European Food Safety Authority

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# Tropane alkaloids (from *Datura* sp.) as undesirable substances in animal feed[1]

## Scientific Opinion of the Panel on Contaminants in the Food Chain

**Question N° EFSA-Q-2003-063**

**Adopted on 9 April 2008**


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## SUMMARY

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. They have in common a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbons 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 natural tropane alkaloids are (-)-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


[1] For citation purposes: 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.

produced. *Datura* plants are toxic for animals if ingested in larger amounts. Their seeds, which contain significant amounts of hyoscyamine and scopolamine, can be found as botanical impurities in feed materials, particularly in soybean and linseed products.

Very little information on the actual contamination of feed materials is available, and previous reports on adverse health effects in animals refer in most cases to accidental intoxications following the consumption of *Datura* plants rather than to the contamination of linseed and sobean contaning feed materials. Hence no conclusive exposure assessment could be presented for farm animals.

Overall, pigs have been shown to be among the most sensitive species to *Datura* poisoning. A worst case exposure estimate indicated that adverse pharmacological effects in pigs following exposure to *Datura ferox* seeds, mainly containing scopolamine, can not be entirely excluded at the current statutory limits of 3000 mg/kg feed. However, the limited data also suggested that it is not likely that the presence of *Datura stramonium* impurities in animals feed up to the current statutory level of 1000 mg/kg would present a risk to animal health.

The mechanism of action of tropane alkaloids relates to their competitive antagonism at muscarinic acetylcholine receptors, preventing the binding of acetylcholine. According to the specificity and selectivity of muscarinergic acetylcholine receptors in different organs, the functions of smooth muscles and exocrine gland cells, as well as the heart rate, respiration and functions in the central nervous system are modulated. Certain tropanes such as atropine (the racemic mixture of (-)- and (+)hyoscyamine) as well as scopolamine (mainly as butylscopolamine bromide) are used in human and veterinary therapy for a variety of related indications. Reports of poisoning of livestock and experimental feeding studies describe as most common symptoms associated with tropane alkaloids exposure dryness of the mucosa in the upper digestive and respiratory tract, constipation and colic (in horses), pupil dilation (mydriasis), alterations in the heart rate and central nervous effects such as restlessness, irritability, ataxia, seizures and respiratory depression.

Tropane alkaloids are readily absorbed following oral ingestion, but have a short biological half-life and are rapidly biotransformed or excreted. Moreover, typical pharmacological signs precede a toxic syndrome, and hence exposed animals would not be slaughtered. Therefore, the CONTAM Panel concluded that it is unlikely that residues of tropane alkaloids in edible tissues, milk and eggs constitute a risk for consumers.

**Keywords:** tropane alkaloids, *Datura stramonium*, *Datura ferox* and *Datura innoxi*, *Solanaceae* family, (-)-hyoscyamine, (-)-scopolamine, atropine, toxicity, exposure, carry-over, animal health, human health.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

### 1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[2] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[3].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[4].

It is worthwhile to note that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition[5],

[2] OJ L140, 30.5.2002, p. 10
[3] OJ L 115, 4.5.1999, p. 32
[4] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
[5] OJ L 38, 11.2.1974, p. 31

which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[6] provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex.

## 2. Specific background

Tropane alkaloids are produced by *Datura stramonium* and *Datura ferox,* which have been detected as contaminant in soybean. The tropane alkaloids present in *Datura spp* are hyoscyamine, atropine and scopolamine, the highest alkaloid concentration found in the seed.

*Datura stramonium* is listed as an undesirable substance in the Annex of Directive 2002/32/EC. A maximum level for seeds and unground and uncrushed fruits of *Datura stramonium* of 1000 mg/kg is established for all feeding stuffs.

SCAN concluded[7] that risk assessments could be made for some of the compounds presumed responsible for their toxicity and consequently maximum limits for botanical contaminants of particular concern should be set on the basis of their known toxicants.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the EFSA to provide a scientific opinion on the presence of tropane alkaloids produced by *Datura sp.* in animal feed.

This scientific opinion should comprise the

– identification of the tropane alkaloids, relevant as regards animal health and eventually public health, which are present in animal feed as the consequence of the contamination by *Datura sp*.

[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)
[7] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 9.5. Conclusion and point 9.6 Recommendations.

- determination of the toxic exposure levels (daily exposure) of the identified tropane alkaloids for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health)
  - the level of transfer/carry over of identified tropane alkaloids from the feed to the products of animal origin results in unacceptable levels of identified tropane alkaloids or eventually their toxic metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by the identified tropane alkaloids (from *Datura spp.)* and the characterisation, insofar as possible, of the distribution of levels of contamination.
  - assessment of the contribution of the different identified feed materials as sources of contamination by the identified tropane alkaloids (from *Datura spp.)*
  - to the overall exposure of the different relevant animal species to the identified tropane alkaloids,
  - to the impact on animal health,
  - insofar relevant, to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates.
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

## ACKNOWLEDGEMENT

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# ASSESSMENT

## 1. Introduction

A variety of common wild plants and/or weeds produce, as secondary metabolites, tropane alkaloids, which act in mammals as antagonists of central and peripheral muscarinic acetycholine receptors and hence can induce a distinct toxic syndrome. Of special interest are the *Datura* species, which regularly synthesise high amounts of tropane alkaloids. *Datura* species produce numerous small seeds encapsulated in apple-shaped fruit capsules (hence the name thorn apple). Upon release, the small seeds have been found as important impurities in harvested soybean and linseed products introducing a variable amount of tropane alkaloids into these feed materials.

The group of tropane alkaloids comprises more than 200 compounds[8] (Lounasmaa and Tamminen, 1993), characterized by a two-ringed structure with a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbon atoms. The amino group, typical for all alkaloids, is in most cases methylated. Tropane alkaloids have been found in different plant families, *Brassicaceae (Cruciferae), Convolvulaceae, Erythroxylaceae, Euphorbiaceae, Olacaceae, Proteaceae*, and *Rhizophoraceae*, but they are best known for their occurrence in the family *Solanaceae* (Griffin and Lin, 2000). This plant family comprises about 100 genera and 3000 species. Particularly the genera *Datura*, *Brugmansia*, *Hyoscyamus*, *Atropa*, *Scopolia*, *Anisodus*, *Przewalskia*, *Atropanthe*, *Physochlaina*, *Mandragora*, *Anthotroche*, *Cyphantera*, and *Duboisia* are known as being rich in tropane alkaloids (Griffin and Lin, 2000).

The greatest variation in tropane alkaloids is found in the *Datureae*, comprising the two genera, *Datura* and *Brugmansia*. Most plants of the tribe *Datureae* are coarse annual herbs, with large, alternate leaves and large, showy, tubular flowers and inhabiting waste areas, particularly those with rich soils. Whereas *Datura* collects shrub-like weed plants, seldom becoming higher than two meters, *Brugmansia* genus collects tree-like forms, which may reach more than 10 meters in height. Thus, the *Datura* species are much more likely to be problematic weeds in cultivated crops than the *Brugmansia* species. Initially both species were endemic in specific geographical areas. However, due to their beautiful red to pink, yellow or white characteristic trumpet-shaped conspicuous flowers they have been spread as ornamental plants to other parts of the world.

The most well known plant of the genus *Datura* for its content of tropane alkaloids is *Datura stramonium*, commonly called thorn apple or Jimson weed, a plant that is widely distributed in all warm regions of the world. Subsequently, seeds of this plant have been found as impurities in important agricultural crops such as linseed and soybean and products thereof, as mentioned above.

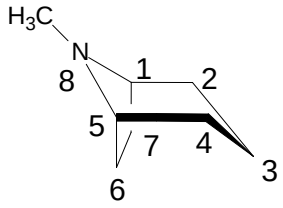
[8]Among these natural tropane alkaloids are mono-, di- and triesters, as well as carboxylated and benzoylated compounds.

The most common tropane alkaloid is **(-)-hyoscyamine**, first isolated in 1833 from *Hyoscyamus niger* (henbane) but is also the most prevalent alkaloid of *Datura stramonium.* By racemisation, **atropine** is formed from (-)-hyoscyamine. Atropine is used in human medicine since the 1500's being first isolated in Europe from the Solanacea species *Atropa belladonna.* Atropine has the same pharmacological activities as (-)-hyoscyamine, but requires twice the amount to be given to achieve an equivalent effect. Atropine is still widely used in medicinal products in human and veterinary medicine for a variety of indications in ophthalmology, as premedication in anaesthesia, and as antidote in cases of intoxications with organic phosphoric acid esters. Hyoscine, better known as **(-)-Scopolamine** was first isolated from *Scopola carniolica* in 1881 and later on identified also in *Hyoscyamus niger*. Scopolamine is the main tropane alkaloid in *Datura metel, Datura wrightii* and *Datura ferox*. Like (-)-hyoscyamine and (-)-scopolamine easily form racemic mixtures when being extracted. Scopolamine is used in medicinal products for a specific range of indications. Particularly its derivative n-butylscopolamine bromide is regularly used in the treatment of intestinal disorders due to its spasmolytic effect, and in the prevention of motion sickness.

## Chemistry of tropane alkaloids

The wide range of tropane alkaloids occurring in the *Solanaceae* family arises from the esterification 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) with various hydroxytropanes (α-tropanol, α– tropane-diol or α-tropane-triol). The alkaloid part of tropane alkaloids is a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbons atoms. Thus, the common structural element of the tropane alkaloids is the bicyclic azabicyclo-octane skeleton shown in Figure 1 (Lounasmaa and Tamminen, 1993).

The most important natural tropane alkaloids hyoscyamine and scopolamine (Figure 1) are esters of tropane-3α-ol (and the 6-7 epoxide of tropane-3α-ol) and tropic acid. The asymmetric α-carbon of tropic acid allows the formation of two stereoisomers. 6β-Hydroxyhyoscyamine (anisodamine), the intermediate between hyoscyamine and hyoscine has been detected in some of the scopolamine-containing plant species.




Common ring system of tropane alkaloids

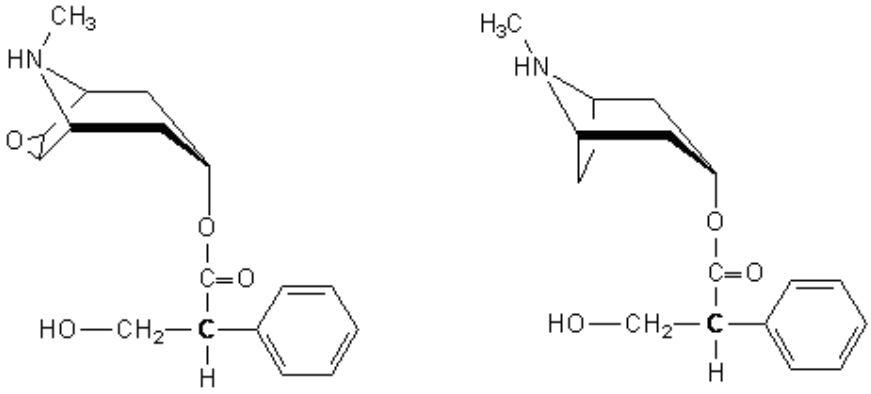



Figure 1. Chemical structure of tropane alkaloids: upper panel: common ring system of tropane alkaloids; lower panel: scopolamine and hyoscyamine with the asymmetric α-carbon of tropic acid marked in bold giving rise to stereoisomers[9].

The physico-chemical data of tropane alkaloids were early reviewed by Boit (1961) and the chemical and physical characteristics of (±)-atropine, (-)-hyoscyamine and (-)-scopolamine, which all are readily soluble in organic solvents and fat, are summarised in Annex I. Available analytical data on the tropane alkaloid content in different plant parts of various *Datura* species are shown in Annex III.

[9] Based on this general formula, the correct chemical nomenclature of prominent tropane alkaloids are: tropine (3α-hydroxytropane), atropine ((±)-3α-tropoyloxytropane), hyoscyamine, noratropine ((±)-3α-tropoyloxynortropane), norhyoscyamine ((-)-3α-tropoyloxynortropane), apotropine (3α-apotropoyloxytropane), aponoratropine (3α-apotropoyloxynortropane), tigloidine (3β-tigloyloxytropane), littorine ((-)-3α-2'-hydroxy-3'-phenylpropionyloxy)tropane, and hyoscine or scopolamine.

## 2. Methods of analysis

Because several tropane alkaloids are constituents of medical preparations and drugs, chemical methods for analyses of these compounds in plants, drugs and exposed individuals have been developed (Bayne *et al.,* 1975; Oshima *et al.,* 1989; Papadoyannis *et al.,* 1993). As many of the tropane alkaloids occur in chiral forms, the chemical methodology should preferably enable the differentiation and quantification of the individual enantiomers.

