with overdosage

In overdosage of atropine, the peripheral effects become more pronounced and other symptoms such as hyperthermia, hypertension, increased respiratory rate, and nausea and vomiting may occur. A rash may appear on the face or upper trunk. Toxic doses also cause CNS stimulation marked by restlessness, confusion, excitement, ataxia, incoordination, paranoid and psychotic reactions, hallucinations and delirium, and occasionally seizures. However, in severe intoxication, central stimulation may give way to CNS depression, coma, circulatory and respiratory failure, and death. There is considerable variation in susceptibility to atropine; recovery has occurred even after 1 g, whereas deaths have been reported from doses of 100 mg or less for adults and 10 mg for children (Martindale, 2011a). Pribilla and Schlosser (1957) reported that accidental oral administration of 3 mg atropine sulphate monohydrate was lethal in a 3 weeks old infant (body weight at the time of birth: 3500 g). Referring to atropine sulphate, for adults an oral dose of about 100 mg and for children a potential oral dose of 2 mg are considered to be lethal (Bracher et al., 2011). Marquardt and Schäfer (2004) indicate that the lethal doses for the base atropine range from 10-20 mg/kg b.w. for adults and from 1 to 10 mg/kg b.w. for children.

[17] No information is available on the form in which atropine was administered.

In chronic intake of (-)-hyoscyamine (base) in daily doses higher than 3.6 mg development of psychological dependence has been reported (Marquardt and Schäfer, 2004).

7.5.1.4. Sensitive subpopulations and interactions in (-)-hyoscyamine and atropine-treatment

Precautions and interactions described for atropine are generally indicated to be also relevant for (-)-hyoscyamine taking into account that (-)-hyoscyamine has about twice the antimuscarinic activity of atropine (Bracher et al., 2006, 2011; Martindale, 2012).

Regarding medical use, atropine is contra-indicated in patients with prostatic enlargement, in whom it may lead to urinary retention, and in those with paralytic ileus or pyloric stenosis. In patients with ulcerative colitis its use may lead to ileus or megacolon, and its effects on the lower oesophageal sphincter may exacerbate reflux. Caution is generally advisable in any patient with diarrhoea. It should not be given to patients with myasthenia gravis except to reduce adverse muscarinic effects of an anticholinesterase. Atropine use may raise intra-ocular pressure in individuals with angle-closure glaucoma or with a narrow angle between the iris and the cornea. Because of the risk of provoking hyperthermia, atropine should not be given to patients, especially children, in case of fever or when the ambient temperature is high. Atropine needs to be used with caution in conditions characterised by tachycardia such as thyrotoxicosis, heart failure, and in cardiac surgery, where it may further accelerate the heart rate. Care is required in patients with acute myocardial infarction, as ischaemia and infarction may be made worse, and in patients with hypertension. Reduced bronchial secretion caused by systemic atropine may be associated with the formation of mucous plugs. The elderly may be particular susceptible to the adverse effects of atropine since the prevalence of certain conditions such as urinary retention, constipation, paralytic ileus, and glaucoma increases with age. Atropine may cause confusion, especially in the elderly (Fachinformation, 2006; Bracher et al., 2011; Martindale, 2011a).

Pregnancy

Atropine sulphate rapidly crosses the placenta when given to pregnant women (Fachinformation, 2006; Martindale, 2011a). Since it may induce tachycardia in the mother and the child, its therapeutic use is contraindicated in the last trimester of pregnancy and during birth. Precautions are recommended during the first and second trimester of pregnancy (Fachinformation, 2006).

Breast feeding

Atropine sulphate is reported to appear in small quantities in breast milk and to impair milk production when given to lactating mothers (Fachinformation, 2006).

Interactions

The effects of atropine and other antimuscarinics may be enhanced by use with other drugs having antimuscarinic properties, such as amantadine, some antihistamines, phenothiazine antipsychotics, and tricyclic antidepressants. Inhibition of drug-metabolising enzymes by monoamine oxidase inhibitors may possibly enhance the effects of antimuscarinics. The reduction in gastric motility caused by antimuscarinics may affect the absorption of drugs. Antimuscarinics may also antagonise the gastrointestinal effects of cisapride, domperidone, and metoclopramide. Antimuscarinics and parasympathomimetics may counteract each others' effects (Martindale, 2011a).

7.5.2. (-)-Scopolamine

7.5.2.1. Pharmacodynamics

(-)-Scopolamine is a tertiary amine which induces typical peripheral and central antimuscarinic actions (see Section 7.3.). It is a more powerful suppressant of salivation and a more powerful mydriatic than atropine, has weaker spasmolytic effects than atropine and usually slows rather than increases heart rate, especially in low doses. Its central action differs from that of atropine in that it depresses the cerebral cortex and produces drowsiness and amnesia in low doses (Bracher et al., 2006; Martindale, 2011b).

7.5.2.2. Therapeutic applications and dosage

(-)-Scopolamine is commonly used for medicinal purposes as (-)-scopolamine hydrobromide trihydrate (Bracher et al., 2006; Martindale, 2011b).

(-)-Scopolamine and (-)-scopolamine hydrobromide trihydrate are effective in the prevention of motion sickness and are among the principal drugs used for this purpose. They may be given orally for short-term protection or by transdermal patch for a prolonged duration of action (Martindale, 2011b).

Orally a dose of (-)-scopolamine hydrobromide trihydrate is taken 20 to 30 minutes before a journey, followed by the same dose every 6 hours if required up to a maximum of 3 doses in 24 hours. The usual single dose in adults is 0.3 mg (Martindale, 2011b). Other authors recommend oral single doses of 0.25 to 1 mg up to a maximum daily dose of 3 mg for adults (Bracher et al., 2006). Children aged 4 to 10 years may be given 0.075 to 0.15 mg and those over 10 years, 0.15 to 0.3 mg. Children aged 3 to 4 years may be given 0.075 mg, repeated once if necessary to a maximum total dose of 0.150 mg in 24 hours. (-)-Scopolamine is also given via a transdermal patch which is placed behind the ear and supplies 1 mg over 3 days. Patches are applied for adults and children aged 10 years and over and should be taken 5 to 6 hours before travelling or on the preceding evening and removed at the end of the journey (Martindale, 2011b).

(-)-Scopolamine hydrobromide trihydrate is used in palliative care to reduce excessive respiratory secretions, although care has to be taken to avoid the discomfort of a dry mouth. While parenteral administration is suggested for adults, oral and sublingual doses have been suggested for children, according to age. (-)-Scopolamine hydrobromide trihydrate in single doses of 0.010 mg/kg b.w. is recommended four times daily for the age of 2 to 12 years and of 0.3 mg/person four times daily for the age of 12 to 18 years. In palliative care (-)-scopolamine hydrobromide trihydrate is also given sublingually to reduce the frequency of spasm in bowel colic in single doses of 0.010 mg/kg b.w. four times daily for children and of 0.3 mg/person four times daily for adults (Martindale, 2011b).

As a premedicant in anaesthesia or to produce mydriasis and cycloplegia (-)-scopolamine hydrobromide trihydrate is given parenterally or directly to the eye, respectively.

7.5.2.3. Adverse reactions, intoxication and clinical studies

Adverse reactions of (-)-scopolamine associated with therapeutical doses

The same kind of peripheral adverse reactions as for atropine is described for (-)-scopolamine varying only with respect to quantitative aspects. It is a more powerful suppressant of salivation and a more powerful mydriatic than atropine. With low doses of (-)-scopolamine (0.1 or 0.2 mg of the base) the cardiac slowing is greater than with low doses of atropine. With higher doses of (-)-scopolamine a transient cardioacceleration may be observed (Brunton et al., 2006). In contrast to atropine, (-)-scopolamine produces central depression at oral therapeutic doses and symptoms include drowsiness and fatigue. (-)-Scopolamine may produce CNS stimulation rather than depression at therapeutic doses if used in the presence of pain without opioid analgesics; symptoms include excitement, restlessness, hallucinations, or delirium (Bracher et al., 2006; Martindale, 2011b). A few cases of angle-closure glaucoma have been associated with the use of transdermal (-)-scopolamine devices. There are also reports of psychotic reactions associated with the transdermal use of (-)-scopolamine (Martindale, 2011b).

The therapeutic oral use of (-)-scopolamine in recommended dose ranges may cause drowsiness and other effects that may impair a patient's ability to perform skilled tasks such as driving or to operate machinery (Bracher et al., 2006; Martindale, 2011b).

Clinical studies

Volunteer subjects (5 males and 1 female, age ranging between 29 and 40 years) were orally exposed to 0.25, 0.50, 1.00 mg (-)-scopolamine[18] in a study already described in Section 7.5.1.3 (Mirakhur, 1978). The administered doses correspond to 5.8, 12 and 23 μg/kg b.w. (-)-scopolamine for the only female subject (based on the actual body weight reported in the publication), and to 3.8, 7.6 and 15 μg/kg b.w. (-)-scopolamine for the male subjects (calculated on the mean of individual body weight reported in the publication). (-)-Scopolamine induced a statistically significant reduction in heart rate 2 and 3 hours after the treatment with the three doses tested. A slight reduction in salivary secretion was observed in subjects treated with 0.25 mg and 0.5 mg (-)-scopolamine, and to a higher extent in subjects treated with 1.0 mg (-)-scopolamine, the effect being lower than that observed with atropine. A decreasing trend in sweat gland activity was recorded for both substances at all doses tested with the effect being more pronounced with atropine in comparison to (-)-scopolamine. No pupillary dilation was observed in subjects treated with (-)-scopolamine.

Eighteen healthy male volunteers were treated with 0.6 mg (-)-scopolamine hydrobromide (corresponding to 470 μg (-)-scopolamine, or 6.8 μg/kg b.w. considering a default b.w. of 70 kg) to study the effectiveness against motion sickness (Golding and Stott, 1997). In another session, the same subjects were administered a placebo. Ninety minutes after the treatments, the subjects were challenged for motion sickness. Heart rate was monitored 5 minutes before the treatment under resting conditions, at 1 hour post treatment and 10 minutes after the end of the motion challenge (approximately 2 hours post treatment). The subjects recorded possible drug side effects in a checklist immediately prior to treatment, and 1 and 2 hours post treatment. In the period comprised between 1 to 1.5 hours following the treatment, visual performance parameters were measured and the subjects were tested for short-term memory performance. Skin conductance was measured during the experiment. A statistically significant decrease in heart rate was observed both at 1 and 2 hours after (-)-scopolamine treatment in comparison to heart rate before treatment and to the treatment with the placebo. No severe side effects symptoms were recorded, although incidence of dry mouth was statistically significantly higher following the treatment with (-)-scopolamine in comparison with the placebo. A statistically significant decrease in skin conductance, related to decreased sweat secretion, was observed following the treatment with (-)-scopolamine in comparison with the placebo. No changes were recorded in the short-term memory performance test, whereas the assessment of visual performance showed a statistically significant difference in one assay only (lower visual pursuit gain at 0.15 Hz following treatment with (-)-scopolamine in comparison with the placebo).

Intoxications and adverse of effects (-)-scopolamine associated with overdosage

Toxic doses of (-)-scopolamine produce stimulation of the CNS in a similar manner to atropine, causing restlessness, disorientation, hallucinations, and delirium. However, (-)-scopolamine does not stimulate the medullary centres and therefore does not produce the increases in respiration rate or blood pressure seen with atropine. At higher doses of (-)-scopolamine, CNS stimulation is followed by central depression and death from respiratory paralysis. The lethal dose is about 100 mg (-)-scopolamine (Bracher et al., 2006).

7.5.2.4. Sensitive subpopulations and interactions in (-)-scopolamine-treatment

Contra-indications and precautions described for atropine are also relevant for (-)-scopolamine (Martindale, 2011b).

Adverse CNS effects have been stated to be more likely in elderly patients and in patients with impaired liver, or kidney function. There have been rare reports of an increase in frequency of seizures in epileptic patients (Martindale, 2011b).

[18] No information is available on the form in which (-)-scopolamine was administered.

Pregnancy

Because (-)-scopolamine readily crosses the placenta, precautions are recommended for therapeutic use of (-)-scopolamine by pregnant women (Renner et al., 2005; Bracher et al., 2006).

Breast feeding

Precautions are recommended for therapeutic use of (-)-scopolamine by breast feeding women (Bracher et al., 2006).

Interactions

Possible interactions described for atropine are also relevant for (-)-scopolamine (Martindale, 2011b). The sedative effect of (-)-scopolamine may be enhanced by alcohol or other CNS depressants (Martindale, 2011b). Pretreatment with grapefruit juice increased the bioavailability of orally administered (-)-scopolamine in healthy volunteers possibly due to inhibition of the cytochrome P450 (CYP) enzyme 3A4, which catalyses *N*-demethylation of tertiary amines (Ebert et al., 2000).

7.5.3. Therapeutic use of plants containing tropane alkaloids

Belladonna leaf - *Belladonnae Folium*

Bracher et al. (2004) note that dried Belladonna leaves may contain alkaloids in the range between 0.2 and 2.0 % (in average 0.3-0.5 %).

The European Pharmacopoeia (7th edition) includes monographs for (i) Belladonna Leaf, (ii) Prepared Belladonna Leaf, (iii) Standardised Belladonna Leaf Tincture, and (iv) Standardised Belladonna Leaf Dry Extract (Ph. Eur.7, 2011). In the existing scientific monographs regarding their therapeutical use it is indicated that recommendations and information for treatment, adverse effects, precautions and intoxications correspond to those for atropine (Bracher et al., 2004; Martindale, 2011c).

Belladonna leaves and their preparations are used to treat gastrointestinal, biliary or renal spasms, (-)-hyoscyamine being considered as active principle (Bracher et al., 2004).

