JOINT FAO/WHO EXPERT MEETING ON TROPANE ALKALOIDS
30 MARCH – 3 APRIL 2020
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### Abbreviations and Acronyms

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<th>Definition</th>
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<tr>
<td>AB</td>
<td>Atropinic burden</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMD</td>
<td>Benchmark dose</td>
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<tr>
<td>BMDL</td>
<td>Benchmark dose lower bound</td>
</tr>
<tr>
<td>BMDU</td>
<td>Benchmark dose upper bound</td>
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<tr>
<td>BMR</td>
<td>Benchmark response</td>
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<tr>
<td>bpm</td>
<td>Beats per minute</td>
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<tr>
<td>bw</td>
<td>Bodyweight</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention (United States of America)</td>
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<tr>
<td>CI</td>
<td>Confidence intervals</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>Maximum concentration (plasma)</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiography</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration (United States of America)</td>
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<tr>
<td>GC-MS</td>
<td>Gas chromatography/mass spectrometry</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GIFT</td>
<td>FAO/WHO Global Individual Food consumption Tool</td>
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<tr>
<td>HBGV</td>
<td>Health based guidance value</td>
</tr>
<tr>
<td>HPLC-DAD</td>
<td>High-performance liquid chromatography diode-array detection</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>i.m.</td>
<td>Intramuscular</td>
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<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram ($10^3$ g)</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>LC-MS</td>
<td>High performance liquid chromatography/ mass spectrometry</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>High performance liquid chromatography/ tandem mass spectrometry</td>
</tr>
<tr>
<td>LD</td>
<td>Lethal dose</td>
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</table>
LNS  Lipid-based nutrient supplements
LOAEL  Lowest observed adverse effect level
LOD  Limit of detection
LOEL  Lowest observed effect level
LOQ  Limit of quantification
µg  Microgram (10^{-6} g)
µL  Microlitre (10^{-6} L)
mg  Milligram (10^{-3} g)
mL  Millilitre (10^{-3} L)
ML  Maximum levels
mm  Millimetre (10^{-3} m)
ng  Nanogram (10^{-9} g)
MOE  Margin of exposure
NOAEL  No observed adverse effect level
NOEL  No observed effect level
OR  Odds ratio
PNS  Peripheral nervous system
QuEChERS  Quick, Easy, Cheap, Effective, Rugged and Safe
R.A.F.  Royal Air Force
RR  Relative risk
s.c.  Subcutaneous
SC  Super cereal
SC+  Super cereal plus
SD  Standard deviation
TA  Tropane alkaloids
T_{max}  Time of maximum concentration observed
UHPLC-MS/MS  Ultra-high-performance liquid chromatography tandem mass spectrometry
WFP  United Nations World Food Programme
WHO  World Health Organization
DECLARATIONS OF INTEREST

All participants in the Joint FAO/WHO Expert Meeting on Tropane Alkaloids completed a declaration of interest form in advance of the meeting.

Following the FAO Guidance Document for Declaration of Interests, the declarations were assessed as to the extent to which any interest could be reasonably expected to affect and exercise influence on the experts’ judgement. The declared interests were considered unlikely to impair the individual’s objectivity or cause significant influences on the impartiality, neutrality, and integrity of the work. The interests of all participants were disclosed to all attendees at the beginning of the expert meeting.
Beneficiaries hold rations of Super Cereal Plus which they received at a WFP food distribution point in Niger.
EXECUTIVE SUMMARY

Assisting 86.7 million people in around 83 countries each year, the United Nations World Food Programme is the leading humanitarian organization that saves and changes lives by delivering food assistance in emergencies and working with communities to improve nutrition and build resilience. The humanitarian organization focuses on the poorest and the most vulnerable people around the world. These include people with high nutrient needs, such as young children, adolescent girls, pregnant women and nursing mothers. The foods distributed vary from common commodities disseminated among the population in general, to Specialized Nutritious Foods (SNF) given to target beneficiaries for the specific purpose of prevention and treatment of malnutrition. One of the SNF products is Super Cereal, which consists of pre-cooked corn, soybean and micronutrients. Approximately 130 000 MT Super Cereal is distributed to 4.9 million people every year with the objective of improving food security and nutrition.