The extraction techniques used to get representative samples for analysis include liquid-liquid extraction, solid phase extraction and column switching. It should be noted that the extraction method might influence the racemization of (-)-hyoscyamine to atropine during isolation and storage (Bunke *et al.,* 1996; Reist *et al.*, 1997). Supercritical fluid extraction with sonication seems to induce less racemization than liquid-solid extraction procedures (Mateus *et al.,* 2000). Alkali solutions are detrimental to ester alkaloids such as hyoscyamine and scopolamine, which are hydrolysed at basic conditions. The extraction is crucial to get reliable qualitative and quantitative data, and needs to be adapted to the matrix and compounds studied. Sample clean-up might be necessary (for review Papadoyannis, 1995, and Dräger, 2002).

Initially gas chromatography (GC) was used for quantification. This method has the advantage of high precision and efficiency in the analysis, but the samples are exposed to rather high temperatures, which may cause decomposition of some alkaloids. At present, high performance liquid chromatography (HPLC) with reversed phase columns are considered as method of choice (Friedman and Levin, 1989; Papadoyannis, 1995) and various individual methods have been described in detail (Paphassarang, 1985; He *et al.,* 1989; Bucher *et al.*, 1989, Papadoyannis *et al*. 1993; Kursinszki *et al.* 2005, and Mroczek *et al.* 2006). HPLC methods have also been developed specifically for the analysis of tropane alkaloid in animal feeds (Bucher *et al.,* 1989), and for tropane alkaloids in biological samples such as serum and urine (Namera *et al.,* 2002). In addition, radioimmunoassays are available for example for atropine in serum (Wurzburger *et al*., 1977).

Since a specific analytical methodology is required to separate the enantiomeric tropane alkaloids, interest focussed on the development of rapid, efficient, and sensitive analytical methods that allow to quantify the individual enantiomers. Besides the classical HPLC and GC, capillary electrophoresis (CE) in combination with mass spectrometry (MS) has been established as an interesting alternative for the enantio-separation of chiral compounds (Mateus *et al.,* 1998, 2000; Cherkaoui *et al.,* 1997, 1999, 2001). This methodology has the advantage that it allows separation of tropane alkaloid enantiomers making use of β-cyclodextrin as a chiral selector. Because of the low sensitivity achieved with ultraviolet (UV) detection, capillary electrophoresis coupled to electrospray ionisation (CE-ESI) has been implemented improving the sensitivity of the methods by a factor of 1000 (Cherkaoui *et al.,* 2001).

It has lately been pointed out that several already published analytical methods do not take into account the potential presence of a littorine (a precursor of hyoscyamine), which often co-elutes with the hyoscyamine peak resulting in an overestimation of the hyoscyamine content. Cherkaoui *et al.* (1997, 1999) and Mateus *et al.* (2000) were able to separate these compounds from each other

using capillary electrophoresis and by adding an organic modifier to the sample, and Bitar and Holzgrabe (2006) recently described an oil-in-water micro-emulsion electro-kinetic chromatography technique (MEEKC) capable to quantify tropane alkaloids in complex mixtures.

In conclusion it can be stated that although only one analytical method has been developed specifically for the analysis of animal feeds, many of the methods developed for analysis of plant materials for pharmaceutical purposes could be adapted and validated easily for the analysis of feed materials.

Currently, the prescribed control of feed materials for contamination with *Datura* seeds is conducted in most cases by simple light microscopy, aiming at the detection of identifiable plant residues from *Datura* species. The successful identification of these botanical impurities depends on several factors including the skills of the operator, the availability of reference material and the degree to which the sampled material has been processed. Milling and combined heat and pressure treatments can destroy much, or all, of the anatomical/histological plant features on which identification is based. Hence this method is time consuming, less accurate than chemical analyses, and predicts inaccurately the actual contamination with tropane alkaloids.

## 3. Current legislation

According to the EU 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 to Council Directive 2002/32/EC contains a list of compounds that are undesirable in animal feed and their maximum levels in different feed commodities. The maximum levels established in the EU for tropane alkaloids are presented in Table 1.

Table 1. EU legislation on tropane alkaloids in feed material.

| **Undesirable substances** | **Product 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<br><br>*Datura stramonium* L. | All feedingstuff | 3000<br><br>1000 |

## 4. Occurrence in feed materials

### 4.1. Biosynthetic pathways and distribution of alkaloids in plants

In the past, there was no general agreement on *Datura* taxonomy. Most authors, like Hammer, Romeike and Tittel (1983), allocate 12-15 species to the genus (the *Datura* species and varieties mentioned by these authors are summarised in Annex II). *Datura* species that have been recognised as frequent contaminants include *D. stramonium*, *D. ferox*, *D. quercifolia*, *D. leichhardtii* ssp. *pruinosa*, *D. leichhardtii*, *D. inoxia*, *D. ceratocaula*, *D. discolor*, D. *metel*, and *D. wrightii*.

Tropane alkaloid biosynthesis in *Datura* mainly takes place in the roots (Conklin, 1976). From the site of synthesis the compounds are translocated to upper parts of the plant. Changes in alkaloid content of leaves follow the fluctuation of roots, with a delay of approximately one month (Demeyer and Dejaegere, 1989). Degradation and transformation seems to take place continuously in stems and leaves during molecule translocation to green plant parts (van de Velde *et al.*, 1988). Within cells, the alkaloids most likely occur in the form of crystals in the vacuoles (Verzár-Petri, 1973). The details of tropane alkaloid biosynthesis have been described in various reviews (Cordell, 1981; Leete, 1989; Robins and Walton, 1993; Humphrey and O'Hagan, 2001).

### 4.2. Alkaloid content in various tissues of *Datura spp.*

Although hyoscyamine and scopolamine are the predominant tropane alkaloids in the green plant parts of *Datura* species several other tropane alkaloids can be formed albeit at lower concentrations. The tropane alkaloid profile differs between species. Around thirty different tropane alkaloids have been found in species/varieties such as *D. ceratocaula*, *D. inoxia*, *D. stramonium* var. *stramonium*, *D. stramonium* var. *tatula* and *D. stramonium* var. *godronii* (Berkov and Zayed, 2004; Berkov *et*

*al.,* 2006). These alkaloids seem to occur in all tissues of the plant, except the capsules and the woody portions of the roots and stems of some *Datura* species, but the largest diversity in constituents is generally found in the roots (Zieli´nska-Sowicka and Szepczy´nska, 1972). The stems contain a lower number of tropane alkaloids, and even less alkaloids are found in seeds, leaves and flowers (Siddiqui *et al.,* 1988; Lounasmaa and Tamminen, 1993; Vitale *et al.,* 1995, Philipov and Berkov, 2002). However, for the main tropane alkaloids, hyoscyamine and scopolamine, levels are higher in flowers and leaves than in the root (Witte *et al.*, 1987) and seeds contain substantial amounts of these alkaloids (Miraldi *et al.,* 2001). Relative amounts of various tropane alkaloids in different tissues (roots, stem, leaves, flowers and seeds) have been reported for *D. ceratocaula* (Berkov, 2003), *D. stramonium* var. *stramonium, D. stramonium* var. *tatuala* and *D. stramonium* var. *godronii* (Berkov *et al.,* 2006).

The ratio between the various tropane alkaloids varies over the lifetime of an individual plant. For example, it has been reported that in very young *D. stramonium* plants scopolamine normally dominates, but at the stage of flowering the hyoscyamine content increases while the relative scopolamine content decreases gradually (Demeyer and Dejaegere, 1989). Moreover, many environmental factors can influence the tropane alkaloid content, including soil composition, soil fertilization, salinity, climate and altitude, application of plant growth regulators and hormones, insect herbivory, and plant health (Karnick and Saxena, 1970; Gupta and Madan, 1975; Stecka *et al.,* 1975; Brachet and Cosson, 1986; van de Velde *et al.,* 1988; Demeyer and Dejaegere, 1989; Shonle and Bergelson, 2000). Of importance is the observation that artificial damage of *D. stramonium* plants had no influence on the tropane alkaloid level.

Available analytical data on the tropane alkaloid content in different plant parts of various *Datura* species is shown in Appendix III. A summary of the tropane alkaloid content of some of these *Datura* species is given in Table 2. One of the difficulties encountered when summarising the available information is the fact that different investigators have used different analytical methods and different units to express the measured alkaloid concentrations found (see Table 2).

Table 2. Compilation of total tropane alkaloid, scopolamine, and hyoscyamine concentrations in various plant tissues of the most important *Datura* species, as reported by different authors to occur in contaminated feed materials, or which have led to intoxications in livestock. Individual authors reported data in various units. The given information did not allow a recalculation and harmonisation of the units. DW = dry weight, FW = fresh weight, and w/w = weight by weight.

| **Datura species** | **Content (mg/kg DW, FW or w/w)** | |
|---|---|---|
| | **Compound** | **Level commonly observed** |
| *Datura stramonium* leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 1900-3600 w/w<br>150-6500 DW<br>1550 DW |
| *Datura stramonium* seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 1600 – 4200 w/w<br>100-900 DW<br>1900 DW |
| *Datura stramonium* var. *stramonium* leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 100 – 1800 FW<br>650 DW<br>700 DW |
| *Datura stramonium* var. *stramonium* seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | <br>1 200 DW<br>700 DW |
| *Datura stramonium* var. *tatula*-leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | <br>600 DW<br>1000 DW |
| *Datura stramonium* var. *tatula*-seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 1400 – 1600 FW<br>500 DW<br>700 DW |
| *Datura stramonium* var. *godronii*-leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | <br>550-600 DW<br>400-450 DW |
| *Datura stramonium* var.*godronii*-seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 1700 FW<br>1300 DW<br>1400 DW |
| *Datura inoxia*-leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 170 – 5700 DW; 300 FW<br>150-1300 DW; 250 FW<br>54 FW |
| *Datura inoxia*-stem | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 3200 DW; 66 FW<br>19 FW<br>25 FW |
| *Datura inoxia*-seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 4100 FW<br>700 DW<br>1400-2900 DW |
| *Datura ferox*-leaves | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 1000 – 4100 DW<br>50-3200 DW |
| *Datura ferox*-stem | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 200 – 2800 DW<br>200-1500 DW |
| *Datura ferox*-seeds | Total tropane alkaloids<br>Scopolamine<br>Hyoscyamin | 800-1250 FW |

Table 2 shows also that dried leaves and stems of several *Datura* species may contain similar or higher amounts of total tropane alkaloids than seeds. Although the total level of tropane alkaloids may be quite similar in several *Datura* species, the ratio of hyoscyamine to scopolamine varies considerably from species to species and in relation to the geographic distribution. In *D. stramonium* hyoscyamine often predominates (varying between 50 and 80% of the alkaloids) but in *D. metel* the two alkaloids are found in quite similar amounts, and in *D. ferox* scopolamine is the major alkaloid (see Table 3). As hyoscyamine and scopolamine have slightly different biological activities and differ for example in their oral bioavailability, it is of clinical relevance to identify which *Datura* species is contaminating a feed. The clearly different alkaloid profile in *Datura stramonium* and *Datura ferox* was confirmed in recent investigations conducted in South Africa (see Table 3). To make this visible, the authors presented a calculation of the ratio between scopolamine and hyoscyamine, which shows close to equal amounts of two alkaloids in *D. stramonium* plants, a ratio of approximately 2:1 in *D. innoxia*, but a ratio of 98:2 for scopolamine:hyoscyamine in *D. ferox*.

Table 3: Tropane alkaloid profile in *Datura* species originating from South Africa. Figures represent the average and range concentrations (mg/kg) detected in plant materials and in seeds. The ratio is calculated from S – scopolamine and H - hyoscyamine (according to Naudé, 2007).

| **Species** | **Hyoscyamine** | | **Scopolamine** | | **Ratio:S/H** |
|---|---|---|---|---|---|
| | **Average** | **Range** | **Average** | **Range** | |
| *D. stramonium* | | | | | |
| Young plants | | | | | |
| Purple stem | 1046 | 531-2291 | 1063 | 296-2844 | 1 |
| Green stem | 587 | 491-742 | 525 | 249-1155 | 0.9 |
| Seeds | 557 | 273-908 | 587 | 254-800 | 1.1 |
| D. *ferox* | | | | | |
| Young plants | 68 | 0-149 | 1048 | 82-2541 | 15 |
| Seeds | 7.5 | 2-19 | 766 | 218-869 | 102 |
| D. *innoxia* | | | | | |
| Young plants | 360 | 175-462 | 790 | 692-869 | 2.2 |
| Seeds | 297 | 127-524 | 454 | 104-815 | 1.9 |

In addition to the major tropane alkaloids hyoscyamine and scopolamine, several minor tropane alkaloids have been identified in *Datura* species. Typical examples of minor alkaoids in *D. stramonium* are tigloidin, aposcopolamine, apoatropin, hyoscyamine N-oxide and scopolamine N-

oxide (Romeike, 1953; Phillipson and Handa, 1975; Bucher and Meszaros, 1989; Bucher *et al.*, 1989, , Gupka, 2006), whereas substantial amounts of 6β-hydroxyhyoscyamine (anisodamine; 1.4-2.5% of total alkaloids) were found in flowers of *Datura metel* collected in China (He *et al.*, 1989), and 6β-[2-methylbutanoyloxy]tropan-3α-ol in *Datura ceratocaula*, a species distributed throughout Mexico and Central America (Beresford and Wooley, 1974). The presence of minor alkaloids might contribute to the toxic syndrome encountered following the ingestion of plant material including seeds.