(i) Belladonna Leaf; *Belladonnae Folium* (Ph. Eur. 7): According to the European Pharmacopoeia Belladonna Leaf consists of the dried leaf, or dried leaf and flowering, and occasionally fruit-bearing, tops of *Atropa belladonna* L. It contains not less than 0.30 % of total alkaloids, calculated as (-)-hyoscyamine. The alkaloids consist mainly of (-)-hyoscyamine with smaller amounts of (-)-scopolamine. The recommended oral single doses for belladonna leaves are 0.05-0.1 g (corresponding to 0.15-0.3 mg of total alkaloids or (-)-hyoscyamine) which may be repeatedly taken up to a maximum of 0.3 g (corresponding to 0.9 mg of total alkaloids or (-)-hyoscyamine) in 24 hours (EMEA, 1998b; Bracher et al., 2004; Ph. Eur.7, 2011). The maximum single dose for belladonna leaves is reported to be 0.2 g (corresponding to 0.6 mg of total alkaloids) resulting in a maximum daily dose of 0.6 g belladonna leaves (corresponding to 1.8 mg of total alkaloids) (EMEA, 1998b).

(ii) Standardised Belladonna Leaf Tincture, *Belladonnae Folii Tinctura Normata* (Ph. Eur. 7): According to the European Pharmacopoeia it contains 0.027-0.033 % of total alkaloids calculated as (-)-hyoscyamine. It is prepared using 70 % (v/v) ethanol. The alkaloids consist mainly of (-)-hyoscyamine together with small quantities of (-)-scopolamine. Considering a concentration of 0.03 % of total alkaloids calculated as (-)-hyoscyamine, the recommended oral single doses for belladonna leaf tincture are 0.6 to 1.0 ml, taken 3 times/day (Bracher et al., 2004; Ph. Eur.7, 2011).

7.5.4. Intoxications associated with plants containing tropane alkaloids

In general plant poisonings can be aggregated into three categories: unintended ingestions, intended ingestions, and poisoning due to abuse of plant material (Beyer et al., 2009). Unintended ingestions of TAs may occur via contamination of food or from a mix-up of edible plants/parts of plants with the TA containing plant material. Intended ingestions are common in homicides and suicides. Increasingly common is the abuse of TA containing plants, such as *D. stramonium* or *D. suaveolens* (synonym: *Brugmansia suaveolens)* for hallucinogenic reasons (e.g. Hall et al., 1977; Tiongson and Salen, 1998). Poisonings of humans by TA containing plants have been reviewed by Adamse and Egmond (2010) and Koleva et al. (2012) and are mostly due to intake of parts of *Brugmansia* spp./*Datura* spp. or of *Atropa belladonna. Brugmansia* belongs to the four plant genera (together with *Laburnum*, *Phaseolus*, and *Thuja*) involved in the most severe poisonings by botanicals in children (Pietsch et al., 2008).

Typical symptoms of anticholinergic toxicity, e.g. after intoxication with parts of *D. stramonium, D. suaveolens, A. belladonna* or related botanicals, are those of atropine intoxication, such as dry skin and mucosa, thirst, difficulty of swallowing and speaking, flushing, mydriasis, sinus tachycardia, hyperpyrexia, decreased bowel sounds, urinary retention, and neurological disorders with ataxia, disorientation, confusion, hallucinations (visual and auditory), psychosis, agitated delirium, seizures, and coma (Vanderhoff and Mosser, 1992; Jaspersen-Schib et al., 1996; Al-Shaikh and Sablay, 2005; Bouziri et al., 2011). In severe forms, respiratory failure, cardiovascular collapse and death have been reported (e.g. Vanderhoff and Mosser, 1992; Chang et al., 1999; Kurzbaum et al., 2001; Spina and Taddei, 2007; Bouziri et al., 2011). *D. stramonium* intoxication usually occurs within 60 minutes after ingestion, and clinical symptoms may persist for 24 to 48 hours because the anticholinergic effects delay gastric emptying, resulting in a prolonged duration of action (Bouziri et al., 2011). Children have a special susceptibility to *D. stramonium* toxicity; even a small intake of *D. stramonium* seeds may produce severe CNS manifestations (and already the ingestion of 15 seeds of *D. stramonium* may be lethal in children (Jaspersen-Schib et al., 1996; Al-Shaikh and Sablay, 2005; Bouziri et al., 2011).

According to Jaspersen-Schib et al. (1996) the fruits of *A. belladonna* have been mistaken for edible berries such as blackberries. The seeds of *D. stramonium* and of *Hyoscyamus niger* may be mixed up with poppy seeds. The roots of *H. niger* may be taken for black salsify (Jaspersen-Schib et al., 1996). The described possibilities of mix-up of edible parts of plants with TA containing plant material due to similarities in appearance may also give reason for contamination of edible fruits, seeds and vegetables which may remain undetected without careful inspection.

In the following sections only case reports are presented which are relevant for the risk assessment of the intake of TAs with food, e.g. giving information on dose effect relationships or indicating intake with food. When analytical data in food samples were reported as atropine, hyoscyamine or scopolamine, the CONTAM Panel cited these data as the corresponding (-)-enantiomers in this opinion since these are the naturally occurring enantiomers.

7.5.4.1. Intoxications associated with contamination of food

In 1949, severe intoxications of the inhabitants of a Turkish village with typical anticholinergic symptoms were reported resulting from the consumption of bread made from flour estimated to contain almost 1 % of seeds from *D. stramonium* (Pulewka, 1949).

In Venezuela during 1984-1998 fifteen persons developed atropine poisoning following consumption of wasp honeycombs (‘matejea’) or wasp honey. Clinical signs, antidotal response and the presence of *D. inoxia* plants near the wasp nests supported the assumption that the intoxications were caused by ingestion of (-)-hyoscyamine-contaminated honey. (-)-Hyoscyamine and (-)-scopolamine are found in the leaves, fruits and flowers of this Venezuelan wild plant. Two deaths occurred from heat stroke associated with the poisoning together with high environment temperatures and intensive physical activity. These two fatalities were in healthy young men of 17 and 18 years of age. They had consumed large amounts of ‘matejea’ (no detailed data given) and developed atropine poisoning symptoms including hallucinations and agitation. Thirteen cases recovered satisfactorily (Ramirez et al., 1999).

In Ethiopia, Aga and Geyid (1992) identified 688 patients who complained about dryness of the mouth and difficulties in swallowing frequently accompanied by blurred vision, flushed face, dry skin, mania and/or talkativeness, associated with corn contaminated with *D. stramonium* seeds. The content of *D. stramonium* seeds found in the flour samples under examination ranged from 30 to 200 g/kg flour, resulting in an alkaloid content of 90-600 mg/kg flour (corresponding alkaloid concentration in the seeds: 0.3 %). The authors calculated that the amount of alkaloid ingested per meal (containing 150 g corn flour) ranged between 13 and 90 mg of alkaloid per person.

In 1998, in Botswana a total of 92 patients were seen with features of *D. stramonium* poisoning due to consumption of contaminated sorghum flour. Visual inspection of the used stock of sorghum grains revealed 1-3 seeds of *D. stramonium* per 20 seeds of sorghum grain (Onen et al., 2002).

In Slovenia, where buckwheat flour is commonly used in the preparation of traditional dishes, 73 cases of domestic food poisoning with a typical syndrome of TA toxicity including dry mouth, hot red skin, blurred vision, tachycardia, urinary retention, ataxia, speech disturbance, disorientation and visual hallucinations, were identified in September 2003. Victims reported ingestion of a dish made of buckwheat flour a few hours prior to the onset of symptoms. Symptoms, which ranged from mild to moderate, ceased spontaneously within 48 hours. Examination of whole buckwheat grain showed up to 190 *D. stramonium* seeds/kg of grain (Perharič, 2005). Based on the results of analyses of the flour products, and recollection after 2 months of the amount of contaminated food consumed, the exposure of 12 of the subjects was estimated to be 0.7-138 µg/kg b.w. for (-)-hyoscyamine and 0.4-64 µg/kg b.w. for (-)-scopolamine (Perharič et al., 2013a).

In 2006, a group of eight employees fell ill with vomiting and nausea within 2 hours of eating lunch in a factory canteen in Austria. All eight persons reportedly affected with gastrointestinal disorders were said to have consumed balls of millet-carrots. One of the eight persons was said to have been hospitalized 12 hours after the incriminated meal because of unconsciousness and auditory hallucinations. The millet left over from the preparation of the millet-carrots balls was provided for testing. Examination of the whole millet grain revealed *D. stramonium* seeds in a concentration of about 50 seeds/kg of grain. The authors assumed an ingestion of 120 g (i.e. including approx. 60 g millet) per patient in the incident and calculated that the average intake would have been three *D. stramonium* seeds per person (no data on the alkaloid concentration of the seeds are available). A dose of only three *D. stramonium* seeds per serving was considered by the authors to be rather small to cause severe toxicity as observed in one of the eight cases. They also state that a time-lag of 12 hours between lunch intake and clinical onset of unconsciousness and auditory hallucinations is unusual for intoxication with (-)-hyoscyamine or (-)-scopolamine, but could be explained by delayed digestion of intact *D. stramonium* seeds. They consider postprandial 'inability to vomit', followed by unconsciousness and auditory hallucinations, as reported by this patient, to be consistent with TA poisoning (Fretz et al., 2007).

In France, adults suffered from blurred vision after the consumption of buckwheat pancakes. The concentration of (-)-hyoscyamine in the pancakes was in the order of 3.5 to 4 mg/kg and (-)-scopolamine in the order of 2.5 to 3 mg/kg (Afssa, 2008). Based on this, it was estimated that the consumption of 2 to 3 pancakes resulted in an intake of 0.1 to 0.6 mg TAs, equivalent to 1.4 to 8.6 µg/kg b.w. assuming a b.w. of 70 kg as recommended by the Scientific Committee (EFSA Scientific Committee (SC), 2012). A second case of intoxication was reported in France in 2007 with persons that had consumed potato pancakes that contained 1 to 3 mg/kg TAs (Afssa, 2008).

In 2008, six adult family members were admitted to a hospital emergency department in Maryland (USA) with hallucinations, confusion, mydriasis, and tachycardia of approximately 3-4 hours duration. About 4-5 hours earlier, all six family members had shared a meal of homemade stew and bread. Subsequent investigation showed that the stew contained Jimson weed (*D. stramonium*) (ingested amount of plant parts is unknown) (Russell et al., 2010).

An intoxication of seven individuals with leaves from *D. innoxia* took place in Athens (Greece) in 2010. The seven individuals, four women and three men, were admitted into two different hospitals of Athens

with anticholinergic syndrome, comprising central and peripheral sighs and symptoms. The intoxication appeared after consumption of boiled blites (vegetables consumed as salad) during their lunch. The patient had bought the vegetables from a food market at the same neighbourhood. Concentrations of (-)-hyoscyamine and (-)-scopolamine in urine samples from all seven patients and in the consumed vegetables were established and reported. Thus, the concentrations of (-)-hyoscyamine and (-)-scopolamine in the cooked vegetables were found to be 0.8 and 1.2 mg/kg, respectively (Papoutsis et al., 2010).

In October 2012, 18 persons exhibited symptoms typical for atropine poisoning after consuming organic buckwheat flour or bread containing organic buckwheat flour in France. Investigations by the regional health agency lead to the conclusion that the organic buckwheat was potentially contaminated by *Datura* (no further details given). Bakeries, stores or crepe places that used or sold buckwheat flour and food products made from buckwheat (bread, crepes, cakes) were informed (ProMED, 2012).

An incident with contaminated marshmallow (*Althaea officinalis*) tea occurred in the Netherlands (January 2013). Four persons were hospitalized after developing typical signs of anticholinergic poisoning (including disorientation, delirium, dilated pupils, irregular or increased heart rate, loss of memory, loss of coordination and difficulty to speak, dry mouth, urinary retention) within 2 hours after drinking tea prepared from marshmallow root that was contaminated with *A. belladonna* root. Analysis of one of the implicated samples showed the presence of 1080 mg/kg (-)-hyoscyamine, 50 mg/kg anisodamine (6-β-hydroxyhyoscyamine) and 20 mg/kg (-)-scopolamine (P. Mulder, personal communication).

7.5.4.2. Intoxications associated with mistaken identity of edible parts of plants

After consumption of a meal of hamburger prepared at home by a couple, the husband collapsed, and the wife also became unconscious. Within 24 hours, the couple regained consciousness and explained the circumstances of their illness. In preparing the hamburger, the wife added what she thought was seasoning but later realised was seeds of Angel's trumpet (*D. suaveolens*) that had been drying for planting the next year as an ornamental flower. After removing most of the seeds from the cooked meat, the husband and wife ate one hamburger patty each. Less than 1 hour later, both began to hallucinate. Other symptoms were tachycardia and severe diarrhoea. Both recovered and were discharged after 3 days of hospitalisation (Anonymous, 1984).

In a 65-year-old man, 3 mg of (-)-hyoscyamine from *Atropa belladonna* leaves, mistaken for burdock (*Arctium lappa*) leaves usually taken to prepare herbal tea in certain regions, produced not only peripheral (-)-hyoscyamine symptoms but also a central anticholinergic syndrome with restlessness, hyperactivity, and dysphasia. Symptoms resolved within 24 hours with symptomatic therapy (Wood and Haq, 1971).

An incident of 14 people with intoxication caused by ingesting *D. suaveolens* mistaken for edible wild vegetables has been described. The incubation period was 15 to 30 min. The symptoms were dizziness, dry mouth, flushed skin, palpitation, nausea, drowsiness, tachycardia, blurred vision, mydriasis, hyperthermia, disorientation, vomiting, agitation, delirium, urine retention, hypertension and coma. Three patients were hospitalized for 2-3 days. All patients recovered with no sequelae (Chang et al., 1999).

7.5.4.3. Intoxications associated with abuse

A 76-year-old Caucasian male ingested 3-4 teaspoons of a homemade wine over a one hour period. Shortly thereafter he experienced loss of coordination in hands and feet, followed by loss of sensation. Approximately 1.5 hours later, he was taken to the emergency room of a local hospital with symptoms of respiratory distress, partial body paralysis and muscular weakness. The plant used in making the wine was Angel's trumpet (*D. suaveolens*). A sample of the wine was analysed for (-)-hyoscyamine and

(-)-scopolamine. (-)-Scopolamine but no (-)-hyoscyamine was detected in the samples. The total ingested dose amounted to 435-580 mg (-)-scopolamine (Smith et al., 1991)[19].