In April 2019, consumption of Super Cereal was associated with five deaths and hospitalisation of approximately 300 people in the Karamoja region of the Republic of Uganda. High concentrations of tropane alkaloids, specifically (−)-scopolamine and (±)-hyoscyamine, from *Datura stramonium*, in soybeans were found to be the source of the intoxication, as determined by a joint investigation by the Government of the Republic of Uganda, United States of America Centers for Disease Control and Prevention (CDC), United States of America Food and Drug Administration (FDA), World Health Organization (WHO), United Nations World Food Programme (WFP) as well as members of academia and independent experts. Moreover, a second contamination incident occurred later in 2019 (albeit with somewhat less severe impact) involving unprocessed sorghum contaminated with *Datura stramonium* seeds, which was distributed as food aid to the Republic of South Sudan.

The issue of tropane alkaloids is a major concern for WFP as the importance of nutritional supplements like Super Cereal has grown significantly with increased global distribution of such products over the years. Moreover, as soy is an essential ingredient and source of protein in various WFP food products and *D. stramonium* is a common weed found among different grains, the issue of tropane alkaloids goes beyond the Super Cereal products for WFP.

Currently there are no international regulations in place for tropane alkaloids, with neither Codex maximum levels (ML) nor a Code of Practice available for these contaminants. While there are some regulations in certain regions of the world that define limits for the presence of noxious seeds in grains, none of these seem to be applicable to the WFP products that are considered in this assessment.¹

¹ The requested scope for FAO/WHO review includes Super Cereal, Specialized Nutritious Foods for infant and young children (i.e. Super Cereal plus, Lipid-based Nutrient Supplements), applicable grains, pulses and their derived products.
Within this context, WFP had requested assistance from the Food and Agriculture Organization of the United Nations (FAO) and WHO to provide scientific advice on tropane alkaloids in WFP products,\(^1\) both processed and unprocessed, to allow for the development of appropriate risk management measures in their supply chains and to prevent intoxication events in the future. To address this request FAO/WHO convened a joint expert meeting on tropane alkaloids from 30th March to 3rd April 2020. The meeting, originally planned as a physical meeting at FAO headquarters in Rome, was held remotely by an electronic platform due to the various travel restrictions in place during the COVID-19 pandemic.
SCOPE

The scope of the Joint FAO/WHO Expert Meeting on Tropane Alkaloids was to:

provide a risk assessment for (-)-scopolamine, (-)-hyoscyamine and (+)-hyoscyamine;

based on the risk assessment, provide guidance for the development of operational limits for hyoscyamine and scopolamine in the relevant WFP products\(^1\), taking into consideration both food safety for WFP’s beneficiaries as well as food security, which is an essential component of the WFP mandate.

Considering the lack of data on other tropane alkaloids (both for the toxicological and exposure assessment), this evaluation was focused on hyoscyamine and scopolamine.
CONCLUSIONS

CHEMICAL AND ANALYTICAL CHARACTERIZATION

> Tropane alkaloids are present in many genera under the Solanaceae family including *Mandragora*, *Brugmansia*, *Duboisia*, *Hyoscyamus*, *Datura*, *Atropa*, and *Scopolia*. Tropane alkaloid content of plant tissue varies according to the plant tissue and species but typically ranges from 0.01 to 3 percent.

> Hyoscyamine and scopolamine have been identified as the main tropane alkaloids in *Datura*, *Brugmansia*, *Hyoscyamus*, *Scopolia*, *Atropa*, and *Duboisia* species. Sixty seven tropane alkaloids were identified by gas chromatography-mass spectrometry in different plant organs from *D. stramonium* cultivated in the Kingdom of Morocco. In seeds, hyoscyamine and scopolamine accounted for 66 percent and 20 percent (percentage estimated using total ion current) of the total tropane alkaloid content, respectively (El Bazaoui, 2011). The assumption that hyoscyamine and scopolamine are the most relevant tropane alkaloids in grain-based food is supported by the results of analysis of food samples collected in nine European countries. Twenty-four different tropane alkaloids were monitored, but hyoscyamine and scopolamine comprised 83 percent of the reported tropane alkaloid content (Mulder et al., 2016).