Available information suggests that tropane alkaloids except for their inclination to racemize are fairly stable during drying or heat treatment of feed materials containing *Datura* plant material. Heat resistance of atropine and scopolamine has been demonstrated in a study where bread was baked from contaminated wheat flour. The baked bread contained between 72 and 100% of the tropane alkaloid content of the flour (Friedman and Levin, 1989).

### 4.3. Tropane alkaloids in feed materials

Contamination of feed with *Datura* seeds is most likely to occur in oil-producing crops, particularly in soybeans and linseed[10]. The degree of contamination, i.e. the number of *Datura* seeds is correlated to the number of seed capsules (the thorn apple) produced per plant, the number of seeds per capsule and the way in which these change with jimsonweed density. Weaver (1986) found that in soybean fields, depending on year, date of planting and distance between the rows of the cultivated soybean, 239-263 seeds were produced per capsule, corresponding to 439-594 seeds per plant or 7830-18,750 seeds per $m^2$, depending on year, date of planting and distance between rows of the major cultivar. As the seeds within the thorn-apple capsule are very small (8 mg per seed) in comparison with soybeans, the seeds can be easily removed during mechanical cleaning of the beans for dirt and small stones before processing.

Van Kempen (1992) identified five sources of tropane alkaloid contamination: *D. stramonium, D. ferox, D. metel, D. wrightii*, and *D. inoxia*. These species are present in America, Asia, Southern Europe and Africa. Based on analytical data from Germany, only *D. stramonium* and *D. ferox* have been estimated to occur in relevant quantities in imported feed materials (Bucher and Meszaros, 1989). *D. stramonium* and other *Datura* species have been identified in soybean products from the United States, whereas *D. ferox* has been identified in soybean products from Argentina (Padula *et al.*, 1976).

Very little information is available regarding recent controls of feed material imported by Member States. As mentioned above, the only well-documented study was published by the feed control authority in Bavaria, Germany, reporting the results of a survey conducted between 1986 and 1988.

[10] Examples of other toxic weed seeds that have been found in soybeans are cocklebur (*Xanthium strumarium*), morning glory (*Ipomea* spp.), castor bean (*Ricinus communis*), pokeweed (*Phytolacca americana*), cowcockle (*Saponaria vaccaria*), corncockle (*Agrostemma githago*), nightshade (*Atropa belladonna*), and crotolaria (*Crotolaria* spp.) (List *et al.*, 1979).

In this survey, 686 feed materials were controlled microscopically for the presence of *Datura* seeds. Between 0% and 38.1% of soybean and linseed products (i.e., crashed soy bean for extraction, crashed linseed for extraction, linseed cake) were contaminated with parts of *Datura* seeds. Soybean-based feeds were less frequently contaminated (0.4%) than linseed products and the contamination of linseed products varied considerably between years (10.7, 29.0 and 51.4%, respectively). Analysis of linseed 'expeller', the remainder left after oil production, showed that 42.4% of the samples taken in 1986 contained more than 1000 mg *Datura* material per kg expeller. The corresponding contamination rate in 1987 and 1988 were 38.1% and 35.2%, respectively. Microscopic examinations revealed that 76% of contaminated products contained *D. ferox*, 14% *D. stramonium* var. *tatula* and 10% a mixture of these two varieties. This observation was confirmed by chemical analysis of various samples of the identified material. It could be shown that 65 of the 66 analysed samples contained only scopolamine at levels between 0.1 and 33 mg/kg. The single exception was one soybean cake containing 2.5 mg total alkaloids (96% scopolamine and 4% hyoscyamine) (Bucher and Meszaros, 1989).

In contrast to these data from Germany, Cooper and Johnson (1984) had previously reported soybeans to be contaminated only up to 0.023%.

More recent data were not reported, and the call for data launched by EFSA in preparation of this Opinion did not identify new data.

## 5. Estimating the intake by farm livestock

Grazing animals are unlikely to consume fresh *Datura* plants voluntarily because the plant has a very unpleasant taste and odour. However, poisoning can occur if *Datura* is present in hay (Naudé , 2007), or its seeds contaminate grain or oilseed products fed to livestock, as animals do not detect these impurities in dried material.

Oil seeds will usually be subjected to processing before being incorporated into feeds for livestock. This processing includes grinding, solvent extraction and heating. Although tropane alkaloids are comparatively stable, it is often not clear to what extent these processes remove or inactivate parts of the alkaloids. Full fat soybeans are processed including de-hulling, followed by heating with steam and extrusion, toasting or micronisation. The intake of tropane alkaloids will therefore be a function of the degree of contamination and the impact of processing.

According to the data provided from the Bavarian investigations (Bucher and Meszaros, 1989; Bucher *et al*., 1989) the rate of *Datura* contamination exceeded in various cases the statutory level set for *Datura stramonium* seeds (1000 mg/kg feed), but also the higher level of 3000 mg/kg feed as set for other weed seeds (see Table 1). Major results of the German study are presented in Table 4, but it needs to be emphasized again, that these data date back to the late 1980s.

Table 4. Maximum and average rate of contamination with *Datura* seeds and estimated total alkaloid[a] concentration (Bucher and Meszaros, 1989)

| Product | *Datura* (mg/kg) average | *Datura* (mg/kg) maximum | Total alkaloids average (mg/kg) | Total alkaloids maximum (mg/kg) |
|---|---|---|---|---|
| Soy extraction meal | 3080 | 12600 | 4.8 | 21.4 |
| Linseed extraction meal | 2450 | 3400 | 3.7 | 5.4 |
| Linseed cake | 4240 | 2950 | 6.8 | 22.0 |
| Linseed expeller | 5130 | 24150 | 8.4 | 41.5 |

[a]: *Datura* seeds (g/kg) = 0.316 + 0.574 mg/kg *x* alkaloid concentration (mg/kg) as measured by HPLC (regression analysis of the correlation between microscopically visible contamination with *Datura* plant material and total alkaloid content as measure by HPLC; Bucher and Meszaros, 1989)

[b]: Feed rations containing 20-40% linseed products

On the basis of these data the following exposure scenarios can be calculated: A diet with 15% soy bean extraction meal given to a high-yielding dairy cow, consuming 12 kg of a concentrate would result in an exposure of 0.02-0.08 mg/kg b.w. In poultry consuming 120 g of feed this would result in an exposure of 0.08-0.36 mg/kg b.w., at a body weight of 1.8 kg, and in pigs (40 kg b.w. and 1600 g feed consumption) of 0.05 to 0.2 mg/kg. Under the same assumptions the exposure from linseed products, which can be given in a percentage of up to 40% in diets for broilers and pigs, can be calculated. In a worst case scenario in which animals obtain contaminated linseed expeller as sole linseed product at an inclusion rate of 40%, exposure rates in broilers could reach 1.28 to 5.7 mg/kg b.w., and in pigs of 0.13-0.66 mg/kg b.w. Normalisation of these data to the statutory maximum of 3000 mg seeds per kg/feed material still results in a possible exposure of pigs from linseed expeller of 0.27 mg/kg b.w. Bucher *et al*. (1989) calculated on the basis of the given formula (see subscript (a) of Table 4) an average total alkaloid concentration of 1.2 mg and 4.7 mg/kg feed, for *D. stramonium* and *D. ferox*, respectively, at the statutory inclusion level of 3000 mg seeds per kg feed material. This would correspond to an intake of 0.08 and 0.3 mg/kg b.w., respectively, in broilers, and 0.04 and 0.2 mg/kg in pigs.

## 6. Adverse effects of tropane alkaloids

### 6.1. Mechanism of action

Tropane alkaloids are commonly described as anti-cholinergic compounds, due to their ability to bind to muscarinic acetylcholine receptors and hence acting as competitive antagonists at these receptors (Brown and Taylor, 2006). According to the organ distribution, different subtypes of muscarinic receptors have been described, denoted M1 to M5, all belonging to the class of G-

protein coupled receptors. M1 represent a population of receptors localized in the central nervous systems, as well as in gastric and salivary glands. M2 receptors occur in the atria of the heart, at smooth muscles of the gastrointestinal tract as well as in the central nervous system. M3 receptors dominate at exocrine glands including the salivary glands, occur in the gastro-intestinal tract as well as in the eye, and on the endothelium of blood vessels. M4 receptors are predominantly found in the central nervous system and M5 receptors are found especially in the Substantia nigra of the central nervous system, in the salivary glands and in the ciliary muscle of the iris of the eye. Atropine is a non-selective antagonist of all classes of muscarinic receptors, but known to have a stimulating effect on the central nervous system, whereas scopolamine is a depressant of the central nervous system (Brown and Taylor, 2006).

Intoxications with tropane alkaloids are characterized by dryness of the mucosa in the upper digestive and respiratory tract, constipation, pupil dilation (mydriasis) and disturbance of vision, photophobia and changes in heart rate, dose-dependent hyper- or hypotension, bradycardia or tachycardia as well as arrhythmias, nervousness, restlessness, irritability, disorientation, ataxia, seizures and respiratory depression. As anticholinergic compounds disturb the balance between cholinergic and adrenergic regulation of organ functions, secondary effects may occur. A prominent example is the effect of atropine on the heart rate, which commences as bradycardia at low (therapeutic) doses, progressing into tachycardia and arrhythmia at higher (toxic) doses.

### 6.2. Toxicity studies in rodents

A 90-day feeding study with ground *D. stramonium* seeds containing 2710 mg atropine and 660 mg scopolamine/kg seed mixed into the feed at 0, 0.5, 1.58, and 5.0% corresponding to 6.85, 53.25 and 168.5 mg tropane alkaloids per kg was conducted with 20 male and female weanling Sprague-Dawley rats (Dugan *et al.,* 1989). All animals survived with the exception of a female rat fed the 5% *Datura* diet. No behavioural differences between groups were observed but a statistically significant and dose-dependent growth inhibition occurred in all groups. The principal effects of exposure to *Datura* was decreased body weight gain, serum albumin and serum calcium, increased liver and testes weights, and elevated serum alkaline phosphatase and blood urea nitrogen levels, with female rats showing more marked responses than males. Female rats showed additional alterations in other serum chemistry and haematological parameters. No histological lesions were associated with ingestion of the highest dose but it increased the size of the pupils. In addition, no indication for a clastogenic activity, as measured by micronucleus frequency, was observed in bone marrow of either sex.

Other studies in rats showed lower body weights and liver weights with diet containing 0.5% ground *D. stramonium* seeds (Crawford and Friedman (1990). Hasan and Kushwaha (1986, 1987) treated rats by intraperitoneal injection with 1.2 mg *Datura alba* extract/kg b.w. per day, given as a single dose or in a subchronic (3 months) toxicity study and showed that acute exposure increased energy metabolism whereas subchronic exposure to *Datura* had opposite effects.

Extrapolation of these results obtained with rats to other species is hampered by the fact that rats seem to hydrolyse tropane alkaloids to a large extent, making them less susceptible to the pharmacological effects of these alkaloids than other species.

Two reproduction studies in mice have been performed: In the 1st study CF-1 albino mice were given a single subcutaneous injection of 50 mg/kg b.w. atropine sulfate on day 8 or 9 of gestation. No teratogenicity was observed (recorded were exencephaly, cryptorchid testes and skeletal malformations) (Arcuri and Gautieri, 1973). In a 2nd study mice (strain not specified) were subcutaneous injected with 1/50 and 1/10 of the $LD_{50}$ dose of scopolamine (4.6 and 59 µg/g b.w., respectively) which induced 3.8 and 8.2% malformed foetuses. However, it is unclear whether or not these malformations indicate a direct embryotoxic effect or just reflect maternal toxicity (Yu *et al.,* 1988).

Both atropine and scopolamine have been intensively tested and found to be non-mutagenic in bacterial assays (Lewis *et al.,* 1996, EMEA, 1998). No DNA-binding potential was detected for atropine at concentrations of 10-100 µM (EMEA, 1998).

In conclusion, these experimental data show that tropane alkaloids, as far as they have been investigated failed to exert specific toxic effects. The alterations observed in the animal studies can be attributed to the pharmacological (anticholinergic) activity of this class of compounds.

### 6.3. Adverse effects in livestock

Although not frequent, *Datura* intoxications, sometimes fatal, have been described in the veterinary literature. Cases of severe poisoning have been reported particularly in horses and cattle, following the consumption of contaminated hay in which (in contrast to fresh material) *Datura* is not detected by animals. Other reports address incidental intoxications in buffaloes, sheep, goats, swine, mules, and ostriches (Cooper and Johnson, 1984). Rabbits, rats, guinea pigs and poultry species are believed to be more resistant to tropane alkaloids, presumably due to the (varying) expression of specific hydrolytic enzymes (atropine-hydrolases) that can cleave and inactivate the majority of tropane alkaloids (Werner and Brehmer, 1967).