Tiongson and Salen (1998) summarise eleven cases of patients, aged 13-21 years, who were presented to an emergency department following oral ingestion of *D. stramonium* seeds in large quantities. All of the patients reported that they had eaten the entire seed pods ranging from one pod to four-and-a-half pods. The authors explain that each pod (or fruit) may contain approximately 100 seeds in total, containing about 6 mg of (-)-hyoscyamine (no indication if analysis of TAs has been performed by the authors). Mydriasis was observed in all cases. From the six patients, who had only consumed one pod, five patients experienced hallucination and four agitation or disorientation. Nine of the eleven patients were admitted for observation. There were no deaths associated with these ingestions and none of the patients required medication for reversal of the severe anticholinergic symptoms (Tiongson and Salen, 1998).

Spina and Taddei (2007) report 2 cases of a 16-year old male and a 15-year-old female who ingested the seeds of 2-3 pods of *D. stramonium* (amounting to 100-300 seeds according to the authors) in combination with alcohol. They were presented to the emergency department with severe acute anticholinergic symptoms including visual hallucinations, disorientation, incomprehensible and nonsensical speech, and dilated sluggish pupils. Both patients required restraints for combativeness until sedation by adequate medication was achieved (Spina and Taddei, 2007).

Hall et al. (1977) describe 10 cases of intoxication by flowers of Angel's trumpet (*D. suaveolens*) in consequence of its use by adolescents and young adults as a hallucinogen. Ingestion of Angel's trumpet flowers or a tea brewed from them resulted in an alkaloid-induced CNS anticholinergic syndrome. Symptom development in the patients observed was rapid: 5-10 minutes after the ingestion of teas or 1-3 hours after the direct ingestion of plant material. The following course of symptom development was typical: intense thirst, visual disturbances, flushing, CNS hyperexcitability, sensory flooding, a delirious incoherent state, hyperthermia, tachycardia, systolic hypertension, visual hallucinations, alternating levels of consciousness and clonus and finally convulsions. Visual hallucinations persisted for up to 4 days after intoxication. Convulsions and flaccid paralysis were observed only in those subjects reporting ingestion of more than 6 flowers. The authors report that the flower of *D. suaveolens* contains approximately 0.20 mg of (-)-hyoscyamine and 0.65 mg of (-)-scopolamine. Information received from the subjects indicated that tea or broth prepared from 1-3 flowers (corresponding to 0.2-0.6 mg of (-)-hyoscyamine and 0.65-1.95 mg of (-)-scopolamine) may produce hallucinations. Tea prepared from 6 flowers (corresponding to 1.2 mg of (-)-hyoscyamine and 3.9 mg of (-)-scopolamine) almost always produced hallucinations. When tea was prepared from 9 flowers (corresponding to 1.8 mg of (-)-hyoscyamine and 5.8 mg of (-)-scopolamine), hallucinations were of considerably longer duration, i.e. 24 to 96 hours, and were often associated with paralysis and/or convulsions (Hall et al., 1977).

According to self-reporting, a student experienced immediately after the ingestion of 2 cups of tea prepared from 7-10 g of leaves of *D. stramonium* dry mouth, blurred vision, tachycardia, urinary retention and hallucination (Dieckhöfer et al., 1971).

7.5.5. Study in human volunteers on food with added atropine and (-)-scopolamine

Perharič et al. (2013a) examined the effects of a mixture of atropine and (-)-scopolamine hydrochloride added to buckwheat-based meal in a double-blind study involving 20 healthy adult males (18-28 years). The volunteers were exposed to control or four doses of atropine and (-)-scopolamine according to a cross-over protocol with a period of at least two weeks between the different meals. All twenty volunteers received meals containing nominal atropine/(-)-scopolamine concentrations of 75.0/37.5, 250.0/125.0,

[19] The CONTAM Panel noted that the reported (-)-scopolamine content seems unlikely high. The published recipe (Smith et al., 1991) reports that 115 flower heads of *Datura suaveolens* were fermented in a final volume of one gallon. Hence, such an infusion should contain 0.5-0.6 flower heads per 15-20 mL dose. Other publications report the (-)-scopolamine content of one flower *Datura suaveolens* to be approximately 0.65 mg (-)-scopolamine (Hall et al., 1977). Using the numbers published by Hall et al. (1977) 15-20 mL of the 'homemade wine' would contain around 300 to 400 µg (-)-scopolamine.

and 2500.0/1250.0 μg per meal, and a control meal. In addition nine of the 20 volunteers received a meal containing 25.0/12.5 μg and the other 11 received a meal containing 750.0/375.0 μg. Thus, all volunteers received within a period of about a maximum of six months the control meal and four TA-containing meals in a random order. The concentrations of the two TAs in the meals were analytically determined following the cooking process and the doses (adjusted for the breakdown during cooking) were estimated to be 0.12, 0.37, 1.22, 3.58 and 12.10 μg/kg b.w. for atropine, and 0.10, 0.29, 0.95. 2.81 and 9.50 μg/kg b.w. for (-)-scopolamine. The cumulative doses were expressed as 0.32, 0.95, 3.12, 9.20 and 31.10 μg/kg b.w. atropine equivalent by the authors (assuming that (-)-scopolamine is twice as effective as atropine). After 30 minutes of rest before each treatment, the baseline measurements of body temperature, heart rate, pupil size, near-point vision, salivary secretion and sweat secretion were taken. The aforementioned parameters were regularly recorded up to 4 hours after administration of the meal. In addition, the electrocardiograms were continuously recorded during the study period. Subjective symptoms were recorded hourly up to 4 hours post treatment, and the subjects were asked to register possible symptoms for at least 24 hours or until the symptoms ceased. The authors reported means (+/- the standard errors of the mean) for these acute effects for all treatment groups and time points of examination without accounting for the intra-individual changes.

Following exposure to TAs, a slight increase in body temperature was observed, without a clear dose-response relationship. Reported mean heart rates were decreased in the 0.95 and 3.12 μg/kg b.w. atropine equivalent groups and increased at the highest dose level of 31.10 μg/kg b.w. atropine equivalent in comparison to controls. Based on the analysis of the individual data obtained by the authors, the CONTAM Panel observed a statistically significant decrease ($p<0.05$ not adjusted for multiplicity) at the level of 0.95 μg/kg b.w. atropine equivalent at 2-4 hour after treatment and at the level of 3.12 μg/kg b.w. atropine equivalent at all examinations after treatment. Heart rate was not statistically significantly different from the control at the level of 9.20 μg/kg b.w. atropine equivalent at all examinations after treatment. In the highest dose treatment group, heart rates were statistically significantly increased at all time points in comparison to controls. The mean salivary secretion decreased with increasing dose of TAs throughout the monitoring period, and statistical significance was reported at 9.20 μg/kg b.w. atropine equivalents and above. The authors reported a similar decreasing trend for sweat secretion and increasing pupil size with administered atropine equivalent dose. CNS symptoms like drowsiness, dizziness, headache and nausea generally increased in a dose related manner. The CONTAM Panel identified the 0.95 μg/kg b.w. atropine equivalent dose as a lowest-observed-adverse-effect level (LOAEL) based on dizziness, headache and nausea reported for one individual who reported also drowsiness at 3.12 μg/kg b.w. More severe symptoms such as speech disturbance or ataxia were reported at 3.12 μg/kg b.w. and above, or 9.20 μg/kg b.w. and above, respectively. The authors concluded that no significant changes were observed at the lowest dose tested (Perharič et al., 2013a). However, the CONTAM Panel questioned whether the statistical analysis in this study was adequate to support this conclusion.

7.6. Dose response assessment

7.6.1. Dose response data in experimental animals

The effect seen at the lowest doses in studies in experimental animals was pupillary dilation, indicating pharmacological activity. This was reported in all animals of all dose groups in 2-year studies with (-)-scopolamine in rats and mice. The lowest tested dose was 0.7 mg/kg b.w. per day (-)-scopolamine. These data cannot be modelled because there are no quantitative measurements of pupil size and the effect occurred with a 100 % incidence at all doses. Taking into account also that human data are available for (-)-scopolamine, (-)-hyoscyamine and atropine, the CONTAM Panel did not further consider the dose response data in animals.

7.6.2. Dose response data in humans

(-)-Hyoscyamine

(-)-Hyoscyamine sulphate dihydrate is prescribed in single oral doses of 0.125-0.3 mg, equivalent to 0.10-0.24 mg (-)-hyoscyamine (base), corresponding to 1.4-3.4 µg (-)-hyoscyamine/kg b.w. assuming a default b.w. of 70 kg (as indicated by EFSA Scientific Committee (SC), 2012), up to 6 times daily in the treatment of visceral spasm. To avoid possible acute severe adverse effects associated with overdosage the oral therapeutic use of (-)-hyoscyamine sulphate dihydrate is limited to a single dose of 0.5 mg (expressed as (-)-hyoscyamine of 0.4 mg corresponding to 5.7 µg/kg b.w. assuming a default b.w. of 70 kg) and a daily dose of 1.5 mg (expressed as (-)-hyoscyamine of 1.2 mg per day corresponding to 17 µg/kg b.w. per day assuming a default b.w. of 70 kg) (Bracher et al., 2011; Martindale, 2012). It has to be taken into consideration that therapeutic doses below the recommended maximum limits may already be associated with less severe acute adverse effects such as slight cardiac slowing or some dryness of the mouth. These dose response data do not apply to individuals with contraindications.

Upon chronic intake of (-)-hyoscyamine (base) in daily doses higher than 3.6 mg development of psychological dependence has been reported (Marquardt and Schäfer, 2004).

Atropine

For most medical indications, such as treatment of bradycardia or as a premedicant in anaesthetic practice atropine is administered by s.c., i.m. or i.v. injection (e.g. Martindale, 2011a). Atropine is given orally in the treatment of gastrointestinal, biliary or renal colics and in order to reduce gastric or pancreatic secretion. For these purposes atropine sulphate monohydrate is administered in single oral doses of 0.5-1 mg (equivalent to 0.42-0.83 mg atropine (base), corresponding to 6-12 µg atropine/kg b.w. assuming a default b.w. of 70 kg) which may be repeated twice up to a daily dose of 1.5-3 mg (equivalent to 1.25-2.5 mg per day atropine, corresponding to 18-36 µg atropine/kg b.w. per day assuming a default b.w. of 70 kg) for adults. For children 2-5 years of age the single oral dose of atropine sulphate monohydrate amounts to 0.25 mg (equivalent to 0.2 mg atropine) and the maximum daily dose to 0.75 mg (equivalent to 0.6 mg atropine) (Fachinformation, 2006; Bracher et al., 2011). Assuming a b.w. of 12-20 kg for children 2-5 years of age (based on EFSA Scientific Committee (SC), 2012), the single oral dose of atropine would range from 10 to 17 µg atropine/kg b.w. and the maximum daily dose from 30 to 50 µg atropine/kg b.w.

Effects of atropine are dose dependent. In an oral dose of 0.5 mg atropine (corresponding to 7 µg atropine/kg b.w. assuming a default b.w. of 70 kg) reported acute side effects are slight cardiac slowing, some dryness of mouth and inhibition of sweating. An oral dose of 1 mg atropine (corresponding to 14 µg atropine/kg b.w. assuming a default b.w. of 70 kg) is associated with definite dryness of mouth, thirst, acceleration of heart, sometimes preceded by slowing and mild dilation of pupils. For an oral dose of 2 mg atropine (corresponding to 28 µg atropine/kg b.w. assuming a default b.w. of 70 kg) indicated adverse effects are rapid heart rate, palpitation, marked dryness of mouth, dilated pupils and some blurring of near vision. At a dose of 5 mg atropine (corresponding to 71 µg atropine/kg b.w. assuming a default b.w. of 70 kg) the previously mentioned symptoms occur in a more pronounced form. Furthermore difficulties of speaking and swallowing, restlessness and fatigue, headache, dry and hot skin, difficulties in micturition and reduced peristalsis are observed. In oral doses of 10 mg atropine (corresponding to 143 µg atropine/kg b.w. assuming a default b.w. of 70 kg) and more, additional symptoms occur such as rapid and weak pulse, practically obliterated iris, very blurred vision, flushed, hot, dry and scarlet skin, ataxia, excitement, hallucinations, delirium, and coma (Brunton et al., 2006; Bracher et al., 2006, 2011).

The MED of atropine in children, measured by the suppression of salivation as onset of effect, ranged from 14 µg/kg b.w. for infants from 1 to 3 years to 22 µg/kg b.w. for children aged 3-6 years and 20 µg/kg b.w. for children aged 6-12 years (Unna et al., 1950).

(-)-Scopolamine

In the prevention of motion sickness (-)-scopolamine hydrobromide trihydrate is administered in single oral doses of 0.25 to 1 mg (equivalent to 0.17-0.69 mg (-)-scopolamine (base), corresponding to 2.5-10 µg (-)-scopolamine/kg b.w. assuming a default b.w. of 70 kg) repeated if required twice in 24 hours up to a maximum daily dose of 3 mg (equivalent to 2.1 mg (-)-scopolamine, corresponding to 30 µg (-)-scopolamine/kg b.w. assuming a default b.w. of 70 kg) for adults (Bracher et al., 2006; Martindale, 2011b). Children aged 4 to 10 years may be given 0.075 to 0.15 mg (equivalent to 0.05-0.1 mg (-)-scopolamine), and those over 10 years, 0.15 to 0.3 mg (equivalent to 0.1-0.2 mg (-)-scopolamine). Children aged 3 to 4 years may be given 0.075 mg (equivalent to 0.05 mg (-)-scopolamine), repeated once if necessary to a maximum total dose of 0.15 mg (equivalent to 0.1 mg (-)-scopolamine) in 24 hours (Martindale, 2011b). Assuming a b.w. of 23 kg for children 4-10 years of age (based on EFSA Scientific Committee (SC), 2012), the single oral dose of (-)-scopolamine would range from 3 to 6 µg (-)-scopolamine/kg b.w.