> The pharmacologically active (−)-hyoscyamine is the predominant enantiomer of hyoscyamine present in plants. However, (−)-hyoscyamine may undergo some enantiomerisation and therefore, both enantiomers of hyoscyamine may be found in plant samples at varying ratios (Eich 2008). In a study reported by Marín-Sáez et al. (2016), about 1 percent of the total hyoscyamine content was identified as (+)-hyoscyamine in the analysis of *Datura* seeds. In this study a stereo-selective analytical method was used.

> Growing season conditions, geographical growing location, plant maturity, plant species and variety, and type of plant tissue affect the concentration and proportion of hyoscyamine and scopolamine in samples. Generally, seeds and flowers of *Datura* species contain more scopolamine and hyoscyamine than other tissues.

> Seeds from species like *Brugmansia*, *Datura*, and *Hyoscyamus* are the likeliest materials to contaminate grains (and subsequently grain-based foods) because their density, size, and shape are similar to grains. Seeds of *D. stramonium* have been reported in linseed/flaxseed, soybean, millet, sunflower and buckwheat. The concentration of hyoscyamine in the analysed food samples is usually expressed as “atropine”. Atropine is defined as the racemic mixture (1:1) of (−)-hyoscyamine and (+)-hyoscyamine. However, the analytical methods used did not measure each enantiomer of hyoscyamine in the samples separately. Therefore, taking in account the potential variability of the enantiomeric fraction, the expert meeting considered it more accurate to express the results as the sum of (−)-hyoscyamine and (+)-hyoscyamine, instead of “atropine”.

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In the study reported by Mulder et al. (2016), 1,709 samples of plant-derived food products, mainly produced in Europe and collected in nine European countries, were analysed for tropane alkaloids. Food samples comprised 268 single component flours from buckwheat, millet and corn, 260 cereal-based foods for young children age 6–36 months (breakfast cereals, biscuits and other cereal-based foods), 219 breakfast cereals, 164 biscuits and pastry, 114 bread, 81 pasta, 121 dry (herbal) teas, 65 legumes and stir-fry mixes. One or more tropane alkaloids were detected in 21.3 percent of single component flours, 20 percent of cereal-based food for young children age 6–36 months, 15.8 percent of bread, 26.2 percent of legumes and stir-fry mixes, and 14.6 percent of biscuits. Due to the large number of samples and broad scope of sampled food matrices this is the most significant study currently available on tropane alkaloid levels in food, though samples were obtained only from markets in European countries.

Caution must be taken when interpreting analytical results of samples exposed to highly alkaline aqueous or alcoholic conditions since hyoscyamine may hydrolyse into tropane and tropic acid. Enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine may also occur.

A validated analytical method using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of the sum of (-)-hyoscyamine and (+)-hyoscyamine, and scopolamine in grain-based foods and grains is available and may be used for monitoring purposes. The limits of quantitation of this method for the determination of the sum of (-)-hyoscyamine and (+)-hyoscyamine; as well as scopolamine ranged from 0.5 to 1.0 µg/kg. Limits of detection of this method ranged from 0.05 to 0.2 µg/kg.

The number of studies reporting data on the fate of tropane alkaloids during food processing is limited. Even among the few studies that are available, most do not consider effects from sample heterogeneity and changes in moisture content, during food processing or do not provide complete analytical method descriptions. Hence, this did not allow for the evaluation of the quality of the data and increased the uncertainty in assessing the fate of hyoscyamine and scopolamine during food processing.

Two studies reported in the literature and relevant to WFP products suggest that hyoscyamine and scopolamine diminish in concentration during cooking processes (Perharić et al., 2013; Marín-Sáez et al., 2019). Decreases reported were 63–70 percent of hyoscyamine and 42–80 percent of scopolamine. However, food products were fortified and the impact of matrix effects on the results was not assessed and nor was the measurement uncertainty reported. These studies also did not identify and quantify all hyoscyamine and scopolamine degradation products, therefore the risk from potential degradation products cannot be assessed.

When processing soybeans, hyoscyamine (90 percent of the content in unprocessed soybeans) and scopolamine (84 percent) will be present in the defatted meal (List and Spencer, 1976). Approximately 0.1 percent will be present
in the unrefined oil, and over 90 percent of this will be lost during alkaline refining and washing of the unrefined oil.