As *Datura* plants may contain a large number of different alkaloids, it remains often difficult to identify the exact compound responsible for the intoxication, as the observed biological effects might be attributable to several compounds. The major symptoms of hyoscyamine intoxications in farm animals are consistent with the well-known anti-cholinergic effects of tropane alkaloids and include hyposalivation, tachycardia, hyperventilation, pupil dilation, restlessness, nervousness, muscle tremor, hypothermia, 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).

Exposure to tropane alkaloids may influence the quality (unpleasant taste) and yields of milk from lactating animals.

### 6.3.1 Adverse effects in pigs

Experimental studies with pigs have been performed with two different *Datura* species and one variety, having different ratios of hyoscyamine:scopolamine. *D. stramonium* (ratio approximately 4:1), *D. stramonium* var. *tatula* (ratio approximately 6:4), and *D. ferox* (ratio 2:98). Feeding a single ration contaminated with up to 548 mg whole or ground *D. stramonium* seeds (containing around 0.4% hyoscyamine) resulted in an exposure of 2.2 mg per kg b.w., but no toxic effects were observed in any of the five German land race pigs (Behrens and Horn, 1961, 1962). However, a dose of 664 mg seeds per kg body weight (2.7 mg hyoscyamine/kg b.w.) resulted in reduced appetite, slight giddiness, and dry faeces. Prolonging the studies by serving ground *D. stramonium* two times a day over eight days at a daily inclusion rate of 365 mg seed/kg body weight (corresponding to 1.38 to 1.46 mg/kg b.w. hyoscyamine per day) did not provoke signs of intoxication during the first two days, but at the third day reduced appetite, lethargy, slight giddiness, dog-like postures, mydriasis, and dry faeces were observed. These symptoms disappeared within 14 days after the end of the experiment. These data were in contrast to the results of a 60-day feeding study with increasing amounts of ground *D. stramonium* seeds mixed into pig feed (total tropane alkaloid dose over the whole period 95-130 mg/kg b.w.), in which no signs of toxic effects were observed.

Hesselbarth (1962) reported an experiment with fattening pigs (32 kg b.w.) fed progressively increasing doses of ground *D. stramonium* (from 0.003 to 0.44 mg/kg b.w) during 39 days. No other adverse effects than a slight reduction of weight gain were observed. In a parallel experiment a daily dose (duration not given) of 25 g *Datura* seeds (14 mg hyoscyamine) had no effects, but diarrhoea was noted at the higher exposure rates of 50 and 100 g seeds per day, respectively.

In more extensive feeding experiments, ground or whole seeds of *D. stramonium*, with a total alkaloid content of 0.18 or 0.2% of the dry weight, were mixed into the feed of Large White x Camborough male pigs, four pigs per dose (Worthington *et al.*, 1981). The amounts of *Datura* seeds were calculated to provide, in the case of ground seeds, a daily exposure of 0, 4, 8 or 12 mg alkaloid/kg b.w. and in the case of whole seeds a dose of 2 or 4 mg/kg b.w., respectively. The feed containing ground *Datura* seeds were fed for four days, whereas the feed with whole seeds were fed during 11 days (2 mg alkaloids/kg b.w.) or 14 days (4 mg/kg b.w.). Inclusion of ground *Datura* in the feed significantly reduced feed intake, with consumption declining with increasing concentration of alkaloid. Whereas control pigs gained 1.9 kg in weight over the four-day period of the experiment, pigs receiving 4 mg/kg b.w. seeds only gained 0.4 kg, and those receiving the two highest doses even lost 0.6 and 1.7 kg, respectively. No effect on rectal temperature or any other clinical alterations were observed during or after the feeding period. The apparent alkaloid exposures in the two dose groups (2 and 4 mg alkaloid/kg b.w.) were 1.0 (first week) and 1.2 (day 8-11) mg/kg b.w. in the lowest dose group and 1.3 and 2.1 mg/kg b.w. in the highest dose group. The latter figure was originally thought to be due to increased consumption and reduced body weight. However, analysis of left over feed and the faeces showed that the pigs tried to reject *Datura* seeds (rejected feed contained 5.3-5.9% *Datura* seeds whereas the original inclusion rate

was 2.4-3.0%). In addition, many seeds appeared undamaged in faeces. Control pigs increased 5.4 kg in weight, the low dose (2 mg alkaloid/kg b.w.) group 1.7 kg, whereas the high dose group lost 4.1 kg.

The nutritional value and potential toxicity of a linseed oil meal contaminated with *D. stramonium* and/or *D. ferox* seeds were investigated in an 84-day feeding study on growing pigs (25-100 kg live weight) (Janssens and De Wilde, 1989). At the beginning of the study the contaminated meal (2.0-2.1 mg scopolamine/kg meal) resulted in a lower daily feed intake and reduced growth rate as compared to the control group. On day 21 the alkaloid intake was 0.048 mg/kg b.w., at day 42 around 0.059 mg/kg b.w., and at day 63 around 0.053 mg/kg b.w. Despite the fact that performance improved with longer exposure, the animals that had received contaminated linseed showed a lower slaughter-weight, a prolonged fattening period, poorer growth rates and feed conversion ratios as compared to the controls. No pathological changes were found in the liver or the kidneys at slaughter.

In the early 1990's, the European Community funded a pig study to determine the threshold toxicity levels for *Datura* alkaloids in feed (Piva, 1993). Forty castrated male or female piglets (20 kg) were fed either a control diet, or were fed a diet with added pure scopolamine and hyoscyamine at a ratio of 98:2, which corresponds to the pattern found in various linseed products contaminated by seeds of *Datura ferox*. Total alkaloid levels of 1.5, 15, 75 or 150 mg/kg feed, respectively, were given. Two pigs in each group of eight were sacrificed after 27 days for gross pathology, whereas the other pigs were given the contaminated feed until the end of the experiment. Feed refusal, particularly during the first two weeks, was noted at inclusion rates of 75 and 150 mg alkaloids per kg feed. A gradual increase in pupil dilatation with increasing alkaloid content in the feed was noted. Performance remained optimal at the level of 1.5 mg alkaloids per kg feed (0.006 mg alkaloids/kg b.w. calculated on initial weight). At the two highest alkaloid concentrations (0.3 and 0.6 mg alkaloids/kg b.w. calculated on initial weight) weight gain was reduced but this reduction did not reach statistical significance. The feed/gain ratio was, however, significantly altered at the highest level of alkaloids in feed. The pigs that received the *Datura*-containing feed had swollen bellies due to constipation and tenesmus. From this study a provisional no-effect level in pigs of 1.5 mg tropane alkaloids per kg feed at a ratio of 98:2 of scopolamine: hyoscyamine could be derived.

In another study (Richter *et al.*, 1992), twenty-four piglets (12-15 kg) of the genotype Pi × DL were fed for five weeks diets with either 35% cake from linseed oil production highly contaminated with *D. ferox*, 35% cake from linseed oil production of good quality and with low concentrations of alkaloids, or 25% of an extracted soybean material, respectively. The test diet with contaminated linseed products contained 17100 mg *Datura* seeds/kg (24.4±1.6 mg scopolamine/kg), which corresponded to 8.7 mg scopolamine per kg feed, whereas the low-contaminated feed contained 650 mg *Datura* seeds/kg, corresponding to 0.28 mg scopolamine per kg feed. The low-level linseed feed, resulting in an exposure of 0.1 mg scopolamine/kg b.w. per day, caused no changes in feed intake. However, the high contaminated linseed feed, corresponding to 0.37 mg scopolamine/kg b.w. per day, resulted in reduced feed intake (7%), energy uptake (11%), final body weight (12%), daily weight gain (22%), feed efficiency (18%), and energy efficiency (13%).

In the short-term study (14 days), feeding 15 mg alkaloid mixture (not specified) per pig significantly decreased the average daily weight gain and feed efficiency. In a 76 days study, 1.5 mg alkaloid mixture in feeds fed to 60 kg pigs decreased their average daily weight gain and the feed efficiency (Piva, 1993). Pupil dilation (mydriasis) was increased in these pigs but not heart rate and breathing frequency. Furthermore, the kidney weight at slaughter was increased and spleen weight decreased in swine receiving the highest amounts of the alkaloid mixture in the feed. The investigators conclude that an intake of 0.7 mg/kg live weight is enough to show effects in 20-25 kg piglets.

In addition to these general feeding studies on pigs, two studies have addressed the possibility that the tropane alkaloids in *Datura* species might have teratogenic effects. In 1971 congenital arthrogryposis (a contracture of one or more joints or one or more limbs) was reported in piglets from Duroc-Hampshire cross sow litters on a farm in Kansas (US) (Leipold *et al.,* 1973). However, the observed teratogenicity could not be directly linked to any of the toxic weeds. In contrast, Keeler (1981) found no teratogenic effect of *Datura* seeds on the joint development in newborn Hampshire piglets after feeding sows 1.2 to 1.7 mg Thorn-apple/kg feed (alkaloid levels not determined).

Based on the available data, Piva and Piva (1995) concluded that the threshold limits for intoxication of pigs (20-60 kg) consuming feed with *Datura* alkaloids is approximately 1.5 mg alkaloids/kg feed, corresponding to approximately 0.06 mg/kg b.w. for a 40 kg pig. Similarly, Worthington *et al.* (1981) had reported that an intake of *Datura stramoium* seeds equivalent to a dose of about 1.5 mg alkaloids/kg feed, resulted in mild toxicity symptoms in pigs, whereas van Kempen (1992) reported a normal performance at this level for *D. ferox* alkaloids. In piglets, mild signs of toxicity could be observed already at levels as low as 0.1 mg scopolamine /kg b.w. (Richter *et al.*, 1992) Piva, 1993).

A comparison with the exposure data (as presented in Chapter 5) indicates that these threshold values can be exceeded at the current statutory level of 3000 mg for seeds (others than those from *Datura stramonium,* see Table 1). Considering the unique alkaloid pattern of *Datura ferox* (98:2; scopolamin:hyoscyamine and minor alkaloids) and the fact that *D. ferox* shows an increasing prevalence in many parts of the world, it would be prudent to limit the rate of contamination also to 1000 mg/kg feed, as for *Datura stramonium* to reduce the risk of undesirable (pharmacological) effects in pigs exposed to contaminated feed material.

### 6.3.2 Adverse effects in poultry

As poultry possess apparently an atropine hydroxylase-like enzyme activity that inactivates tropane alkaloids, they are believed to be more resistant than other farm animals against the exposure to tropane alkaloids (Werner and Brehmer, 1967). In a first study, New Hampshire hens were given increasing amounts of whole *Datura* seeds, up to 2000 seeds per hen/day for ten days (five days for the highest doses), and after withdrawal the animals were observed for an additional week. The

highest dose, corresponding to 15g seeds per bird, induced no adverse effect on health and egg production (Fangauf and Vogt, 1961). In parallel experiments with younger birds, 5 to 100 seeds per day were given to three weeks old broiler chickens for ten days. Feed consumption was influenced only at the highest dose, but no adverse effects were noted on the broiler chickens. A daily dose of 100 seeds resulted in reduced growth but the reduction was not statistically significant (Fangauf and Vogt, 1961). Even younger chickens and higher doses were used in the study of Day and Dilworth (1984) but no adverse effects were observed.

Male one-day old Arbor Acre broiler chickens were fed a starter diet in which 0-6% of the maize in the basal diet had been substituted with *D. stramonium* seeds (tropane alkaloid content unknown) for 21 days. No effects were observed in broiler chickens given 1% seeds. Inclusion rates of 3 and 6% into the diet depressed the body weight gain significantly at three weeks, whereas feed conversion was significantly affected only at the highest dose level.

Flunker *et al.* (1987) performed two studies, one in chickens and one in hens. In the first study three replicates of 8 Cobb × Cobb male broiler chickens were each given a well-characterized feed during 21 days as a combination of *D. stramonium* seeds and filler (washed builders sand) *ad libitum*. The inclusion rates of *D. stramonium* seeds were 0, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0%. Although no difference in feed consumption was noted, growth was better in broilers that received the *Datura* seed in the diet than in those receiving the control diet with 3% sand filler. Feed efficiency was improved at 1% inclusion rate of *Datura* seeds. There was no difference in mortality between treatment groups, and no difference in the moisture content of the excreta.

In the parallel experiments on White Leghorn hens, Flunker *et al.* (1987) studied the influence of substituting parts of the 3% sand filler in the diet with 0, 1, 2 or 3% *D. stramonium* seed for two weeks. The *Datura* seeds induced no significant difference in daily egg production, egg weight, egg specific gravity, Haugh unit scores, change in body weight, and mortality. However, the highest inclusion rate of *Datura* seeds in the diet reduced feed consumption. This reduction was not observed after one week of the study. Is was concluded that up to 3% *D. stramonium* seeds when given together with filler in the diet of broiler chicks and hens had no influence on performance during short-term exposure.