(-)-Scopolamine hydrobromide trihydrate is used orally or sublingually in palliative care of children to reduce excessive respiratory secretions, although care has to be taken to avoid the discomfort of a dry mouth. In single doses of 10 µg/kg b.w. (equivalent to 7 µg (-)-scopolamine/kg b.w.) it is recommended four times daily for the age of 2 to 12 years and of 0.3 mg/person (equivalent to 0.2 mg (-)-scopolamine/person) four times daily for the age of 12 to 18 years. In palliative care (-)-scopolamine hydrobromide trihydrate is also given sublingually to reduce the frequency of spasm in bowel colic in single doses of 10 µg/kg b.w. (equivalent to 7 µg (-)-scopolamine/kg b.w.) four times daily for children and of 0.3 mg/person (equivalent to 0.2 mg (-)-scopolamine/person, corresponding to 3 µg (-)-scopolamine/kg b.w. assuming a default b.w. of 70 kg) four times daily for adults (Martindale, 2011b).

With low acute doses of (-)-scopolamine (0.1 or 0.2 mg of the base, corresponding to 1.4 or 3 µg (-)-scopolamine/kg b.w. assuming a default b.w. of 70 kg), the cardiac slowing is greater than with low acute doses of atropine. With higher doses of (-)-scopolamine a transient cardioacceleration may be observed (Brunton et al., 2006).

Belladonna Leaf*; Belladonnae Folium (Ph. Eur. 7)*

Belladonna Leaf (Ph. Eur. 7) contains not less than 0.30 % of total alkaloids, calculated as (-)-hyoscyamine. The alkaloids consist mainly of (-)-hyoscyamine with smaller amounts of (-)-scopolamine. The recommended single oral doses for belladonna leaves are 0.05-0.1 g (corresponding to 0.15-0.3 mg of total alkaloids or (-)-hyoscyamine/person and 2-4 µg of total alkaloids or (-)-hyoscyamine/kg b.w. assuming a default b.w. of 70 kg) which may be repeatedly taken up to a maximum of 0.3 g (corresponding to 0.9 mg of total alkaloids or (-)-hyoscyamine/person and 13 µg of total alkaloids or (-)-hyoscyamine/kg b.w. assuming a default b.w. of 70 kg) in 24 hours (EMEA, 1998b; Bracher et al., 2004; Ph. Eur.7, 2011). The maximum single dose for belladonna leaves is reported to be 0.2 g (corresponding to 0.6 mg of total alkaloids/person and 9 µg of total alkaloids/kg b.w. assuming a default b.w. of 70 kg) three times a day resulting in a maximum daily dose of 0.6 g belladonna leaves (corresponding to 1.8 mg of total alkaloids/person and 26 µg of total alkaloids/kg b.w. assuming a default b.w. of 70 kg) (EMEA, 1998b).

Atropine/(-)-scopolamine mixture

In the double-blind, placebo-controlled cross-over study of Perharič et al. (2013a), described in Section 7.5.5, a mixture of atropine and (-)-scopolamine hydrochloride was given to up to 20 young healthy male volunteers at atropine/(-)-scopolamine doses of 25.0/12.5 µg, 75.0/37.5 µg, 250/125 µg, 750/375 µg and 2500/1250 µg in a boiled buckwheat dish. Perharič et al. (2013a) estimated the ingested doses of atropine and (-)-scopolamine, taking into account the breakdown during cooking, and expressed the total dose as atropine (Table 13). The CONTAM Panel noted that atropine is a racemic mixture of (+)- and (-)-hyoscyamine, and that only the (-) enatiomer has anticholinergic activity. Therefore the CONTAM Panel made a further adjustment to convert the dose to the active alkaloid constituents (Table 13).

Table 13: Dose groups in the volunteer study of Perharič et al. (2013a).

DOSE GROUPS						
Number of subjects	**Atropine added (µg/meal)**	**(-)-Scopolamine added (µg/meal)**	**Atropine µg/kg b.w.[(a)]**	**(-)-Scopolamine µg/kg b.w.[(a)]**	**Total dose of atropine equivalent (µg/kg b.w.) as expressed by Perharič et al. (2013a)**	**Total dose (µg/kg b.w.) expressed as sum of (-)-scopolamine plus (-)-hyoscyamine[(b)]**
20	0	0	0.00	0.00	0.00	0.00
9	25.0	12.5	0.12	0.10	0.32	0.16
20	75.0	37.5	0.37	0.29	0.95	0.48
20	250	125	1.22	0.95	3.12	1.56
11	750	375	3.58	2.81	9.20	4.60
20	2500	1250	12.1	9.50	31.1	15.55

b.w.: body weight.
(a): As reported by Perharič et al. (2013a), taking into account the breakdown during cooking.
(b): As calculated by the CONTAM Panel, taking into account that atropine is a racemic mixture (50/50) and assuming that (+)-hyoscyamine has a negligible anticholinergic activity, expressed as sum of (-)-scopolamine plus (-)-hyoscyamine.

Several peripheral antimuscarinic effects including changes in heart rate, decreased salivary and sweat secretion (resulting in increased body temperature) and pupil dilation, and also CNS effects such as drowsiness, dizziness, headache and nausea were reported. For heart rate, there was a biphasic response, with a decrease at lower doses and an increase at higher doses. The decreased heart rate occurred at a lower dose than the other peripheral antimuscarinic effects, and appeared to be the critical effect in this study. The CONTAM Panel concluded that because of the biphasic nature of the response, the data were not suitable for dose-response modelling. Based on their statistical analysis of the heart rates, the authors concluded the lowest dose was the NOAEL, which was equivalent to 0.16 µg/kg b.w. expressed as sum of (-)-scopolamine plus (-)-hyoscyamine (see Table 13). The CONTAM Panel noted that the study design and statistical analysis did not preclude that a small decrease in heart rate could also occur at this dose. The next higher dose of 0.48 µg/kg b.w. resulted in a transient lowering of the heart rate, which is not adverse in healthy volunteers, but could be adverse in more susceptible individuals, such as those with bradycardia. For the subjective CNS effects the lowest dose of 0.16 µg/kg b.w. is the NOAEL. Intake of the next higher dose of 0.48 µg/kg b.w. resulted in dizziness, headache and nausea in one individual out of twenty.

7.7. Derivation of a Health-based Guidance Value

The toxicological effects of (-)-hyoscyamine and (-)-scopolamine relate to their pharmacological effects, which are mediated by their antagonistic action on muscarinic ACh receptors in the CNS and autonomic nervous system (ANS). The effects of these two TAs are similar in the ANS, resulting in decreased secretions from the salivary, bronchial and sweat glands, dilation of the pupils, paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion. (-)-Hyoscyamine and (-)-scopolamine differ in their ability to affect the CNS, with (-)-scopolamine having more prominent depressing central effects at therapeutic doses.

The pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration, and therefore the CONTAM Panel concluded that it was appropriate to establish an ARfD[20] for these substances. Since they are not bioaccumulative or genotoxic and do not exhibit chronic toxicity, the ARfD would also protect against effects of long-term exposure. Due to the common mode of

[20] The acute reference dose is the estimate of the amount of substance in food, normally expressed on a body-weight basis (mg/kg or µg/kg of body weight), that can be ingested in a period of 24 hours or less without appreciable health risk to the consumer on the basis of all known facts at the time of evaluation (JMPR, 2002)

action through receptor interaction, the CONTAM Panel considered it appropriate to establish a group ARfD for (-)-hyoscyamine and (-)-scopolamine.

The lowest single therapeutic doses are 1.4 µg/kg b.w. for (-)-hyoscyamine, 6 µg/kg b.w. for atropine (corresponding to about 3 µg/kg b.w. (-)-hyoscyamine) and 2.5 µg/kg b.w. for (-)-scopolamine. These doses can be associated with adverse side effects, such as cardiac slowing or dryness of the mouth, and do not apply to individuals with contraindications who are likely to be more sensitive to some effects.

Information on the lowest doses associated with pharmacological activity of (-)-hyoscyamine and (-)-scopolamine is available from the human volunteer study of Perharič et al. (2013a). This study provided dose response information for a relevant mixture of (-)-hyoscyamine and (-)-scopolamine in food, with no statistically significant effect at the lowest dose of 0.16 µg/kg b.w. expressed as sum of these two TAs. The CONTAM Panel concluded that this dose level provided the preferred basis for establishing a group ARfD. The Panel noted that the next higher dose of 0.48 µg/kg b.w. resulted in a transient statistically significant lowering of the heart rate, which is not adverse in healthy individuals but could be in more susceptible individuals, such as those with bradycardia. Also regarding CNS effects, 0.48 µg/kg b.w. was a LOAEL resulting in dizziness, headache and nausea only in one individual out of twenty. Thus the overall NOAEL from this study was 0.16 µg/kg b.w. expressed as the sum of (-)-hyoscyamine and (-)-scopolamine. The Panel decided to apply an uncertainty factor of 10 for inter-individual differences to allow for the fact that this was a small study in young healthy male volunteers. The Panel divided the NOAEL of 0.16 µg/kg b.w. by the uncertainty factor of 10 and established a group ARfD of 0.016 µg/kg b.w. expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. The group ARfD is approximately two orders of magnitude lower than the lowest single doses of (-)-hyoscyamine and (-)-scopolamine used therapeutically.

8. Risk characterisation

8.1. Human health risk characterisation

Adequate occurrence data were only available for the food group for infants and young children 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' and the relevant consumption data were only sufficient for exposure assessment for the toddler age class. Therefore, exposure estimates were only performed for this food and age class. Risk characterisation was not possible for other age classes.

Because TAs have acute pharmacological effects, it is necessary to consider estimates of acute exposure based on high-level consumption and high-level occurrence data, a scenario that could apply to some consumers on some eating occasions, and is the most likely to result in effects.

The estimates of dietary exposure are based on the few available dietary surveys for toddlers, which are from Germany and Finland, and not necessarily representative of all European countries. Although the data represent only the food group for infants and young children "Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids", these are the foods for toddlers most likely to contain TAs, and taking into account that other food samples did not contain detectable concentrations, the total exposure from all food sources is unlikely to be much higher.

For the German surveys, the estimates of acute dietary exposure assessment carried out using the deterministic approach with the average of the last quartile of the occurrence data for the sum of (-)-hyoscyamine and (-)-scopolamine were approximately twice the group ARfD of 0.016 µg/kg b.w. for mean consumption and five times the group ARfD for high level consumption. For the Finnish survey the estimated mean exposure was six to seven times higher than the group ARfD, and the number of consumption days in the survey was too few to calculate a robust exposure for high level consumption.

In a refinement of the dietary exposure assessment (see Section 6.1.2.) the number of cases above the group ARfD and its related probability were calculated for each individual combining individual

consumption data with each of the reported occurrence data. Following this approach, the average probability of being above the group ARfD for all consumers and high consumers was estimated. For all consumers the average probability was 11.6 % and 15.9 % in the German surveys and the Finnish survey, respectively. For high consumers (95th percentile consumption) the probability was only calculated for the merged German surveys, with an estimated average probability of being above the group ARfD of 17.9 %.

The CONTAM Panel concluded that, based on the limited available information, the dietary exposure of toddler consumers could exceed the group ARfD in approximately 11 to 18 % of the consumption days.

8.2. Animal health risk characterisation

There are considerable differences between species in risks associated with consuming plant materials derived from *Datura* spp. Since these plants may contain (-)-hyoscyamine and (-)-scopolamine as well as a number of other TAs in varying proportions and concentrations, it is difficult to relate the observed biological effects to a specific TA-composition.

Ruminant livestock are sensitive to *Datura* alkaloids, but in practice toxicosis is relatively rare because of the unpalatable nature of TA-containing plants. However, since TA-containing plants retain their toxicity after drying and storage, contaminated forages conserved as hay represent a potential risk of toxicosis. Based on the limited available data, EFSA (2008) concluded that cattle are sensitive to Datura alkaloids, and that although signs of toxicity are likely to occur at levels exceeding 500 μg (-)-hyoscyamine plus 100 μg (-)-scopolamine per kg b.w., levels of up to 300 μg /kg b.w. (total alkaloids) may be safe. Since no data have been published since 2008 to alter this conclusion, the CONTAM Panel proposes this as a NOAEL for cattle. In ruminants, the highest exposure was in the category 'cereal-fed beef cattle', for which an UB exposure of 0.25 μg /kg b.w. was estimated (Table 9), which is 1000 times lower than the NOAEL.

Based on the data of Piva et al. (1997) the CONTAM Panel identified a LOAEL of 1500 μg/kg feed in the ratio of 98:2 of (-)-scopolamine:(-)-hyoscyamine, corresponding to approximately 60 μg/kg b.w. for a 60 kg pig (Piva and Piva, 1995). Based on assumptions on feed intake and diet composition reported above, the CONTAM Panel estimated an UB dietary exposure for the sum of (-)-hyoscyamine and (-)-scopolamine of 0.15 μg/kg b.w. per day.

Poultry are considered to be more resistant than other farm animals to TAs (Werner and Brehmer, 1967). In studies with both fattening and laying hens, the intake of *D. ferox* (containing (-)-scopolamine as the major alkaloid) at an inclusion rate of 150 mg alkaloid/kg feed showed no adverse effects (EFSA, 2008). This compares with estimated UB diet concentrations of 4.97 and 5.49 μg/kg (sum of (-)-hyoscyamine and (-)-scopolamine) for fattening and laying hens, respectively.

Insufficient data are available to identify a NOAEL for horses. However, Naudé et al. (2005) concluded that the toxic dietary concentration of TAs for the horse is approximately 100 μg of (-)-hyoscyamine/kg feed (corresponding to 200 μg atropine/kg). Using upper bound estimates ((-)-hyoscyamine plus (-)-scopolamine) in feeds, the CONTAM Panel estimates likely maximum dietary concentrations of 2.3 μg/kg.

No data are available to identify NOAELs or LOAELs for cats and dogs.

In summary, TA toxicosis in livestock and companion animals is relatively rare because TA-containing plant products appear to be unpalatable and animals try to avoid them where possible. Furthermore, compared to other livestock, poultry and rabbits are considerably less sensitive to exposure to TAs due to the expression of specific hydrolysing enzymes that inactivate the alkaloids. NOAELs have been proposed for ruminants and pigs, but these are significantly higher than estimated exposure.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to TAs 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, and model input (parameters).

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 EFSAs request to submit occurrence data on TAs in food and feed, results on atropine and scopolamine, considered in this opinion as (-)-hyoscyamine and (-)-scopolamine, in 124 food and 611 feed samples were received. The samples were collected in Germany and the Netherlands. Around 83 % of the food samples and 91 % of the feed samples were left-censored. Most of the food samples with quantified TA concentrations belonged to only one group, 'Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids' (FoodEx Level 3). As the type of food commodities sampled is limited and the samples were only collected in two Member States, there is substantial uncertainty over possible regional differences in TA contamination of food and feed, and the CONTAM Panel recognised that the dataset is not representative of TA occurrence in food and feed across the EU market.

With respect to food consumption data, the selection of only one food category implied that only few consumption days from two countries were available in the Comprehensive Database. In addition, only one age class was represented with a significant number of subjects (toddlers, $\geq$ 12 months to $<$ 36 months old) and therefore a reliable acute dietary exposure assessment in the other age classes (infants, other children, adults, elderly and very elderly) was not possible. As with the occurrence data, the representativity of the exposure assessment at European level is rather limited as only four surveys (from two countries) were used to calculate the acute dietary exposure to TAs for toddlers. It is important to mention that dietary exposure for high consumers was only calculated for one country since in the survey with the highest exposure for the average consumers the consumption data were too few to calculate a robust exposure for high consumers. As several of the contaminated samples in the food group "Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids" are cereal products also indicated for infants, this age group could also be exposed to TA through the diet, specially in countries with high consumption of these cereal products. However, the lack of consumption data for infants prevented calculation of their dietary exposure to TA to confirm this assumption. All this together introduces a substantial uncertainty in the exposure assessment.

Although more than 200 different TAs were so far identified, due to lack of toxicity and occurrence data in food and feed, this risk assessment only covers (-)-hyoscyamine and (-)-scopolamine. Thus, the exposure to total TAs may be substantially underestimated.

TAs are susceptible to racemisation and degradation under conditions used for food and feed preparation (e.g. pH, temperature). For the moment there are not sufficient data on how to take those changes into account. Furthermore, there is insufficient information addressing effects such as *in vivo* racemisation or toxicity of TA degradation products.

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 TAs 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 TAs and laboratories can use any appropriate method of analysis, provided it can be demonstrated in a traceable manner that they fulfil the requirements according to ISO 17025. However, this may have only added slightly to the uncertainty in the analytical results as all analyses were performed by only one laboratory.

9.4. Other uncertainties

The CONTAM Panel considered it appropriate to establish a group ARfD for (-)-hyoscyamine and (-)-scopolamine due to the common mode of action through receptor interaction.

The ARfD was derived from a study in a small number (20) of healthy male volunteers exhibiting signs of pharmacological effects following a single dose of atropine and (-)-scopolamine in food, which was an order of magnitude lower than the doses reported to have similar effects in studies conducted in relation to pharmaceutical use. The NOAEL for CNS effects was based on reports of subjective symptoms. The CONTAM Panel noted that the anticholinergic activity of atropine has been historically attributed to its naturally occurring form (-)-hyoscyamine, but that estimates of the relative affinities of the enantiomers for muscarinic receptors vary and may be dose-dependent. The CONTAM Panel therefore took a conservative approach of assuming that the activity of atropine in this study was due to (-)-hyoscyamine. The effects reported at the LOAEL in this study were unlikely to be adverse in healthy people, but could be in more susceptible individuals. The CONTAM Panel assumed that an uncertainty factor of 10 would be adequate to allow for inter-individual differences. In setting the group ARfD based on the sum of (-)-hyoscyamine and (-)-scopolamine, the CONTAM Panel assumed equivalent potency of these TAs.

There is a lack of data on toxicity of TAs other than those covered in this opinion.

A number of species, particularly poultry and rabbits, are considerably less sensitive to exposure to TAs. This is due to the expression of specific hydrolysing enzymes that inactivate the alkaloids. However, significant inter-individual and inter-breed differences in susceptibility have been noted, leading to uncertainty of the effects of exposure in these species.

9.5. Summary of uncertainties

Table 14: Summary of the qualitative evaluation of the impact of uncertainties on the risk assessment of human and animal exposure to TAs through consumption of food and feed.

Sources of uncertainty	Direction
Uncertainty in analytical results	+/-[(a)]
Very low number of food and feed samples available for exposure assessment	+/-
Extrapolation of occurrence data from only two European countries over a limited period of time to the whole of Europe	+/-
Lack of occurrence data on TAs other than those considered in this opinion	-
Influence of food and feed processing on TA stability and toxicity of potential degradation products	+/-
Use of very limited food consumption data from two countries only	+/-
Limited data on feed consumption across Europe	+/-
Human dietary exposure estimates for only one age class	+/-
Lack of data on toxicity on TAs other than those covered in this opinion	-
Assumption that transient lowering of the heart rate is adverse for susceptible individuals	+
The NOAEL for CNS effects was based on reports of subjective symptoms (e.g. dizziness, headache and nausea)	-
Appropriateness of the uncertainty factor of 10 to allow for inter-individual variability	+/-
Assumption of equivalent potency of (-)-hyoscyamine and (-)-scopolamine	+/-

(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 (-)-hyoscyamine and (-)-scopolamine through consumption of food and feed is substantial. Risk assessment for other TAs was not possible due to the lack of information.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Background

- Tropane alkaloids (TAs) are secondary metabolites which naturally occur in plants of several families including Brassicaceae, Solanaceae and Erythroxylaceae. More than 200 TAs have been identified so far.

- TAs contain an azabicyclo[3.2.1]octane ring structure. The common structural element is the tropane skeleton, (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octane.

- The most studied TAs are (-)-hyoscyamine and (-)-scopolamine. Atropine is the racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine of which only the (-)-hyoscyamine enantiomer exhibits anticholinergic activity.

- The genus *Datura* is long known for its content of TAs. *Datura stramonium* is widely distributed in temperate and tropical regions and for this reason seeds have been found as impurities in linseed, soybean, millet, sunflower and buckwheat and products thereof.

- Due to the lack of data on other TAs, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) could only perform a risk assessment on (-)-hyoscyamine and (-)-scopolamine.

Methods of analysis

- The control on the current provisions in the European Union (EU) on *Datura* spp. in feed legislation can only be achieved by visual inspection of unprocessed raw feed material.

- Current analytical methods in use focus mainly on the content of (-)-hyoscyamine and/or atropine and (-)-scopolamine in food and feed and comprise gas chromatography – mass spectrometry (GC-MS) and high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS). The reported limits of quantification (LOQ) are 1-6 µg/kg by GC-MS and 3 µg/kg by HPLC-MS/MS.

- Biosynthesis results in (-)-hyoscyamine and (-)-scopolamine, but it is known that extraction and other work-up procedures can lead to some degree of racemisation. So far, methods in use for food and feed analysis generally do not apply enantioseparation of the individual TAs.

- Reference standards for (-)-hyoscyamine and (-)-scopolamine are available. Isotopically labelled standards (atropine-d_3 and (-)-scopolamine-d_3), useful for HPLC-MS/MS approaches are available from a limited number of commercial suppliers.

- So far, none of the methods for TAs in food or feed have been fully validated by inter-laboratory studies. In addition, no certified reference materials or proficiency studies are currently available for the determination of TAs in food or feed.

Occurrence

- As the biosynthesis of TAs leads to (-)-hyoscyamine and (-)-scopolamine, any analytical result where no stereoselective separation is achieved is regarded in this opinion as 100 % (-)-hyoscyamine or (-)-scopolamine. When analytical results were reported as atropine, the CONTAM Panel used these data as (-)-hyoscyamine.

- A total number of 124 food samples were available with analytical data on (-)-hyoscyamine and (-)-scopolamine. Sampling was mainly carried out in the Netherlands (112 samples), although a few samples were also collected in Germany (12 samples). Samples were collected between 2010 and 2012.

- Only four food groups were represented, of which 93 samples belonged to the food group "Cereal-based food for infants and young children".

- Most of the data on food were left-censored (103 out of 124 samples, 83 %), where neither (-)-hyoscyamine nor (-)-scopolamine were quantified. Almost all the quantified samples (19 out of 21) were reported for the food category "Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids", at FoodEx Level 3.

- The ingredients in these samples included wheat, maize, rye, oats and rice, indicating the possibility of contamination of different cereals.

- A total of 611 feed samples were available with analytical data on (-)-hyoscyamine and (-)-scopolamine. All feed samples were collected in the Netherlands between 2006 and 2011.

- Most of the data on feed were left-censored (557 out of 611 samples, 91 %). Almost half of the samples belonged to the feed group "Forage and roughage, and products derived thereof", and they were all left-censored. The highest levels of TAs were reported in samples of millet grains.

- More than half of the quantified samples were reported for compound feed.

Human dietary exposure

- The assessment of the acute dietary exposure was considerably influenced by the lack of occurrence data and consumption data relevant to the available occurrence data. Exposure to TAs could only be evaluated in one age class, "Toddlers" (≥ 12 months to < 36 months old), and only in two surveys (two countries).

- Using a deterministic approach, acute exposure to the sum of (-)-hyoscyamine and (-)-scopolamine for average consumers was 0.039 µg/kg body weight (b.w.) per day for Germany and 0.107 µg/kg b.w. per day for Finland due to the different consumption data for these two countries.

- A reliable acute dietary exposure for high consumers could only be estimated in the German survey, which was 0.081 µg/kg b.w. per day. A further refinement using a probabilistic approach was made to estimate the probability of exceeding the acute reference dose (ARfD; see below).

Animal dietary exposure

- Plants containing TAs are generally unpalatable, and will be avoided by most livestock unless other feed is unavailable. Therefore, animal exposure to the sum of (-)-hyoscyamine and (-)-scopolamine is primarily from consuming feed contaminated with TA-containing plant material.

- For lactating and fattening ruminants, the estimated lower bound (LB) and upper bound (UB) exposure ranged from 0.002 to 0.30 µg/kg b.w. per day, respectively.

- For piglets, fattening pigs and sows, the estimated LB and UB exposures ranged from 0.011 to 0.28 µg/kg b.w. per day, respectively.

- Exposure estimates for poultry were also low, with LB and UB estimates for laying hens, broilers, turkeys and ducks ranging from 0.012 to 0.33 µg/kg b.w. per day, respectively.

- For rabbits, the estimated LB and UB exposures were 2.04 and 2.47 µg/kg b.w. per day, respectively.

- For horses, the estimated LB and UB exposures were 0.001 and 0.046 µg/kg b.w. per day, respectively.

- The LB and UB exposure estimates of 0.005 and 0.047 µg/kg b.w. per day, respectively, were calculated for farmed fish.

- For dogs and cats, estimated LB and UB exposure ranged from 0.001 to 0.025 µg/kg b.w. per day.

Hazard identification and characterisation

Toxicokinetics

- Atropine, (-)-hyoscyamine and (-)-scopolamine are readily absorbed from the gastrointestinal tract, quickly and extensively distributed into tissues, and excreted predominantly in the urine.

- *N*-demethylation and Phase II conjugation of atropine, (-)-hyoscyamine and (-)-scopolamine are known metabolic pathways in humans.

- Internal exposures to, and effects of, atropine and (-)-hyoscyamine appear to be greater in children and the elderly.

- A polymorphic carboxylesterase catalysing the hydrolysis of TAs into inactive products has been identified in rabbit serum, which confers reduced sensitivity. This enzyme activity does not appear to be present in many other species, including humans.

Toxicity of tropane alkaloids

- (-)-Hyoscyamine and (-)-scopolamine are antimuscarinic agents which are antagonists of the muscarinic acetylcholine receptors primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves but also in the central nervous system (CNS).

- Based on their peripheral and/or CNS effects (-)-hyoscyamine/atropine and (-)-scopolamine are used therapeutically in veterinary and human medicine.

- The toxicological effects of TAs in experimental animals relate to their pharmacological activity, particularly pupillary dilation and neurobehavioural effects. At higher doses more severe effects appear to be secondary to inhibition of salivary gland secretion and swallowing, and dehydration. In 2-year studies of (-)-scopolamine in rats and mice pupillary dilation was observed in all treated animals of all dose groups including the lowest dose tested of 0.7 mg/kg b.w. per day.

- Atropine sulphate did not show any mutagenic activity in the Ames assay. Two limited studies did not show carcinogenic potential of atropine.

- No evidence of genotoxicity emerged from the studies reported for (-)-scopolamine. In a two-year gavage study there was no evidence of carcinogenic activity of (-)-scopolamine hydrobromide trihydrate in male or female F344/N rats or B6C3F1 mice administered 1, 5, or 25 mg/kg b.w. per day.

- In humans, the predominant peripheral antimuscarinic effects are decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion.

- (-)-Hyoscyamine and (-)-scopolamine differ in their antimuscarinic actions, particularly in their ability to affect the CNS. (-)-Hyoscyamine rarely has effects on the CNS in doses that are used clinically. In contrast, (-)-scopolamine has prominent depressing central effects at low therapeutic doses.

- The lowest therapeutic doses are 1.4 µg/kg b.w. for (-)-hyoscyamine, 6 µg/kg b.w. for atropine (corresponding to about 3 µg/kg b.w. (-)-hyoscyamine) and 2.5 µg/kg b.w. for (-)-scopolamine. These doses can be associated with adverse side effects, such as cardiac slowing or dryness of the mouth, and do not apply to individuals with contraindications who are likely to be more sensitive to some effects.

- The lowest dose clearly associated with biological activity of a mixture of (-)-hyoscyamine and (-)-scopolamine in food is 0.48 µg/kg b.w. which resulted in a transient lowering of the heart rate in healthy volunteers, an effect that could be adverse in susceptible individuals, such as those with bradycardia. The no-observed-adverse-effect level (NOAEL) in this study of 0.16 µg/kg b.w. expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, was considered to be the most appropriate basis for deriving an ARfD.