Egg-laying hens of a meat-producing strain were for three months fed a standard diet supplemented with purified scopolamine and hyoscyamine in the ratio 98:2 (the ratio that is typical for *D. ferox*), starting when the hens were 28 days old (Kovatsis *et al.,* 1993). Inclusion rates were 0, 1.5, 15, 75, and 150 mg total tropane alkaloids/kg feed and feed consumption was limited to around 150 g/day. With exception of mild diarrhoea persisting for 1-2 day in the tropane alkaloid-fed hens, no clinical signs were observed in exposed hens during the entire study. No feed refusal and no influence on the body weight of hens were noted. At the highest inclusion level, egg production was reduced during the initial stages of alkaloid feeding but egg production regained levels comparable to that in other groups after a delay of approximately 5-6 weeks. No influence was observed on egg weight and eggshell thickness. Whereas traces of scopolamine could be detected in eggs of hens given the highest rate of tropane alkaloids in the feed (150 mg), no scopolamine was detected in eggs of hens receiving the two lowest alkaloid doses. Cardiac rates were influenced only after 3 months of

alkaloid feeding and only in hens that received the highest dose. No influence on breathing frequency was observed. Determination of a number of diagnostic enzymes activities in the plasma of hens provided no clear indication of toxic effects. No treatment related macroscopic and microscopic lesions were noted at necropsy (Kovatsis *et al.,* 1994).

In parallel experiments broiler chickens received analogous treatments for 90 days, but in this case the tropane alkaloids were given in the form of *D. ferox* seeds (scopolamine: hyoscyamine ratio 98:2) (Kovatsis *et al.,* 1993). Four male and sixteen female broilers were used for each intended dose level (0, 1.5, 15, 75, and 150 mg total alkaloids/kg feed). Exposure to the tropane alkaloids caused initially a significant reduction in body weight gain, especially at the higher dose levels. These effects were transient, however, as no changes in body weight gain were noted after two months of feeding the material containing *D. ferox*. The amounts of feed consumed were not controlled. At the end of the second and third month of feeding, the heart rate and breathing frequency of the broilers were controlled, but no changes were found. At the end of the experiments, all groups fed a *Datura*-containing diet showed reduced plasma activity of leucine aminopeptidase, whereas the activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were not influenced. Three broilers of each dose group were randomly selected for macroscopic and microscopic examination. No treatment-related effects were observed.

Limited and contradictory information is available on the influence of tropane alkaloids on chicken development (McBride, 1980; Bueker and Platner, 1956; Magras *et al.,* 1993). However, the relevance of these findings is, in this context, limited because they refer to direct exposure of fertilized eggs by injection and not to feed contamination.

In conclusion, the available data indicate that levels up to 3% *D. stramonium* seeds (containing predominantly (-)-hyoscyamine) in the diet of broiler chicks and hens have no influence on performance during short-term exposure. Furthermore, *D. ferox* (containing scopolamine as major alkaloid) contamination at an inclusion rate of 150 mg alkaloid/kg feed showed no adverse effects in poultry.

### 6.3.3. Adverse effects in ruminants

Several contradictory field reports have been published on *Datura* poisoning in cattle. Early reports implicated *Datura* poisoning as the reason for loss of cattle (Hansen, 1924; Singh and Singh, 1971). West and Emmel (1952) reported that cattle are the livestock most frequently poisoned by *Datura* species. They claim that 240-400 g of green parts, or corresponding amounts of dried material of the plant will produce fatalities in cattle. Cows that ingest *Datura* plant material experience an alkaloid-dependent relaxation of the forestomach, which accelerated the feed aversion caused by the odd taste and flavour of *Datura* plant material limiting under normal circumstances the consumption of high amounts of these plants.

The first controlled feeding study on the effect of tropane alkaloids via *Datura* contaminated feed was published in the 1940's. Following a preliminary fast of 24 hours, Kehar and Rau (1944) fed

chopped leaves, flowers, fruit and soft stalk of a hill variety of *D. stramonium* mixed in green fodder for 3½ to 4 weeks to two bulls. Bulls fed 2.2-4.4 kg a a feed contaminated with *Datura* (no details are given) showed constipation, bulging of the eye-balls with red discolouration of the lens, dilation of the pupils, slight drowsiness and an accelerated pulse. In another early study, Hesselbarth (1961) fed nine ox calves (220-430 kg) a predefined feed containing various levels of milled *D. stramonium* seeds with 0.25% hyoscyamine per kg seed. No effects were observed below an inclusion rate of 58.5 g *Datura* seeds per kg feed (which would correspond to a level of approximately 14.6 mg hyoscyamine/kg feed).

In another experiment, 11 one year-old heifers were given a feed on average containing various amounts of *D. stramonium* seeds (0, 8.8, 881, or 4408 seeds per kg diet) for 14 days (Nelson *et al.*, 1982). Heifers given the highest dose, never consumed the entire daily ration, and exhibited anorexia from the first day of the experiment. In some animals, bloat and dry mucosal surfaces developed already on the second day, followed by miosis and constipation. No differences in serum chemistry and haematological parameters were found in heifers following exposure to the diets containing *D. stramonium* seeds. Also no difference in feed efficiency was observed between treatments. The toxic dose (4408 seeds/kg diet) was calculated to correspond to 2.49 mg atropine and 0.5 mg scopolamine per kg body weight.

In conclusion, the available data suggest that ruminating cattle are sensitive to *Datura* alkaloids. Following exposure to seeds in feed concentrates for ruminants, signs of toxicity are likely to occur at levels exceeding 0.5 mg hyoscyamine plus 0.1 mg scopolamine per kg b.w., whereas levels of up to 0.3 mg /kg b.w. (total alkaloids) are tolerated.

### 6.3.4. Adverse effects small ruminants

Sheep and goats appear to be less sensitive to tropane alkaloids than many other livestock species. Already in 1931 Steyn reported that no effects were observed in individual sheep receiving 500 g ripe *D. stramonium* seeds, 500 g *D. stramonium* var. *tatula* seeds, or 500 g *D. stramonium* x *D. tatula* hybrid seeds on each of two consecutive day, or 750 g of fresh leaves of *D. tatula* on each of two consecutive days. Steyn (1931) concluded that it was impossible to induce lethality in sheep by drenching them with large amounts of the fresh green leaves and ripe seed of *D. stramonium*, *D. stramonium* var. *tatula* or a hybrid between these two species. Case (1955) noted that goats respond with feed refusal when exposed to *Datura* plants.

In one experiment, two sheep and two goats were fed chopped leaves, flowers, fruits and soft stalk of two varieties of *D. stramonium* (one from the hills and one from the plains) mixed in green fodder (Kehar and Rau, 1944). As no symptoms appeared during the first 8 days of feeding, and the animals took the feed voluntarily, in the second part of the experiment, the plant material was drenched in water for around 2½ weeks before being given to the animals. Still no symptoms appeared. In another study (El Dirdiri *et al.*, 1981), dessert sheep received 10 g/kg per day of fruits or leaves of *D. stramonium* and died within 38 days. In the same study, Nubian goats dosed daily with fresh *Datura* leaves or fruits at the rate of 2.5 and 10 g/kg per day in the diet showed

unthriftiness and lethargy and death occurred within 136 days. These findings are difficult to interpret, as no details of the diet are given. However, these findings may point towards differences between breeds in the sensitivity to tropane alkaloids, which have been observed also in rabbits (often used as a reference species due to their atropine hydroxylase expression).

### 6.3.5. Adverse effects in horses

There are several reports on accidental intoxications of horses by *Datura* plants, which are not recognized by the animals when present in hay or preserved feeds. In horses the clinical signs include anorexia, diarrhoea, hyperexcitability, mydriasis, polyuria, staggering and general in-coordination, intermittent muscular spasms and rigors (Barney and Wilson, 1963; Williams and Scott, 1984). The most important complication in the horse is the occurrence of (often fatal) spastic constipation colic, with and without an ileus, which is related to the unique anatomic and physiologic features of the equine intestinal tract. Schulman and Bolton (1998) reported a *Datura*-induced ileus of the colon in two horses supplied a feed supplement containing 25% *Datura* seeds. One study reported coma and death occurred within 48 hours (Barney and Wilson, 1963). Recent studies have tried to identify the toxic dose level in horses of tropane alkaloids in *Datura*. Galey *at al.* (1996) dosed 4 adult mares with multiple doses of air-dried *D. wrightii* (=*D. meteloides*). Clinical signs were only observed in the mare receiving the highest dose (0.5 g/kg, equivalent to 0.275 mg scopolamine, 0.185 mg atropine/kg feed) comprising severe gastrointestinal atonia, tachycardia, sweating and colic. Clinical signs were evident two hours after dosing and had not resolved by 72 hours. Naudé *et al.* (2005) reported an outbreak of impaction colic in eighteen of eighty-three riding horses at a riding school. The outbreak was traced to hay that was introduced in the riding school. Analysis of this hay showed contamination with *D. stramonium and D. ferox.* Naudé and co-workers (2005) concluded that the toxic oral dose of tropane alkaloids in the horse is approximately 0.1 mg/kg of hyoscyamine (corresponding to 0.2 mg/kg atropine). In a second case report, spontaneous intoxication in 34 horses after ingesting freshly harvested maize heavily contaminated with young *Datura stramonium* plants has been described. The clinical status of all horses was monitored for 7 days and was accompanied by mild hyperthermia, tachycardia, polypnoea, dyspnoea and shallow breathing, mydriasis, dry oral, rectal, vaginal and nasal mucosae, acute gastric dilatation and severe intestinal gas accumulation, anorexia to complete refusal of feed, decreased or absent thirst, absence of defecation and urination. Necropsies and pathological studies performed on two horses that died, revealed toxic liver dystrophy, cardiac lesions and

As atropine is used as medicinal product, a few studies on horses focused on specific toxic effects (e.g. Ducharme and Fubini, 1983; Adams *et al.,* 1984; Malone *et al.*, 1996, 1999). These studies with atropine generally confirm the symptoms in animals involuntarily intoxicated by hyoscyamine-containing plants. However, as the oral bioavilability of hyoscyamine is not known in horses, and for therapeutic use atropine is injected, a direct comparison of the dose-response-relation of atropine and hyoscyamine can be not be presented.

The available data suggest that horses show signs of toxicity already following exposure to 0.1 mg hyoscyamine/kg feed.

### 6.3.6. Adverse effects in other animal species

**Rabbits**

Rabbits have been identified to be comparatively resistant to atropine. Glick and co-workers described already in 1940 the existence of a specific atropine esterase activity in the blood of rabbits (Glick, 1940; Glick and Glaubach, 1941). The insensitivity to atropine is hence assumed to be due to an effective cleavage of the alkaloid into the non-active compounds tropine/scopine and tropic acid (Ammon and Savelsberg, 1949). The tropine esterase is approximately two orders of magnitude more active against the natural constituents (-)-hyoscyamine, (-)-scopolamine, and (±)-homatropine than against (+)-hyoscyamine and (+)-scopolamine (Werner, 1961; Werner and Brehmer, 1967; Werner, 1967). The esterase, now known as (-)-hyoscyamine acylhydrolase, seems to be related to an allel in a polymorphic locus, as only 30-70% of the rabbits are (-)-hyoscyamine acylhydrolase positive (Bernheim and Bernheim, 1938; Werner, 1961, Werner and Brehmer, 1967). Rabbits identified as having (-)-hyoscyamine acylhydrolase in the serum also have this enzyme activity in other tissues of the body.

An equal enzymatic activity has also been identified in guinea pigs, and poultry hens (Werner and Brehmer, 1967). It should also be noted that guinea pigs and chicken have also a homatropine esterase activity that interestingly prefer the (+)-homatropine as substrate instead of the (-)-homatropine (Werner and Brehmer, 1959).

**Cats**

The only tropane alkaloid-related poisoning described in cats is a case when a cat was given the veterinary preparation Lomotil, which contains atropine. The cat showed toxic effects typical of the excitement reaction of narcotic analgesics (Ormerod *et al.,* 1978).

**Dogs**

Two observations on intoxicated dogs are available. In the first case a one-year-old poodle arrived at the veterinary clinic with toxicity symptoms appearing 2-3 hours after consumption of *Datura* seeds. The symptoms included hyperaesthesia, high agitation, tachycardia, tachypnoea and mydriasis, which were followed by coma and circulatory and respiratory failure, resulting in death (Tostes, 2002). According to previous 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 motility and tachycardia (EMEA, 1997).

In the other case mydriasis of one of the eyes was reported in a five year-old Griffon dog (Hansen and Clerc, 2002). The authors performed experimental studies on dogs, which showed that contact with any part of *D. stramonium* could induce this condition. The same condition, a syndrome called anisocoria (an unilateral mydriasis) is also know in human patients that have been in contact with

plant material from (ornamental) *Datura* species (Firestone and Sloane, 2007) indicating a high rate of absorption of tropane alkaloids through mucosal membranes.

## 7. Toxicokinetics, metabolism and tissue distribution

### 7.1. Absorption

Several research groups have investigated the absorption, distribution, metabolism and excretion of tropane alkaloids in different mammals (Bernheim and Bernheim, 1938; Gosselin *et al.,* 1955; Evertsbusch and Geiling, 1956; Kalser *et al.,* 1957; Werner, 1961; Werner and Schmidt, 1968a), but only one single oral study with the pure alkaloids is available. However, as tropane alkaloids are readily absorbed through the skin and through mucous membranes (Lewis and Elvin-Lewis, 1977), a relatively high oral bioavailability may be assumed and data from intravenous/parenteral injections may provide a reliable indication of the fate of atropine after oral ingestion. In contrast to atropine, scopolamine has a limited oral bioavailability ranging from 10 to 50% (Putcha *et al.,* 1991; Ali-Melkkilä, 1993). Following absorption, both alkaloids have a high volume of distribution (EMEA, 1997, 1998).

### 7.2. Metabolism and excretion

Following absorption, the extent of the expression of hepatic hyoscyamine acylhydrolase that cleaves the hyoscyamine into an alkamine and tropic acid, determines the internal dose and the individual sensitivity to tropane alkaloids (Glick and Glaubach, 1941; Krantz *et al.,* 1954; Wada *et al.,* 1991). Recent investigations have shown that hyoscyamine acylhydrolase (atropine esterase EC 3.1.1.10) is likely to be enantiomer-specific with a preference to (-) forms of the alkaloids. Plasma from individual rabbits, and plasma from all other species (dog, goat, guinea pig, pig, rhesus monkey, and humans, proved capable of hydrolysing atropine at a very low rate, which exceeds, however, that of non-specific breakdown. It remains to be determined whether this effect is due to a low expression of atropine esterase or an alternative hydrolysing enzyme (Harrison *et al.*, 2006).

The metabolism and excretion of scopolamine has been investigated using liquid chromatographic-tandem mass spectrometric (LC-MS/MS) or gas chromatography coupled to mass spectrometry. Scopolamine undergoes oxidative demethylation and first-pass metabolism mediated by cytochrome P450 3A so that only a small fraction (<5%) of the dose is excreted as the parent compound (Putcha *et al.,* 1991; Renner *et al.*, 2005). However, the oxidation products have not yet been identified. In contrast, atropine is excreted unchanged in the urine (60%) and the remaining metabolites are hydrolysis and conjugation products (sulphation and glucuronidation) (Chen *et al.*, 2006, EMEA, 1998; Ali-Melkkilä, 1993). Scopolamine and atropine both have short serum half lives (<5 hours) (Putcha *et al.*, 1991). The renal clearance of scopolamine is low (<10 mL/min) (Renner *et al.*, 2005) whereas that of atropine is high (660 mL/min), suggesting significant tubular secretion (Hinderling *et al.*, 1985; EMEA, 1998). Butylscopolamine (the medicinal product is mostly given by parenteral administration) is mainly excreted in the urine, whereas after oral

administration about 90% is excreted in the faeces, reflecting its limited oral bioavailability and biliary excretion. (EMEA, 1997). More detailed studies with the medicinal formulations of atropine (given IM or SC) to sheep, pigs (IV and endobronchial installation) and for butylscopolamine in pigs (IV), cattle (IV) and horses (IV) are reported by EMEA (1997, 1998).

### 7.3. Residue formation

In one study in the horse, radio-labelled butylscopolamine was administered intravenously. The measurable label showed the highest concentration in liver and kidney, amounting to 14000 µg/mL and 21000 µg/mL, respectively, at 30 minutes, and declining rapidly to 500 and 50 µg/mL within 48 hours. In muscle and fat levels were less than 30 µg/mL at 30 minutes and less than 10 µg/mL by 48 hours after injection. Within 24 hours after administration more than 50% of the given dose was excreted in urine and more than 20% in faeces, the latter increasing to 45% of the total dose within the next 48hrs. The largest fraction of the radioactivity in urine consisted of unmetabolised butylscopolamine, and unmetabolised butylscoploamine bromide was also the major component of the residues in liver and kidney (EMEA, 1997).

### 7.4. Kinetic studies in rodents

Due to the use of atropine and (butyl)scopolamine as medicinal products in human therapy, their kinetic parameters have been studied in detail in rodents.

Gossellin and co-workers studied the excretion and metabolite pattern in urine after injection of radiolabelled **atropine** and tropic acid into rats and mice. Tropic acid was excreted into urine quicker and more complete than was atropine (Gosselin *et al.*, 1955, Kalser *et al.*, 1957). No radioactivity could be detected in the expired air.

The excretion pattern in urine of mice, intravenously injected with atropine (labelled in the α-carbon), revealed insignificant differences between sexesApproximately 25% of the injected dose was excreted as atropine, and over 50% as conjugates with glucuronic acid. The remaining 20-25% were intermediate oxidation products (probably p-hydroxyatropine and m,p-dihydroxyatropine), tropine-modified atropines, and tropic acid. The latter compound did not occur in urine until 6-8 hours after injection, and only at very low levels (Gabourel and Gosselin, 1958). Rats produced similar metabolites with 39% of the administered radio-label representing unchanged atropine.

Rats were treated by gavage with 25 mg/kg atropine and urinary excretion of atropine and eleven metabolites was assessed using LC-MS ion trap with electrospray inonization. Atropine, five phase I metabolites (*N*-demethyltropine, tropine, *N*-demethylatropine, *p*-hydroxyatropine and *p*-hydroxyatropine N-oxide) and six phase II metabolites (glucuronide conjugates, sulfate conjugates of *N*-demethylatropine, atropine and *p*-hydroxyatropine) were detected in the urine from one hour after treatment and up to 106 hours (Chen *et al.*, 2006). No quantitative assessment of the metabolites was performed.

One of the species comparably insensitive to atropine, the guinea pig, excreted radio-labelled atropine quicker than rats, but slower than mice. Around 25% were unchanged atropine and close to 54% tropic acid. Cats and kittens excreted atropine in a similar manner as rats (Gosselin *et al.*, 1955).

The tissue levels and excreta have also been studied in mice up to 48 hours after subcutaneous, and up to 60 hours after intravenous and intraperitoneal administration of radiolabelled $^{14}$C-labelled atropine (Evertsbusch and Geiling, 1956; Werner and Schmidt, 1968a). Immediately after administration the drug could be found in all of the tissues analysed, except those of the central nervous system (Evertsbusch and Geiling, 1956). Eighty percent of the injected radiolabel had been excreted during the first four hours and around 95% within 60 hours. Approximately 82-85% of the injected radioactivity was excreted in the urine, 11-13% in the faeces and 1.5-7% in the expired air. The main site of retention in the body was the gastrointestinal tract.

Kalser *et al.* (1957) studied the distribution of atropine by following the level of radioactivity in various tissues of the mouse, rat, guinea pig, and cat at 0.5 and 4 hours after intravenous injection of radiolabelled atropine. Four hours after injection of the labelled atropine, the mouse had excreted 76% in urine, 0% in faeces, and retained around 5% in the colon. The corresponding figures were for the rat 39, 0 and 0.3%, for the guinea pig 61, 0 and 1%, and for the cat 35, 0 and 0.4%, respectively. After 24 hours mice had excreted 82% of the dose in urine and 11.5% in faeces, and 7.0% in the colon content. Corresponding figures for the rat were 44, 18 and 16.6%, and for the cat 44, 21 and 16.9%, respectively.

The metabolism of injected **(-)-scopolamine** was investigated by Werner and Schmidt (1968b) in NMRI mice. The radiolabelled compounds in urine were to 78% (-)-scopolamine-9´-glucuronide, 12% unchanged (-)-scopolamine, 5% scopine (and tropic acid), 5% 6-hydroxy-(-)-hyoscyamine, some aposcopolamine, and very small levels of (-)-norscopolamine and (-)-norscopolamine-9´-glucuronide. No less than 23% of the radiolabel was expired as carbon dioxide by the mouse. This portion was 5.9% in Sprague Dawley rats, 12.5% in guinea pigs and 4.5% in monkeys (*Callithrix jacchus*). No racemization of scopolamine or any of its optically active metabolites was detected during passage of the drug through the body. Separate studies showed that (-)-scopolamine-9´-glucuronide may be metabolised to (-)-norscopolamine-9´-glucuronide under the production of carbon dioxide.

## 8. Carry-over and residues

As reported in section 6.2., traces of scopolamine have been detected in eggs laid by hens of a meat-producing strain that for three months were fed a standard diet supplemented with 150 mg purified scopolamine and hyoscyamine/ kg feed in the ratio 98:2 (the characteristic ratio of scopolamine and hyoscyamine in *Datura ferox*) (Kovatsis *et al.*, 1993). At this dose level egg production was reduced during the initial stages of alkaloid feeding but egg production reached levels comparable to that in other groups after a delay of approximately 5-6 weeks. No influence was observed on egg

weight and eggshell thickness. Whereas traces of scopolamine could be detected in eggs of hens given 150 mg total tropane alkaloids per kg feed dose, no scopolamine was detected in eggs of hens receiving 1.5 or 15 mg/kg feed. The identification of scopolamine in eggs is in agreement with the finding that no (-)-hyoscyamine acylhydrolase activity has been detected in fresh egg white and egg yolk (Glick and Glaubach, 1941).

Tropane alkaloids have never been identified in the milk, although an off-flavour of milk has been reported from cows that had consumed *Datura*-contaminated feed. EMEA reported residue depletion studies in horses and cattle, receiving either a single intravenous dose (0.2 mg butylscopolamine bromide/kg b.w.) or an intramuscular dose (0.3 mg/kg b.w.). Residues in horse tissues were below the limit of detection of 100 µg/kg at the first sampling day (i.e. 6 days p.a.). Residues in cattle tissue including the injection site were below 100 µg/kg tissue at the 1st sampling day, which was in this study day 9 after application. Comparable results were obtained in pigs, where all tissues were below the limit of detection by the first slaughter time (9 days p.a.). In cattle, which received a single intramuscular dose of 0.25 mg butylscopolamine/kg b.w., at the first day of sampling (9 days after application) none of the tissue samples were found to exceed the limit of detection. Milk samples were taken as well, but residue concentrations in all samples were below the limit of quantification of the applied HPLC method (0.10 µg/L). No residue studies are available for atropine but EMEA considered this not to be of importance, because of the rapid absorption and elimination of the substance, which precludes a risk of significant residue deposition in edible animal products.

## 9. Human intoxications

There is a large database from poison information centres reporting *Datura* intoxications in children, teenagers and adults. Such data indicate that nearly all recent reports on exposure to *Datura* plant material are relating to abuse (due to the hallucinogenic effects) or experimentation with the plants (Klein-Schwartz and Oderda, 1984; Torbus *et al.*, 2002; Al-Shaikh and Sabley, 2005; Spina and Taddei, 2007; Montcriol *et al.,* 2007; De Germont-Buquier, 2008). Accidental exposure is rare, but has been reported in very young children, who have ingested the fruits or the seeds (Mitchell and Mitchell, 1955; Rosen and Lechner, 1962: Arena, 1963; Rumack, 1973; Taha and Mahdi, 1984) and in teenagers who by mistake collected and consumed leafs of *Datura* as rhubarb leafs (Forno and Terry, 1998). In Taiwan, *Datura* intoxication was recorded in 14 people and caused by the ingestion of wild *Datura suaveolans* mistaken for edible vegetables (Chang *et al.,* 1999). The herbal use of this plant has led to intoxications for example in Tunisia (Hamouda *et al.*, 2000).

Intoxications have been registered also after dietary exposure to other *Datura* species, such as *Datura aurea* (Belton and Gibbons, 1979, Wilhelm *et al.*, 1991), *Datura candida* (McHenry and Hall, 1978; Wilhelm *et al.*, 1991; Greene *et al.,* 1996), *Datura cornigera* (Belton and Gibbons, 1979), *Datura inoxia* (Pekdemir *et al.*, 2004; Raman and Jacob, 2005), *Datura rosei* (Hudson, 1973), *Datura sanguinea* (Belton and Gibbons, 1979; Wilhelm *et al.,* 1991), and *Datura suaveolens*

(McHenry and Hall, 1978; Wilhelm *et al.,* 1991; Greene *et al.,* 1996; Havelius and Åsman, 2002). Some of these species or varieties are known under the name Angels Trumpet and are common ornamental garden plants as well as indoor plants because of their beautiful trumpet-shaped blossoms. Furthermore, two cases of *D. stramonium* contaminated food have been reported; one in mine boys in South Africa eating contaminated beans and one report on contaminated hamburgers (MMWR, 1984).