- The Panel applied an uncertainty factor of 10 for inter-individual differences to allow for the fact that this was a small study in young healthy male volunteers. The Panel divided the dose level of 0.16 µg/kg b.w. by the uncertainty factor of 10 and established a group ARfD of 0.016 µg/kg b.w. expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency.

- The CONTAM Panel considers that the ARfD would also protect against effects of long-term exposure.

Adverse effects in livestock, fish and companion animals

- The adverse effects in livestock and companion animals were reviewed by EFSA in 2008; since then little new information has been published to alter their conclusions.

- There are considerable inter-species differences in the adverse effects of consuming plant material derived from *Datura, Hyoscyamus* or *Atropa* spp. Since these plants may contain (-)-hyoscyamine and (-)-scopolamine as well as a number of other TAs in varying proportions and concentrations, it is difficult to relate the observed biological effects to a specific TA-composition.

- Several cases of toxicity and deaths in livestock as a result of consuming *Datura*, *Hyoscyamus* or *Atropa* spp. have been described, particularly in horses and cattle following the consumption of contaminated hay.

- The predominant symptoms of intoxications in livestock include hyposalivation, tachycardia, hyperventilation, pupil dilation, restlessness, nervousness, muscle tremor, convulsions, delirium and death from asphyxia. In small ruminants, such as goats and sheep, typical symptoms also include drowsiness and reduced ability to stand.

- The European Agency for the Evaluation of Medicinal Products (EMEA; now the European Medicines Agency (EMA)) and the European Food Safety Authority (EFSA) concluded in their evaluations in 1997 and 2008 respectively, that residues of TAs in edible tissues (milk, meat or eggs) were unlikely to constitute a risk for consumers following the legal use of *Atropa belladonna* and atropine as authorised veterinary medicines, and no information has subsequently been published to alter these conclusions.

- Outside the EU, studies were recently performed to evaluate the effect on goat meat quality of atropine sulphate injected pre-slaughter. Injection with atropine sulphate improved tenderness, increased pH and water capacity and reduced drip loss of goat meat. The CONTAM Panel noted that such use of atropine is illegal in the EU but has the potential to become an emerging risk from imports from outside the EU.

Human health risk characterisation

- Risk characterisation was only possible for the toddlers age class because a reliable exposure assessment was not possible for other age classes.

- Using a deterministic approach, for the German consumption surveys, the estimates of acute dietary exposure assessment carried out for the sum of (-)-hyoscyamine and (-)-scopolamine were approximately twice the group ARfD of 0.016 µg/kg b.w. for mean consumption and five times the group ARfD for high level consumption. For the Finnish consumption survey the estimated mean exposure was six to seven times higher than the group ARfD, but a reliable estimate of high level exposure was not possible.

- Using a probabilistic approach, the acute exposure calculations showed an average probability of being above the ARfD for all consumers that ranged between approximately 11 % and 16 %. For

high consumers in the merged German surveys the average probability of being above the ARfD was 18 %.

Animal health risk characterisation

- TA toxicosis in livestock and companion animals is relatively rare because TA-containing plant products appear to be unpalatable and animals try to avoid them where possible.
- Certain livestock (poultry, rabbits and certain breeds of small ruminants) are considerably less sensitive to TAs due to the expression of specific hydrolysing enzymes that inactivate the alkaloids.
- A NOAEL has been proposed for ruminants and a lowest-observed-adverse-effect level (LOAEL) for pigs, but these are significantly higher than estimated exposure.

RECOMMENDATIONS

- There is a need for better characterisation of TAs occurring in food and feed either naturally or as contaminants.
- Analytical data should be collected on occurrence of TAs in cereals and oilseeds, including TAs not considered in this opinion occurring in food and feed commodities.
- There is a need for investigations into the agricultural conditions under which TAs occur in cereals and oilseeds.
- There is a need for defined performance criteria for the analysis of TAs in food and feed.
- There is a need for certified reference materials containing TAs at levels of interest as well as proficiency tests.
- There is a need for data on relative potency of (-)-hyoscyamine and (-)-scopolamine and for data on endogenous formation of (+)-hyoscyamine and its biological relevance.
- There is a need for toxicity data for TAs occurring in food and feed commodities other than those covered in this opinion.
- There is a need for information on stability of TAs during food and feed processing and the identity and toxicity of potential degradation products.

DOCUMENTATION PROVIDED TO EFSA

1. Heart rate data of all subjects as reported by Perharic et al. (2013a) and further information on the study design. June 2013. Submitted by Perharič L.

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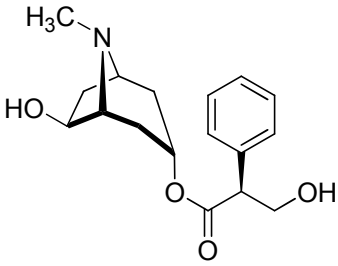
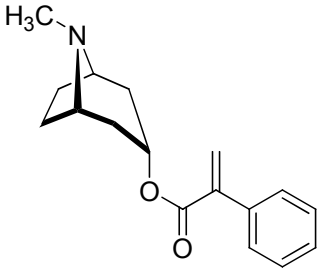
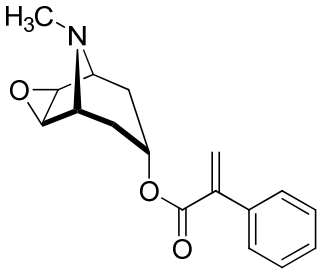
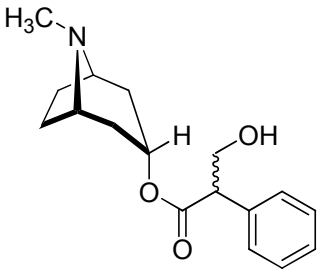
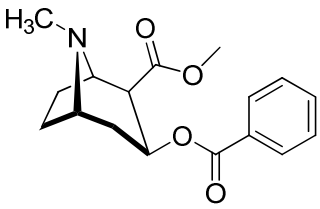
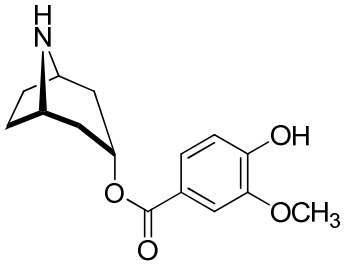
APPENDICES

Appendix A. Chemical structure of tropane alkaloids

name / CAS number / chemical formular / molecular weight	synonyms	structure
anisodamine CAS: 55869-99-3 chemical formula: $C_{17}H_{23}NO_4$ molecular weight: 305.37 g/mol	6-hydroxyhyoscyamine 6β-hydroxyhyoscyamine	
apoatropine CAS: 500-55-0 chemical formula: $C_{17}H_{21}NO_2$ molecular weight: 271.35 g/mol	atropyltropeine atropamine apohyoscyamine	
aposcopolamine CAS: 535-26-2 chemical formula: $C_{17}H_{19}NO_3$ molecular weight: 285.34 g/mol	oscine atropate apohyoscine	
atropine CAS: 51-55-8 chemical formula: $C_{17}H_{23}NO_3$ molecular weight: 289.37 g/mol	DL-hyoscyamine tropine (±)-tropate	
cocaine CAS: 50-36-2 chemical formula: $C_{17}H_{21}NO_4$ molecular weight: 303.35 g/mol	ecgonylbenzoate nose candy benzoylmethylecgonine	

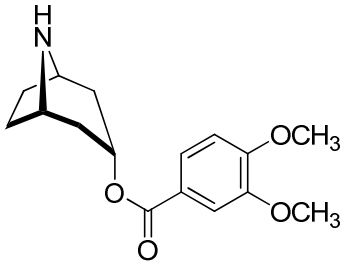
convolidine
CAS: 63911-32-0
chemical formula: $C_{15}H_{19}NO_4$
molecular weight: 277.32 g/mol

vanilloylnortropine



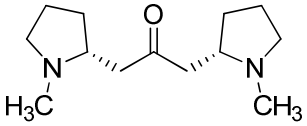
convolvine
CAS: 537-30-4
chemical formula: $C_{17}H_{23}NO_4$
molecular weight: 291.34 g/mol

norconvolamine
norconvolvamine
veratroylnortropine



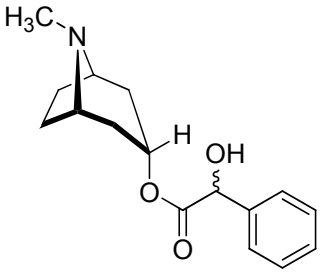
cuscohygrine
CAS: 454-14-8
chemical formula: $C_{13}H_{24}N_2O$
molecular weight: 224.34 g/mol

bellaradine
cuskhygrine
meso-cuscohygrine



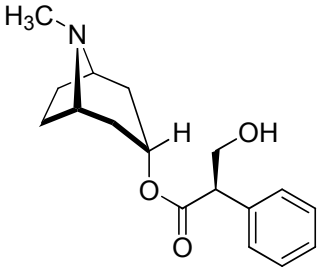
homatropine
CAS: 87-00-3
chemical formula: $C_{16}H_{21}NO_3$
molecular weight: 275.34 g/mol

homoatropine
mandelyltropeine
tropine (±)-mandelate



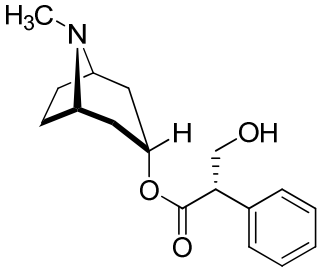
(+)-hyoscyamine
CAS: 101-31-5
chemical formula: $C_{17}H_{23}NO_3$
molecular weight: 289.37 g/mol

(*R*)-(+)-hyoscyamine
D-(+)-hyoscyamine
(+)-atropine



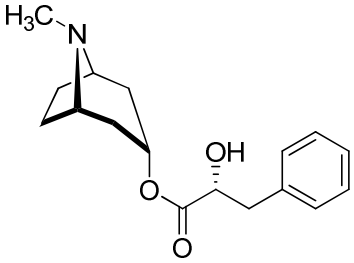
(-)-hyoscyamine
CAS: 101-31-5
chemical formula: $C_{17}H_{23}NO_3$
molecular weight: 289.37 g/mol

(*S*)-hyoscyamine
L-(-)-hyoscyamine
daturine



littorine
CAS: 21956-47-8
chemical formula: $C_{17}H_{23}NO_3$
molecular weight: 289.37 g/mol

(*R*)-(-)-littorine



phygrine
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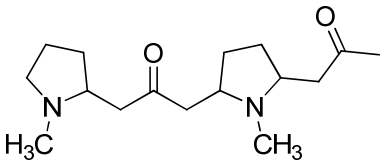
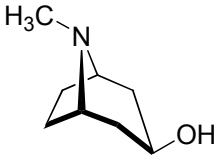
chemical formula: $C_{16}H_{28}N_2O_2$
molecular weight: 280.41 g/mol



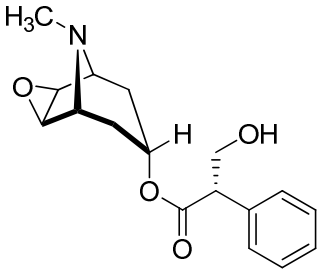
pseudotropine
CAS: 135-97-7
chemical formula: $C_8H_{15}NO$
molecular weight: 141.21 g/mol

pseudotropanol
ψ-tropine
3β-tropanol



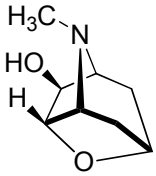
(-)-scopolamine
CAS: 51-34-3
chemical formula: $C_{17}H_{21}NO_4$
molecular weight: 303.35 g/mol

levo-duboisine
hyoscine

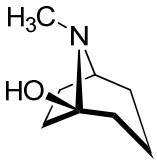


scopoline
CAS: 487-27-4
chemical formula: $C_8H_{13}NO_2$
molecular weight: 155.19 g/mol

oscine

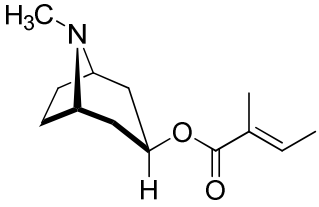


secotropane; example physoperuvine
CAS: 60723-27-5
chemical formula: $C_8H_{15}NO$
molecular weight: 141.21 g/mol



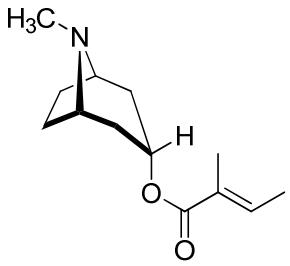
tigloidine
CAS: 495-83-0
chemical formula: $C_{13}H_{21}NO_2$
molecular weight: 223.31 g/mol

3β-tigloyloxytropane
tigloyl pseudotropine
tiglylpseudotropine
tiglyssin

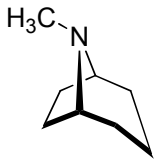


3α-tigloyloxytropane
CAS: 533-08-4
chemical formula: $C_{13}H_{21}NO_2$
molecular weight: 223.31 g/mol

tigloyltropine
tropigline
tropine tiglate
tropyl 2,3-dimethylacrylate

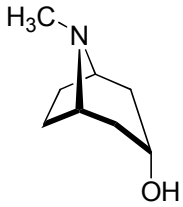


tropane
CAS: 529-17-9
chemical formula: $C_8H_{15}N_1$
molecular weight: 125.21 g/mol



tropine
CAS: 120-29-6
chemical formula: $C_8H_{15}NO$
molecular weight: 141.21 g/mol

tropanol
tropane-3α-ol



tropinone
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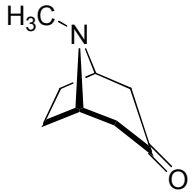
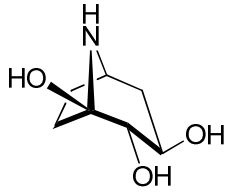
chemical formula: $C_8H_{13}NO$
molecular weight: 139.19 g/mol

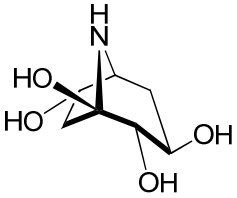
tropionone



Naturally occurring calystegines



calystegine A_3



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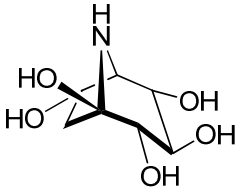

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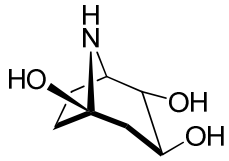

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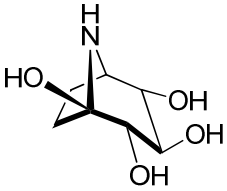

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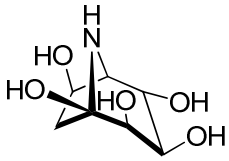

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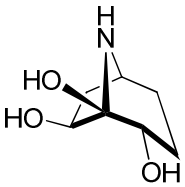
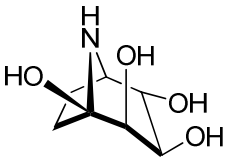


calystegine A_6



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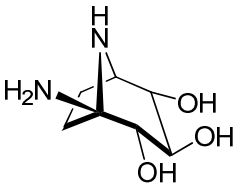
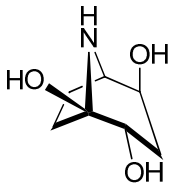


calystegine N_1



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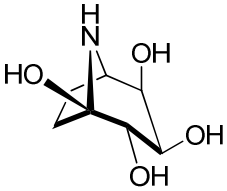
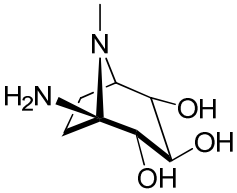
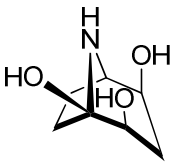


calystegine B_4



N-methyl-calystegine B_2



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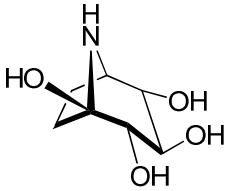

N-methyl-calystegine C_1

Appendix B. Occurrence

Table B1: Summary statistics for (-)-hyoscyamine concentrations (µg/kg) in the different food samples. Concentration data were rounded to two significant figures.