## 10. Human dietary exposure

Beside the ingestion of plant materials, no data are available that refer to the exposure of humans by products from animal origin.

## CONCLUSIONS

- The tropane alkaloids represent a group of over 200 compounds occurring primarily as metabolites produced by members of the *Solanaceae* family comprising over 100 genera and 3000 plant species. Tropane alkaloids have in common a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbons atoms. In *Datura* spp., the most common tropane alkaloids are (-)-hyoscyamine and (-)-scopolamine (also known as hyoscine), which are produced predominantly by *Datura stramonium, Datura ferox* and *Datura innoxi.* The actual alkaloid concentration differs in individual plants depending on the genotype and environmental conditions during growth.
- For the detection of hyoscyamine and scopolamine in plant materials various analytical methods have been described, but most of these methods have not been validated for feed materials. The same applies for atropine, the racemic mixture of (-)- and (+)-hyoscyamine.
- Tropane alkaloids act as competitive antagonists at muscarinic cholinergic receptors with different specificity for individual receptor subclasses. Symptoms associated with tropane alkaloids include dryness of the mucosa in the upper digestive and respiratory tract, constipation and colic (in horses), pupil dilation (mydriasis), alterations in the heart rate and central nervous effects such as restlessness, irritability, ataxia, seizures and respiratory depression.
- Pigs have been shown to be the most sensitive species to *Datura* poisoning. Model calculations revealed that it is unlikely that pigs exhibit signs of intoxications at levels below the statutory level set for seeds of *Datura stramonium* as a botanical impurity in imported feed materials. However, a comparison of exposure data and tolerated scopolamine levels indicated that it can not be excluded that feed contamination with *Datura ferox* at or below the current statutory level would cause undesirable pharmacological (anticholinergic) effects in pigs.

- Levels of individual tropane alkaloids tolerated by other target animal species can be derived from the available data only to a limited extent as many studies, for example those in horses and cattle, represented case reports without a complete description of exposure levels.
- A number of species, including poultry and particularly rabbits, are considerable less sensitive to the exposure to tropane alkaloids, presumably due to the expression of specific hydrolysing enzymes that inactivate the alkaloids. However, significant inter-individual and inter-breed differences in the susceptibility have been noted.
- There is no information available on carry-over of tropane alkaloids from feed into animal derived products under normal livestock conditions. Traces of alkaloids have been found in eggs, but no data were available on residues in milk or tissues from exposed animals.
- The available kinetic data and their longstanding clinical use provide no evidence of an accumulation of atropine or butylscopolamine in animal tissues and hence it is unlikely that residues of the corresponding natural tropane alkaloids hyoscyamine and hyoscine (scopolamine) will reach concentrations in animal tissues that are pharmacologically active in consumers.

## RECOMMENDATIONS

- Analytical methods for the major alkaloids have been developed for plant materials. These methods should be validated for use in the analysis of feed materials to allow the control of total and individual tropane alkaloid content in feed materials and feed.

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## ANNEX I. CHEMICAL AND PHYSICAL PROPERTIES OF (±)-ATROPINE, (-)-HYOSCYAMINE AND (-)-SCOPOLAMINE (Merck, 2001).

| Property | (±)-atropine | (-)-hyoscyamine | (-)-scopolamine |
|---|---|---|---|
| Chemical Abstracts Registry Number | [51-55-8] | [101-31-5] | [51-34-3] |
| Synonyms | α-(Hydroxymethyl)-benzeneacetic acid (*3-endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester; *1αH,5αH*-tropan-3α-ol (±)-tropane (ester); dl-hyoscyamine; tropic acid ester with tropine, dl-tropyl tropate, tropine tropate | [*3(S)-endo*]-α-(Hydroxymethyl)-benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester; *1αH,5αH*-tropan-3α-ol (-)-tropane (ester);3α-tropanyl S-(-)-tropate; l-tropic acid ester with tropine, l-tropine tropate, duboisine, l-hyoscyamine | (αS)-α-(Hydroxy-methyl)benzeneacetic acid (1α,2β,4β,5α,7β) 9-methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]non-7-yl ester; 6β,7β-epoxy-*1αH,5αH*-tropan-3α-ol (-)-tropane; 6β,7β-epox-3α-tropanyl S-(-)-tropate; 6,7-epoxytropine tropate, scopine tropate, tropic acid aster with scopine, hyoscine, l-scopolamine |
| Molecular formula | $C_{17}H_{23}NO_3$ | $C_{17}H_{23}NO_3$ | $C_{17}H_{21}NO_4$ |
| Molecular weight | 289.37 | 289.37 | 303.35 |
| Biological activity | Anticholinergic | Anticholinergic | Anticholinergic |
| Chrystals | Long orthorhomibic prisms from acetone | Silky tetragonal needles from alcohol | Viscous liquid, forms crystal monohydrate |
| Melting point | 114-116°C | 108.5°C | 59°C |
| $[\alpha]_D^{20}$ | | -21.0° (alcohol) | -28.0° |
| pKa | 4.35 | (21°C) 9.7 | |
| Solubility | Best in alcohol, ether, glycerol, less good in water | Freely soluble in alcohol and diluted acids, good in chloroform, and not so good in ether, benzene and water | Freely soluble in hot water, alcohol, ether, chloroform, acetone, and to some extent in benzene |
| Stability | | Easily racemizes | Decomposes on standing |

**ANNEX II. THE VARIOUS DATURA SPECIES AND THEIR VARIETIES** (Hammer *et al.*, 1983).

| **Species (synonyms)** | **Habitat and botany** |
|---|---|
| **Section Dutra** | |
| *Datura wrightii* hort. ex Regel. (= *D. meteloides* Dun. f. *wrightii* (hort. ex Regel) Danert) | Wright's Datura is found in Western United States<br>Originally from southern North-America and Mexico, now occurring in warm areas all around the world.<br>Rank-smelling foliage and large (20 cm) white flowers. Sprawling perennial with a large taproot (60 cm) |
| *Datura inoxia* Mill. (identical to *D. inoxia*) (=*D. metel* L.; *D. metel* Ucria; *D. guyaquilensis* H.B.K.; *D. meteloides* Dun.; and *D. velutinosa* Fuentes) | Originates from Latin and South America, spred to Canary Islands, Northern Africa and India, and can now be found more or less in every warm country. |
| *Datura discolor* Bernh. (=*D. thomasii* Torr; *D. kymatocarpa* Barclay; and *D. reburra* Barclay | Originates from Mexico, southwestern United States and the Caribean Islands, where it occurs in desert washes and riverbeds |
| *Datura discolor* Bernh. ssp. *leichhardtii* | Australia |
| *Datura leichhardtii* F. Muell. ex. Benth. (=*D. pruinosa* Greenm.) | Originates from Mexico to Guatemala, but can now be found also in Australia |
| *Datura leichhardtii* F. Muell. ssp. *pruinosa* (Greenm.) Barclay ex Hammer (*D. pruinosa* Greenm.) | Latin America |
| *Datura metel* L. (=*D. fastuosa* L., *Stramoium fastuosum* (L.) Moench, *D. hummatu* Bernh., *D. alba* Nees, *D. nilhummatu* Dunal) | Comes form Asia and Africa, but can now be found all over the world as it is cultivated for drug production |
| *Datura metel* L. var. *metel* (=*D. alba* Nees; *D. fastuosa* L. var. *alba* (Nees) Clarke; and *D. hummatu* Bernh.) | |
| *Datura metel* L. var. *muricata* (Bernh) Danert (=*D. fastuosa*, L.; *D. muricata* Bernh.; and *D. hummatu* Bernh. var. *muricata*) | |
| *Datura metel* L. var. *rubra* (Bernh) Danert (=*D. hummatu* Bernh. var. *rubra* Bernh.) | |
| *Datura metel* L. f. *rubra* Danert (=*D. hummatu* Bernh. var. *rubra* Bernh.; and *D. hummatu* Bernh. var. *dubia* (Pers.) Bernh.) | |
| *Datura metel* L. f. *sanguinea* Danert (=*D.* | |

| Species (synonyms) | Habitat and botany |
|---|---|
| *nilhummatu* Dun.) | |
| *Datura metel* L. var. *obscura* Danert (=*D. fastuosa* L.; *D. hummatu* Bernh. var. *rubra* Bernh.; and *D. nilhummatu* Dun.) | |
| *Datura metel* L. f. *obscura* Danert (=*D. hummatu* Bernh. var. *rubra* Bernh.) | |
| *Datura metel* L. f. *atropurpurea* Danert (=*D. fastuosa* L.; and *D. nilhummatu* Dun.) | |
| *Datura metel* L. var. *fastuosa* (Bernh.) Danert (=*D. fastuosa* L.; *D. hummatu* Bernh. var *dubia* (Pers.) Bernh.; and *D. hummatu* Bernh. var. *fastuosa* Bernh.) | |
| *Datura metel* L. f. *fastuosa* (Bernh.) Danert (=*D. fastuosa* L.; and *D. hummatu* Bernh. var. *fastuosa* Bernh.) | |
| *Datura metel* L. f. *malabarica* Danert (*D. hummatu* Bernh. var. *dubia* (Pers.) Bernh.) | |
| | |
| **Section *Ceratocaulis Bernh.*** | |
| *Datura ceratocaula* Ort. (=*D. macrocaulos* Roth; *Brugmansia ceratocaula* (Ort.) Dum.; *Apemon crassicaule* Raf.; *Ceratocaulos daturoides* Spach.; and *Datura sinuata* Sessé et Moc.) | Originates from Mexico and Cuba |
| | |
| **Section *Datura Bernh.* (section *Stramonium Bernh.*)** | |
| *Datura stramonium* L. (=*D. tatula* L.; *Stramonium foetidum* Scop.; *D. inermis* Jac.; *Stramonium spinosum* Lam.; *D. laevis* L.; *Stramonium vulgatum* Gaertn.; *Stramonium tatula* Moench; *D. lurida* Salisb.; *D. parviflora* Salisb.; *D. bertolonii* Parlat. ex Guss.; and *D. wallichii* Dun.) | Cosmopolitan weed and prolific seed-producer. Four varieties have been described, one of which is sometimes described as an own species, *D. tatula*. Originates from southeastern United States but can now be found all over the world in temperate regions.<br>Erect habit, relatively small flowers, and distinctive angular or prismatic calyx |
| *Datura stramonium* L. var *stramonium* (=*Stramonium vulgatum* Gaertn.; *Stramonium vulgare* Moenh.; and *D. wallichii* Dun.) | |
| *Datura stramonium* L. f. *stramonium* (=*Stramonium vulgatum* Gaertn.; *Stramonium* | |

| Species (synonyms) | Habitat and botany |
|---|---|
| *vulgare* Moenh.; and *D. wallichii* Dun.) | |
| *Datura stramonium* L. f. *labilis* Hammer | |
| *Datura stramonium* L. var *inermis* (Jacq.) Lundstrt. (=*D. inermis* Jacq.; *D. laevis* L.; *Stramonium laeve* (L.f.) Moench; and D. *bertolonii* Parlat. ex Guss.) | |
| *Datura stramonium* L. var *tatula*(L.) Torr. (=*D. tatula* L.; and *Stramonium tatual* L.) | |
| *Datura stramonium* L. f. *tatula* Danert (=*D. tatula* L.; *Stramonium tatula* L.; *D. stramonium* L. var. *chalybaea* K; and *D. praecox* Godr.) | |
| *Datura stramonium* L. f. *bernhardii* (Lundstr.) Danert (=*D. bernhardii* Lundstr.) | |
| *Datura stramonium* L. var. *godroniii* Danert (=*D. tatula* var. *inermis* Godr.) | |
| *Datura ferox L.* | Comes from southeastern China and came to Europa via Sicily and Spain. Nowadays it can be found in warmer regions all over the world |
| *Datura quercifolia* H.B.K. (=*D. villosa* Fernald) | Comes from Mexico and southwestern United States |

## ANNEX III. AMOUNT OF TROPANE ALKALOIDS DETECTED IN VARIOUS TISSUES OF DIFFERENT *DATURA* SPECIES.

The following units for content have been used:-*weight by weight, **mg/kg dry weight, and ***mg/kg fresh weight (% of total tropane alkaloids).

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. stramonium* (Russia) | Roots during vigours growth | (18.5%) | (66.2%) | | 1200* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (Russia) | Roots during flowering | (16.3%) | (61.1%) | | 1900* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (Russia) | Roots during fruit bearing | (15.5%) | (55.2%) | | 2200* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (Italy) | Roots, young plants | 140** | | 1210** | | Miraldi *et al*., 2001 |
| *D. stramonium* (Italy) | Stem, young plants | 1290** | | 9150** | | Miraldi *et al*., 2001 |
| *D. stramonium* (Italy) | Stem, adult plants | n.d.** | | 10** | | Miraldi *et al*., 2001 |
| *D. stramonium* (Russia) | Epigeal parts during vigours growth | (19.5%) | (73.5%) | | 3600* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (Italy) | Leaves, young plants | 350-730** | | 1560-8310** | | Miraldi *et al*., 2001 |
| *D. stramonium* (Russia) | Epigeal parts during flowering | (17.2%) | (69.3%) | | 2600* | Mirzamatov and Lutfullin, 1986# |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. stramonium* (Russia) | Epigeal parts during fruit bearing | (16.5%) | (61.2%) | | 1900* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (Italy) | Leaves, adult plants | 160-440** | | 1340-1650** | | Miraldi *et al*., 2001 |
| *D. stramonium* (USA) | Leaves | 1550** (500–6 500) | 1550** | | | Shonle and Bergelson, 2000 |
| *D. stramonium* (Italy) | Flowers | 2700-2990** | | 660-1060** | | Miraldi *et al*., 2001 |
| *D. stramonium* (Russia) | Seeds | (20.5%) | (72.5%) | | 4200* | Mirzamatov and Lutfullin, 1986# |
| *D. stramonium* (USA) | Seeds | 530±130* (360-690) | | 2270±360* (1690-2710) | | Friedman and Levin, 1989 |
| *D. stramonium* (USA) | Seeds | 660* | | 2710* | | Dugan *et al*., 1989 |
| *D. stramonium* (Italy) | Seeds | 120-890** | | 1700-3870** | | Miraldi *et al*., 2001 |
| *D. stramonium* (not stated) | Seeds (n=1) | traces** | 1900** | | | Duez *et al*., 1985 |
| *D. stramonium* (USA) | Seeds | | | 4000* | | Klein-Schwartz and Oderda, 1984 |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. stramonium* (USA) | Seeds | 500* (200-1100) | | 2900* (1900-4300) | 3400* (2200-4800) | List *et al*., 1979 |
| *D. stramonium* var. *godronii* (greenhouse in Bulgaria) | Roots | (2.2%) | (32.0%) | | | Berkov *et al*., 2006# |
| *D. stramonium* var. *godronii* (greenhouse in Bulgaria) | Leaves | (11.4%) | (60.5%) | | | Berkov *et al*., 2006# |
| *D. stramonium* var. *godronii* (Poland) | Leaves | 571** | 427** | | | Mroczek *et al*., 2006 |
| *D. stramonium var. godronii* (from feed) | Seeds (n=1) | (12%) | (88%) | | 1 675 | Bucher *et al*., 1989 |
| *D. stramonium* var. *godronii* (Poland) | Seeds | 1275** | 1382** | | | Mroczek *et al*., 2006 |
| *D. stramonium* var. *godronii* (greenhouse in Bulgaria) | Seeds | (16.0%) | (69.6%) | | | Berkov *et al*., 2006# |
| *D. stramonium var. inermis* (from feed) | Seeds (n=2) | (31-41%) | (59-69%) | | 1645±790*** (1085-2205) | Bucher *et al*., 1989# |
| *D. stramonium* var. *stramonium* (greenhouse in Bulgaria) | Roots | (1.9%) | (21.4%) | | | Berkov *et al*., 2006# |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. stramonium* var. *stramonium* (Poland) | Leaves | 713** | 658** | | | Mroczek *et al*., 2006 |
| *D. stramonium* var. *stramonium* (greenhouse in Bulgaria) | Leaves | (12.8%) | (62.3%) | | | Berkov *et al*., 2006# |
| *D. stramonium var. stramonium* (from feed) | Seeds (n=4) | (6-35%) | (65-94%) | | 1040±727*** (74-1790) | Bucher *et al*., 1989# |
| *D. stramonium* var. *stramonium* (Poland) | Seeds | 1 192** | 722** | | | Mroczek *et al*., 2006 |
| *D. stramonium* var. *stramonium* (greenhouse in Bulgaria) | Seeds | (36.0%) | (55.5%) | | | Berkov *et al*., 2006# |
| *D. stramonium* var. *tatula* (greenhouse in Bulgaria) | Roots | (<1.0%) | (7.6%) | | | Berkov *et al*., 2006# |
| *D. stramonium* var. *tatula* (greenhouse in Bulgaria) | Leaves | (18.4%) | (57.7%) | | | Berkov *et al*., 2006# |
| *D. stramonium* var. *tatula* (Poland) | Leaves | 625** | 1 047** | | | Mroczek *et al*., 2006 |
| *D. stramonium var. tatula* (from feed) | Seeds (n=2) | (39-47%) | (53-61%) | | 1485±135*** (1390-1580) | Bucher *et al*., 1989# |
| *D. stramonium* var. *tatula* (greenhouse in Bulgaria) | Seeds | (18.1%) | (50.4%) | | | Berkov *et al*., 2006# |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. stramonium* var. *tatula* (Poland) | Seeds | 522** | 711** | | | Mroczek *et al*., 2006 |
| *D. stramonium* + *D. stramonium var. tatula* | Seeds (n=3) | (17-47%) | (53-83%) | | 1425±110*** | Bucher and Meszaros, 1989# |
| *D. ferox* (Argentina) | Root | 40-120** (3-28%) | | | 800-3 600** | Padula *et al*., 1976 |
| *D. ferox* (Argentina) | Stem | 300-1500** (1.7-95%) | | | 200-2800** | Padula *et al*., 1976 |
| *D. ferox* (Argentina) | Leave | 40-3200** (2-84%) | | | 1000-4100** | Padula *et al*., 1976 |
| *D. ferox* (Argentina) | Fruit | 130-210** (12-15%) | | | 1100-2300** | Padula *et al*., 1976 |
| *D. ferox* | Seeds (n=2) | (91%) | (9%) | | | Papadoyannis, 1995 |
| *D. ferox* (from feed) | Seeds (n=4) | (98-100%) | (<1-2%) | | 936±217*** (800-1260) | Bucher *et al*., 1989# |
| *D. ferox* (Argentian) | Seeds (n=4) | (76.3%) | | | | Vitale *et al*., 1995 |
| *D. inoxia* (greenhouse in Germany) | Roots | 100*** | 172*** | | 1123*** | Witte *et al*., 1987 |
| *D. inoxia* (France) | Roots | | | | 6700** | Brachet and Cosson, 1986 |
| *D. inoxia* (greenhouse in Germany) | Stem | 19*** | 25*** | | 66*** | Witte *et al*., 1987 |
| *D. inoxia* (France) | Stem | | | | 3200** | Brachet and Cosson, 1986 |
| *D. inoxia* (USA) | Stems and leaves (n=12) | 612±278** (340-1350) | | 187±103** (110-369) | | Galey *et al*., 1996 |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. inoxia* (not stated) | Leaves (n=3) | 120-770** | traces** | | | Duez *et al*., 1985 |
| *D. inoxia* (greenhouse in Germany) | Leaves | 250*** | 54*** | | 314*** | Witte *et al*., 1987 |
| *D. inoxia* (France) | Basal leaves | | | | 1700** | Brachet and Cosson, 1986 |
| *D. inoxia* (France) | Leaves (7+8) | | | | 5700** | Brachet and Cosson, 1986 |
| *D. inoxia* (greenhouse in Germany) | Flower | 520*** | 42*** | | 550*** | Witte *et al*., 1987 |
| *D. inoxia* (not stated) | Fruit (n=1) | 190** | 20** | | | Duez *et al*., 1985 |
| *D. inoxia* (from feed) | Seeds (n=1) | (62%) | (38%) | | 4095*** | Bucher *et al*., 1989 |
| *D. inoxia* (not stated) | Seeds (n=1) | 710** | 1400** | | | Duez *et al*., 1985 |
| *D. inoxia* (USA) | Seeds (n=1) | 29** | | 26** | | Galey *et al*., 1996 |
| *D. inoxia* (Poland) | Seeds | 672** | 2 862** | | | Mroczek *et al*., 2006 |
| *D. ceratocaula* (greenhouse in the Netherlands) | Roots | 700** (2.2%) | 1600** (26.3%) | | | Berkov, 2003 |
| *D. ceratocaula* (greenhouse in the Netherlands) | Stem | 2000** (6.3%) | 4200** (44.2%) | | | Berkov, 2003 |
| *D. ceratocaula* (greenhouse in the Netherlands) | Leaves | 2900** (9.2%) | 4700** (34.4%) | | | Berkov, 2003 |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. ceratocaula* (greenhouse in the Netherlands) | Flowers | 3400** (19.3%) | 4100** (49.1%) | | | Berkov, 2003 |
| *D. ceratocaula* (greenhouse in the Netherlands) | Seeds | 700** (2.0%) | 2500** (50.6%) | | | Berkov, 2003 |
| *D. metel* (India) | Roots | | | | 2700-8900* | Karnick and Saxena, 1970 |
| *D. metel* (Nigeria) | Root towards end of season | 4900* | | | | Abo *et al*., 1993* |
| *D. metel* (India) | Stem | | | | 1900-4600* | Karnick and Saxena, 1970 |
| *D. metel* (India) | Leaves | | | | 2500-5800* | Karnick and Saxena, 1970 |
| *D. metel* (Poland) | Leaves | 1110** | 904** | | | Mroczek *et al*., 2006 |
| *D. metel* (India) | Leaves | 1810* | | 2200* | 4480* | Shah and Khanna, 1963 |
| *D. metel* (Nigeria) | Leaves at peak flowering | 5200* | | | | Abo *et al*., 1993 |
| *D. metel* (India) | Herb | 1830* | | 1550* | 3770* | Shah and Khanna, 1963 |
| *D. metel* (India) | Flowers | | | | 6900-9900* | Karnick and Saxena, 1970 |
| *D. metel* (Nigeria) | Flowers at peak flowering | 6400* | | | | Abo *et al*., 1993 |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D.metel* (India) | Flowers | 5510* | | 3200* | 9390* | Shah and Khanna, 1963 |
| *D. metel* (Nigeria) | Fruits at fruit dehiscence | 2700* | | | | Abo *et al*., 1993 |
| *D. metel* (India) | Fruits | | | | 600-970* | Karnick and Saxena, 1970 |
| *D. metel* (India) haploid | Fruit | | | | 1500* | Karnick and Saxena, 1970 |
| *D. metel* (India) diploid | Fruit | | | | 3300* | Karnick and Saxena, 1970 |
| *D. metel* (India) tetraploid | Fruit | | | | 4200* | Karnick and Saxena, 1970 |
| *D. metel* (India; herbarium) | Seeds | 1250* | 650* | | 1910* | Shah and Khanna, 1964 |
| *D. metel* (India) | Seeds | | | | 900-1900* | Karnick and Saxena, 1970 |
| *D. metel* (Poland) | Seeds from 2000<br>Seeds from 2002 | 756**<br>425** | 914**<br>1248** | | | Mroczek *et al*., 2006 |
| *D. metel* (India; herbarium) | Pericarps | 480* | 230* | | 860* | Shah and Khanna, 1964 |
| *D. metel* var. *metel* (Japan) | Leaves (n=2) | 2280–2530** | | | | Hiraoka *et al*., 1996 |

| Species | Tissue | Scopolamine (mg/kg) (% of total) | Hyoscyamine (mg/kg) (% of total) | Atropine (mg/kg) | Total tropane alkaloids (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| *D. metel* var. *rubra* (Japan) | Leaves (n=2) | 460-590** | | | | Hiraoka *et al*., 1996 |
| *D. metel* var. *muricata* (Japan) | Leaves (n=2) | 810-820** | | | | Hiraoka *et al*., 1996 |
| *D. metel* var. *fastuosa* (Japan) | Leaves (n=2) | 690-1200** | | | | Hiraoka *et al*., 1996 |
| *D. metel* var. *fastuosa* (India) | Leaves | 2610* | | 3300* | 6600* | Shah and Khanna, 1963 |
| *D. metel* var. *fastuosa* (India) | Herb | 3060* | | 2720* | 6370* | Shah and Khanna, 1963 |
| *D. metel* var. *fastuosa* (India) | Flowers | 4770* | | 3210* | 8900* | Shah and Khanna, 1963 |
| *D. metel var. fastuosa* (India; herbarium) | Seeds | 3700* | 420* | | 4160* | Shah and Khanna, 1964 |
| *D. metel* var. *fastuosa* (Poland) | Seeds | 773** | 1027** | | | Mroczek *et al*., 2006 |
| *D. metel var. fastuosa* (from feed) | Seeds (n=1) | (76%) | (24%) | | 3240*** | Bucher *et al*., 1989# |
| *D. metel var. fastuosa* (India; herbarium) | Pericarp | 1200* | 570* | | 1930* | Shah and Khanna, 1964 |
| *D. wrightii* (from feed) | Seeds (n=1) | (75%) | (25%) | | 2205*** | Bucher *et al*., 1989# |
| *D. quercifolia* (Poland) | Seeds | 387** | 1929** | | | Mroczek *et al*., 2006 |

#additional tropane alkaloids have been measured;