FoodEx Level 1	FoodEx Level 2	FoodEx Level 3	N	NLC	(-)-Hyoscyamine			
					Mean		Average last quartile	
					Lower bound	Upper bound	Lower bound	Upper bound
Fruiting vegetables	Berries and small fruits	Berries and small fruits	1	1	0.0	0.30	-	-
Food for infants and small children	Cereal-based food for infants and young children	Biscuits, rusks and cookies for children	27	27	0.0	0.30	-	-
		Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid	10	10	0.0	0.60	-	-
		Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids	56	39	3.6	3.8	18	18
Grains and grain-based products	Breakfast cereals	Cereal flakes (wheat, oat, mix)	7	7	0.0	0.30	-	-
	Grain milling products	Grain milling products	11	10	2.8	3.1	-	-
	Grains for human consumption	Buckwheat, millet, quinoa grains	5	5	0.0	0.30	-	-
Legumes, nuts and oilseeds	Legumes, beans, dried	Lupins (*Lupinus* spp.)	2	2	0.0	0.30	-	-
	Oilseeds (unspecified)	Oilseeds (unspecified)	5	4	0.060	0.30	-	-

N: number of samples; NLC: number of left-censored data.

Table B2: Summary statistics for (-)-scopolamine concentrations (µg/kg) in the different food samples. Concentration data were rounded to two significant figures.

FoodEx Level 1	FoodEx Level 2	FoodEx Level 3	N	NLC	(-)-Scopolamine			
					Mean		Average last quartile	
					Lower bound	Upper bound	Lower bound	Upper bound
Fruiting vegetables	Berries and small fruits	Berries and small fruits	1	1	0.0	0.30	-	-
Food for infants and small children	Cereal-based food for infants and young children	Biscuits, rusks and cookies for children	27	27	0.0	0.30	-	-
		Cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid	10	10	0.0	0.60	-	-
		Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids	56	44	0.86	1.1	18	18
Grains and grain-based products	Breakfast cereals	Cereal flakes (wheat, oat, mix)	7	7	0.0	0.30	-	-
	Grain milling products	Grain milling products	11	10	1.5	1.8	-	-
	Grains for human consumption	Buckwheat, millet, quinoa grains	5	5	0.0	0.30	-	-
Legumes, nuts and oilseeds	Legumes, beans, dried	Lupins (*Lupinus* spp.)	2	2	0.0	0.30	-	-
	Oilseeds (unspecified)	Oilseeds (unspecified)	5	5	0.0	0.30	-	-

N: number of samples; NLC: number of left-censored data.

Table B3: Summary statistics for (-)-hyoscyamine concentrations (µg/kg) in the different feed samples. Concentration data were rounded to two significant figures.

N			N	NLC	(-)-Hyoscyamine									
					Mean		Min		Max		Median		95th percentile	
					LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Compound feeds	66	Ruminants	29	16	5.7	6.8	0.0	2.0	44	44	0.0	2.0	-	-
		Poultry	20	17	1.9	3.6	0.0	2.0	26	26	0.0	2.0	-	-
		Equines	1	0	4.0	4.0	-	-	-	-	-	-	-	-
		Porcine	16	14	8.3	10	0.0	2.0	69	69	0.0	2.0	-	-
Cereal grains, their products and by-products	122	Millet grains	4	1	260	260	0.0	4.5	900	900	-	-	-	-
		Other cereals (barley, maize, oats, rice, rye, sorghum, triticale, wheat)	118	117	0.030	2.3	0.0	2.0	4.0	4.5	0.0	2.0	0.0	4.5
Forages and roughage, and products derived thereof			301	301	0.0	4.5	-	-	-	-	-	-	-	-
Legume seeds and products derived thereof			13	13	0.0	4.5	-	-	-	-	-	-	-	-
Oil seeds, oil fruits, and products derived thereof	71	Sunflower seeds	6	2	70	71	0.0	5.0	320	320	22	22	-	-
		Other seeds (soya, palm kernel, rape and linseed)	65	65	0.0	4.5	-	-	--	-	-	-	-	-
Other plants, algae and products derived thereof			32	28	13	17	0.0	4.5	250	250	0.0	4.5	-	-
Other seeds and fruits, and products derived thereof			5	4	18	22	0.0	4.5	91	91	-	-	-	-
Tubers, roots, and products derived thereof			1	1	0.0	4.5	-	-	-	-	-	-	-	-

LB: lower bound; Max: maximum; Min: minimum; N: number of samples; NLC: number of left-censored data; UB: upper bound.

Table B4: Summary statistics for (-)-scopolamine concentrations (µg/kg) in the different feed samples. Concentration data were rounded to two significant figures.

N			N	NLC	(-)-Scopolamine									
					Mean		Min		Max		Median		95th percentile	
					LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Compound feeds	66	Ruminants	29	15	2.8	3.8	0.0	2.0	15	15	0.0	2.0	-	-
		Poultry	20	16	0.90	2.5	0.0	2.0	7.0	7.0	0.0	2.0	-	-
		Equines	1	0	0.0	2.0	-	-	-	-	-	-	-	-
		Porcine	16	11	3.6	5.0	0.0	2.0	21	21	0.0	2.0	-	-
Cereal grains, their products and by-products	122	Millet grains	4	1	190	190	0.0	4.5	710	710	-	-	-	-
		Other cereals (barley, maize, oats, rice, rye, sorghum, triticale, wheat)	118	117	0.10	2.3	0.0	2.0	10	10	0.0	2.0	0.0	4.5
Forages and roughage, and products derived thereof			301	301	0.0	4.5	-	-	-	-	-	-	-	-
Legume seeds and products derived thereof			13	13	0.0	4.5	-	-	-	-	-	-	-	-
Oil seeds, oil fruits, and products derived thereof	71	Sunflower seeds	6	1	66	67	0.0	4.5	230	230	40	40	-	-
		Other seeds (soya, palm kernel, rape and linseed)	65	55	1.9	5.7	0.0	4.5	20	20	0.0	4.5	16	16
Other plants, algae and products derived thereof			32	28	6.3	10	0.0	4.5	110	110	0.0	4.5	-	-
Other seeds and fruits, and products derived thereof			5	4	6.0	9.6	0.0	4.5	30	30	-	-	-	-
Tubers, roots, and products derived thereof			1	1	0.0	4.5	-	-	-	-	-	-	-	-

LB: lower bound; Max: maximum; Min: minimum; N: number of samples; NLC: number of left-censored data; UB: upper bound.

Appendix C. Human consumption

Table C1: Dietary surveys considered for the acute dietary exposure assessment with the available number of days in the different age classes.

Code[(a)]	Country	Dietary survey[(b)]	Method	Days	Age	Number of days[c]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
AT	Austria	ASNS	24-hour recall	1	19-65					2 123		
BE/1	Belgium	Diet National 2004	24 h dietary recall	2	15-105				1 187	2 648	1045	1 448
BE/2	Belgium	Regional Flanders	Food record	3	2-5		108	1 875				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1-5	1 720	867	856				
BG/2	Bulgaria	NSFIN	24-hour recall	1	> 16				162	691	151	200
CY	Cyprus	Childhealth	Dietary record	3	11-18				909			
CZ	Czech Republic	SISP04	24-hour recall	2	4-64			798	596	3 332		
DE/1	Germany	DONALD 2006	Dietary record	3	1-10		276	633				
DE/2	Germany	DONALD 2007	Dietary record	3	1-10		255	678				
DE/3	Germany	DONALD 2008	Dietary record	3	1-10		252	669				
DE/4	Germany	National Nutrition Survey	24-hour recall	2	14-80				2 022	20 838	4 012	980
DK	Denmark	Danish Dietary Survey	Food record	7	4-75			3 426	3 398	19 722	2 159	140
EL	Greece	Regional Crete	Dietary record	3	4-6			2 508				
ES/1	Spain	AESAN	24-hour recall	2	18-60					828		
ES/2	Spain	AESAN-FIAB	Food record	3	17-60				226	2 748		
ES/3	Spain	NUT INK05	24-hour recall	2	4-18			798	1 302			
ES/4	Spain	enKid	24-hour recall	2	1-14		34	312	418			
EE	Estonia	NDS_1997	24-hour recall	1	19-64					1 866		
FI/1	Finland	DIPP	Food record	3	1-6		1 486	2 773				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25-74					3 150	926	
FI/3	Finland	STRIP	Food record	4	7-8			1 000				
FR	France	INCA2	Food record	7	3-79			3 315	6 728	15 727	1 824	571
HU	Hungary	National Repr Surv	Food record	3	18-96					3 222	618	240
IE	Ireland	NSFC	Food record	7	18-64					6 706		
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1-98	48	108	579	741	6 939	870	684
LV	Latvia	EFSA_TEST	24-hour recall	2	7-66			377	949	2 655		
NL/1	The Netherlands	DNFCS 2003	24 h dietary recall	2	19-30					1 500		
NL/2	The Netherlands	VCP kids	Food record	3	2-6		644	1 914				
PO	Polonia	IZZ_FAO_2000	24-hour recall	1	1-96		79	409	666	2 527	329	124
SE/1	Sweden	RIKSMATEN 1997-98	Food record	7	18-74					8 466		
SE/2	Sweden	NFAn	24-hour recall	4	3-18			5 875	4047			
SK	Slovakia	SK_MON_2008	24-hour recall	1	19-59					2 763		
Sl	Slovenia	CRP_2008	24-hour recall	1	18-65					407		
UK	United Kingdom	NDNS	Food record	7	19-64					12 068		

(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 'Use of the EFSA Comprehensive European FoodConsumption Database in Exposure Assessment' (EFSA, 2011b);
(c): Number of available days for acute exposure assessment in each age class.

Appendix D. Composition of diets used in estimating animal exposure to tropane alkaloids

This Appendix gives data used in estimating feed intakes for different livestock, fish and companion animals used in this Scientific Opinion. The composition of diets for each of the major farm livestock species are based on published guidelines on nutrition and feeding (e.g. AFRC, 1993; Carabano and Piquer, 1998; NRC 2007a,b; Leeson and Summers, 2008; EFSA, 2009; McDonald et al., 2011). They are therefore estimates made by the Panel on Contaminants in the Food Chain (CONTAM Panel), but are in agreement with common practice. Based on these estimates of intake, the lower bound (LB) and upper bound (UB) mean concentrations of tropane alkaloids (TAs) in the estimated diets for the farm livestock species and companion animals have been calculated and are given in this Appendix.

D1. Feed intake

D1.1. Cattle, sheep and goats

The diets of cattle, sheep and goats consist predominantly of forages supplemented mainly with cereal grains and vegetable proteins and other by-products of food production as necessary (see Section 5.2.). As discussed in Section 5.2, the leaves and stems of plants containing TAs have a pungent odour and taste, making them unpalatable to most livestock; as a result, animals generally avoid these plants where they are present as weeds, and will only consume them when other forages are unavailable. Because of this, the CONTAM Panel has assumed that forages make no significant contribution to exposure, and exposure has been estimated on intake of non-forage feeds only. Live weights, feed intakes and growth rates/productivity are from AFRC (1993) and NRC (2007a). The live weights, feed intakes, the proportion of the daily ration that is non-forage feed and growth rates/productivity for cattle, sheep and goats used in this Scientific Opinion are given in Table D1.

Table D1: Live weights, growth rate/productivity, dry matter intake for cattle, sheep and goats, and the proportions of the diet as non-forage.

	Live weight (kg)	Growth rate or productivity	Dry matter intake (kg/day)	% of diet as non-forage feed	Reference
Dairy cows, lactating	650	40 kg milk/day	20.7	40	AFRC (1993)
Fattening cattle: beef[(a)]	400	1 kg/day	9.6	15	AFRC (1993)
Fattening cattle: intensive cereal beef	400	1.4 kg/day	8.4	85	AFRC (1993)
Sheep: lactating	80	Feeding twin lambs	2.8	50	AFRC (1993)
Goats: milking[(b)]	60	6 kg milk/day	3.4	65	NRC (2007a)
Goats: fattening	40	0.2 kg/day	1.5	40	NRC (2007a)

(a): housed castrate cattle, medium maturing breed;
(b): months 2-3 of lactation.

D1.2. Pigs, poultry and fish

Data for feed intake and live weight of pigs, poultry and fish from EFSA (2009) and of ducks from Leeson and Summers (2008) are used in this Scientific Opinion (Table D2). Although some pigs (e.g. outdoor sows) and poultry (e.g. geese) may consume forages, in this Opinion it has been assumed that non-forage feeds represent 100 % of the diet.

Table D2: Live weights and feed intake for pigs, poultry and fish (EFSA, 2009) and ducks (Leeson and Summers, 2008).

	Live weight (kg)	Feed intake (kg dry matter/day)
Pigs: piglets	20	1.0
Pigs: fattening pigs	100	3.0
Pigs: lactating sows	200	6.0
Poultry: broilers[(a)]	2	0.12
Poultry: laying hens	2	0.12
Turkeys: fattening turkeys	12	0.40
Ducks: fattening ducks	3	0.14
Salmonids	2	0.04

(a): chickens for fattening

D1.3. Rabbits

A daily intake of 75 g/kg b.w. for a 2 kg rabbit is used in this Scientific Opinion to estimate exposure (derived from Carabano and Piquer, 1998).

D1.4. Horses

In this Scientific Opinion, exposure to TAs has been estimated for a mature horse (450 kg live weight) with a moderate level of activity and a dry matter intake of 9 kg/day, of which half is non-forage feeds (NRC, 2007b).

D1.5. Companion animals (dogs and cats)

The amount of food consumed is largely a function of the mature weight of the animal, level of activity, physiological status (e.g. pregnancy or lactation) and the energy content of the diet. In this Scientific Opinion, the CONTAM Panel estimated daily intake of dogs and cats based on NRC (2006). Intakes for a 25 kg dog and a 4 kg cat given below in Table D3 have been used in this Scientific Opinion to estimate exposure.

Table D3: Estimates of total food and intake, derived from NRC (2006) and the proportion that might consist of cereals and cereal by-products for adult dogs and cats[21].

	Dogs	Cats
Body weight (kg)	25	4
Feed intake (g/day)	360	60
% cereals and cereal by-products	65	55

D2. Diet composition and concentration estimates

Many livestock in the European countries are fed proprietary commercial compound feeds. However, in the absence of any reliable data on levels of TAs in compound feeds provided by the European countries (Section 4.2.4.2.), it has not been possible to estimate exposure based on compound feed intakes. As discussed in Section 5.2, a wide range of cereals and cereal by-products are used, in varying proportions, in livestock diets in Europe. However, in the absence of suitable data on levels of TAs in individual feed materials, the CONTAM Panel decided to use the LB and UB values for the feed group 'cereal grains, their products and by-products' of 11 and 43 µg/kg, respectively. Inclusion rates of cereal grains, their products and by-products in the non-forage component of the diet for

[21] J.M. Fremy, 2011, personal communication.

different livestock and companion animals are given below, and these have been used to calculate the TAs concentrations in the diets.

As noted above, levels of TAs in forage crops are low and it has been assumed that with the exception of maize silage they make no contribution to the exposure. Therefore, for cattle, sheep, goats and horses exposure has only been estimated for non-forage component of the ration. An exception to this is maize silage for which mean LB and UB values of 80 and 173 µg/kg, respectively have been reported (Section 4.2.4.2), and these values have been used in estimating the exposure for livestock fed maize silage-based diets.

D2.1. Cattle, sheep and goats

Assumed inclusion rates of cereal grains, their products and by-products in the non-forage component of diets for cattle, sheep and goats are given in Table D4, together with the calculated mean LB and UB concentrations of TAs based on the mean LB and UB concentrations above.

Table D4: Assumed inclusion rates (%) of cereal grains, their products and by-products in the non-forage component of diets for cattle, sheep and goats, and the calculated mean lower-bound and upper-bound concentrations of tropane alkaloids in these diets.

	Dairy cow: High yielding	Beef cattle: Cereal beef	Beef cattle: Fattening	Sheep: Lactating	Goats: Dairy	Goats: Fattening
Cereal grains and cereal by-products[(a)]	55 %	75 %	60 %	55 %	60 %	70 %
Total tropane alkaloids concentration (µg/kg dry matter)						
Lower bound	0.22	5.94	0.07	3.57	0.35	0.19
Upper bound	2.46	9.92	0.87	6.38	3.86	2.13

(a): The percentage of the non-forage component of the diet as reported in Table D1.

D2.2. Pigs and poultry

Pig and poultry diets consist predominantly of cereals and vegetable proteins. Pig diets may also include more fibrous feeds, particularly for older animals. The assumed inclusion rates of cereal grains, their products and by-products in the non-forage component of diets for pigs and poultry are presented in Table D5 together with the calculated mean LB and UB concentrations of TAs assuming LB and UB concentrations above.

Table D5: Assumed inclusion rates (%) of cereal grains, their products and by-products in diets of pigs and poultry, and the calculated mean lower-bound and upper-bound concentrations of the tropane alkaloids in these diets.

	Piglets	Pigs for fattening	Lactating sow	Broilers	Laying hens	Turkeys for fattening	Ducks for fattening
Cereal grains, their products and by-products	74 %	72 %	75 %	74 %	65 %	65 %	72 %
Tropane alkaloids concentration (µg/kg dry matter)							
Lower bound	0.56	0.38	0.39	0.38	0.50	0.36	0.61
Upper bound	5.56	4.97	4.98	4.97	5.49	5.24	6.27

D2.3. Rabbits

Although there are no published standard rations for rabbits, in a typical French commercial rabbit compound feed, cereal grains, their products and by-products account for 36 % of the total diet (T. Gidenne, 2011, personal communication), while sunflower meal was included at 20 % of the feed. Assuming LB and UB concentrations given in Section 4.2.4.2., estimated dietary LB and UB TAs concentrations are 27.9 and 33.5 µg/kg, respectively.

D2.4. Horses

Forages (fresh or conserved) account for 50 % or more of the total diet, but cereal grains – principally oats - and cereal by-products are widely used as feeds for horses. In this Scientific Opinion, it is assumed that cereal grains, their products and by-products represent 80 % of the non-forages component of the diet. In this calculation of exposure it is also assumed that beans are included in the ration (10 % of the non-forage feeds). Assuming that non-forage feeds account for half of the total feed dry matter intake, the calculated LB and UB TAs concentrations in this diet are 0.05 and 2.30 µg/kg, respectively.

D2.5. Farmed fish

A wide range of diets is used for commercially farmed fish in Europe. However, fishmeal and fish oils are usually the main ingredients. The salmon feed composition described by Berntssen et al. (2010) has been used as being representative of commercial feed producers, and in this formulation cereal grains and cereal by-products accounted for 25 % by weight of all ingredients. On this basis when cereal grains, their products and by-products contain 1.8 (LB) and 6.2 (UB) µg TAs/kg, the calculated mean LB and UB TAs concentrations in this diet are 0.26 and 2.34 µg/kg, respectively.

D2.6. Companion animals (dogs and cats)

In the absence of any general data on the composition of dog and cat food in the EU, information on ingredients used in typical French commercial pet foods has been used. According to data compiled from six and seven different food brands for dogs and cats, respectively, collected from pet food stores and veterinary clinics (J.M. Fremy, 2011, personal communication), the cereals used are wheat, maize, barley, rice, maize gluten meal. The amounts of cereals in the premium quality and the standard quality dog food were 45 % and 65 %, respectively; in cat foods cereals and cereal by-products represented 40 % in premium quality food and 55 % in the standard quality food (B.M. Paragon, 2011, personal communication)[22].

Assuming 65 and 55 % cereal grains, their products and by-products in standard dog and cat foods, respectively, and the above LB and UB concentrations, the mean LB and UB concentrations of TAs are estimated as 0.05 and 1.76 µg/kg in dog food, and 0.04 and 1.49 µg/kg in cat food, respectively.

[22] Based on statistics of 2010 of the French association of pet food manufacturers (FACCO), http://www.facco.fr/

Appendix E. Animal dietary exposure

Table E1: Dietary concentrations (µg/kg) and intake (µg/day and µg/kg b.w) of farm livestock and companion animals for (-)-hyoscyamine (Hyos.) and (-)-scopolamine (Scop.).

		Diet concentration (µg/kg)		Intake (µg/day)		Intake µg/kg b.w.	
		Hyos.	Scop.	Hyos.	Scop.	Hyos.	Scop.
Dairy: high yielding	LB	0.29	0.30	5.92	6.21	0.009	0.010
	UB	1.45	1.40	30.10	28.94	0.046	0.045
Beef: intensive cereal	LB	3.80	3.20	38.04	32.05	0.095	0.080
	UB	5.82	5.19	58.23	51.94	0.15	0.130
Beef: fattening	LB	0.12	0.10	1.14	0.99	0.003	0.002
	UB	0.54	0.50	5.15	4.79	0.013	0.012
Sheep: lactating	LB	2.06	1.91	5.76	5.35	0.096	0.089
	UB	3.50	3.30	9.79	9.24	0.16	0.154
Goats: lactating	LB	0.68	0.55	2.32	1.86	0.039	0.031
	UB	2.51	2.27	8.54	7.73	0.14	0.129
Goats: fattening	LB	0.36	0.29	0.55	0.44	0.014	0.011
	UB	1.38	1.25	2.06	1.87	0.052	0.047
Pig starter	LB	0.88	0.82	0.88	0.82	0.044	0.041
	UB	3.51	3.26	3.51	3.26	0.18	0.163
Pig finisher	LB	1.00	0.67	3.00	2.01	0.030	0.020
	UB	3.37	2.93	10.11	8.80	0.101	0.088
Lactating sow	LB	0.98	0.68	5.85	4.07	0.029	0.020
	UB	3.35	2.94	20.07	17.62	0.100	0.088
Chickens for fattening	LB	1.00	0.67	0.12	0.08	0.060	0.040
	UB	3.37	2.93	0.40	0.35	0.20	0.176
Laying hens	LB	0.85	0.74	0.10	0.09	0.051	0.045
	UB	3.45	3.19	0.41	0.38	0.21	0.191
Turkeys for fattening	LB	0.85	0.61	0.34	0.24	0.028	0.020
	UB	3.36	3.02	1.34	1.21	0.11	0.101
Ducks for fattening	LB	0.87	0.87	0.12	0.12	0.041	0.040
	UB	3.83	3.63	0.54	0.51	0.18	0.169
Horses	LB	0.53	0.21	4.80	1.85	0.011	0.004
	UB	1.66	1.33	14.94	11.99	0.033	0.027
Salmonids	LB	0.32	0.36	0.013	0.014	0.006	0.007
	UB	1.42	1.37	0.06	0.05	0.028	0.027
Cats	LB	0.43	0.17	0.026	0.010	0.006	0.002
	UB	1.16	0.89	0.069	0.053	0.017	0.013
Dogs	LB	0.51	0.20	0.18	0.070	0.007	0.003
	UB	1.37	1.05	0.49	0.38	0.020	0.015
Rabbits	LB	14.47	13.38	2.17	2.01	1.09	1.00
	UB	17.31	16.22	2.60	2.43	1.30	1.22

b.w.: body weight; hyos.: (-)-hyoscyamine; LB: lower bound; scop.: (-)-scopolamine; UB: upper bound.

ABBREVIATIONS

ACh	Acetylcholine
Afssa	Agence française de securité sanitaire des aliments
AGP	α_1-acid glycoprotein
ANS	Autonomic nervous system
Anses	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
APCI	Atmospheric pressure chemical ionization
ARfD	Acute Reference Dose
AUCs	Areas under the curve
BfR	German Federal Institute for Risk Assessment
b.w.	Body weight
cAMP	Cyclic adenosine monophosphate
CE	Capillary electrophoresis
CNS	Central nervous system
CONTAM Panel	Panel on Contaminants in the Food Chain
CSP	Chiral stationary phases
CYP	Cytochrome P450
CZE	Capillary zone electrophoresis
DAD	Diode array detection
DAO	Diamine oxidase
DART	Direct analysis in real time
DCM Unit	Dietary and Chemical Monitoring Unit
DESI	Desorption electrospray ionization
d.w.	Dry weight
EFSA	European Food Safety Authority
EI	Electron-ionization
EI-MS	Electron-ionization mass spectrometry
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
ESI+	Positive ion electrospray ionization
EU	European Union
FID	Flame ionization detection
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detection
GC-SIM-MS	GC-MS in selected ion monitoring mode
GC-MS	Gas chromatography-mass spectrometry
GI	Gastrointestinal
HPLC	High-performance liquid chromatography
HPLC−MS	High-performance liquid chromatography-mass spectrometry
HPLC-MS/MS	high performance liquid chromatography-tandem mass spectrometry
HPLC-MS/(MS)	High performance liquid chromatography-(tandem) mass spectrometry
HPLC−UV	High-performance liquid chromatography-ultra violet detection
hyos	(-)-hyoscyamine
H6H	Hyoscyamine 6β-hydroxylase
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
LB	Lower bound
LC	Left-censored

LLE	Liquid-liquid extraction
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
LOD	Limit of detection
LOQ	Limit of quantification
MCX	Mixed-mode reversed-phase cation-exchange
MED	Minimal effective dose
MEEKC	Microemulsion electrokinetic chromatography
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
ML	Maximum level
MRL	Maximum Residue Level
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSPD	Matrix solid phase dispersion
N	Number of samples
NACE	Non-aqueous capillary electrophoresis
n.d.	Not detected
NLC	Number of left-censored data
NOAEL	No-observed-adverse-effect level
NTP	National Toxicology Program
QuEChERS	Quick, easy, cheap, effective, rugged and safe
RASFF	Rapid Alert System for Feed and Food
RIA	Radioimmunoassay
RP	Reversed phase
scop	(-)-scopolamine
SCX	Strong cation exchange
SIM	Selected ion monitoring
SPE	Solid phase extraction
s.c.	Subcutaneously
TAs	Tropane alkaloids
TLC	Thin layer chromatography
TOF	Time of flight
UB	Upper bound
USA	United States of America
UV	Ultra violet
v/v	Volume/volume