> The small number of cooking studies considered relevant, the uncertainty surrounding the results from these studies, the absence of information on degradation products in these studies as well as the lack of information on how food matrix and specific food processing techniques will impact the degree of hyoscyamine and scopolamine loss, prevented the expert meeting from estimating degradation factors for use in dietary exposure assessments. The expert meeting agreed as a default to assume that there was no loss of hyoscyamine and scopolamine due to food processing for the purpose of the dietary exposure assessments in order to maximize protection of consumers.

> Enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine is possible, but unlikely under most food processing conditions. In a study using plant material, enantiomerisation was favoured only in aqueous alkaline solution (pH > 9) combined with elevated temperatures (>80 °C). After one day under these conditions, approximately 30 percent of (-)-hyoscyamine converted to (+)-hyoscyamine. Under milder conditions more relevant to food processing, minimal enantiomerisation occurred. At a lower pH of 5, less than 10 percent of (-)-hyoscyamine was converted to (+)-hyoscyamine after one day. Therefore, in most conditions used for food processing minimal enantiomerisation is expected to occur.

> In literature, there are a limited number of studies that has reported the enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine in pharmaceuticals and plant extracts. Very few of these these studies are relevant for grain-based food processing, as experimental conditions are extremely alkaline and unlikely to be encountered during processing of grain-based foods, aside from nixtamalisation of corn/maize.

> Toxicological Evaluation of Hyoscyamine and Scopolamine

> As the toxicological and exposure information on tropane alkaloids is generally limited to hyoscyamine and scopolamine, the hazard characterization described herein necessarily focuses on these two compounds. Clinical and experimental studies typically use commercial atropine which is the 1:1 racemic mixture of (-)- and (+)-hyoscyamine. The anticholinergic activity of atropine is predominantly associated with the (-) enantiomer. Even though the true enantiomeric fraction was not assessed in these studies, where atropine was administered and a non-enantiomeric specific method was used for analysis, the results were expressed as atropine.
Atropine is readily absorbed from the gastrointestinal tract (up to 90 percent) with a lower bioavailability observed for (-)-scopolamine (27 percent or lower; range 12 to 48 percent). Both tropane alkaloids are extensively distributed into tissues and excreted predominantly via the renal system. Only 2.6 percent of parent (-)-scopolamine is excreted in urine whereas up to 50 percent of an atropine oral dose has been reported to be excreted unchanged. Maximum blood levels after oral dosing are typically reached within 30 minutes to 2 hours and peak plasma concentrations of the parent molecules are significantly higher for atropine compared to scopolamine. Atropine and scopolamine can cross the placenta and blood brain barrier. There is no quantitative information regarding the concentration of atropine and (-)-scopolamine transferred to breast milk.

Phase 1 (CYP3A mediated N-demethylation and dehydroxylation) and Phase II (glucuronide and sulphate conjugation) reactions have been reported as metabolic pathways in humans. Half-lives of both atropine and scopolamine in humans are typically in the range of 1 to 4 hours.

Clearance of both atropine and scopolamine is age dependent. For example, following intravenous administration of atropine, the mean half-life ($t_{1/2}$) was reported to be longer in paediatric subjects under two years (6.9 ± 3.3 hours) and in geriatric patients 65–75 years (10.0 ± 7.3 hours), compared to in children over two years of age (2.5 ± 1.2 hours) and in adults 16–58 years old (3.0 ± 0.9 hours). Based on data in healthy adults (ages 18 to 78 years), older adults appear more sensitive to the effects (i.e. cognitive function tests) of (-)-scopolamine than younger adults due to lower clearance, rather than to any difference in pharmacodynamics. Depending on health status, clearance of atropine and scopolamine in individuals may vary widely.

Effects caused by (-)-hyoscyamine and (-)-scopolamine are related to their competitive inhibition of acetylcholine binding to muscarinic receptors (M1–M5). Muscarinic receptors bind acetylcholine, a neurotransmitter in both the peripheral and central nervous systems. Competition binding assays indicate that (-)-hyoscyamine and (-)-scopolamine have a high affinity for muscarinic acetylcholine receptors. The antimuscarinic activity of hyoscyamine is stereospecific, with the (-) enantiomer estimated to be approximately 30 to 300-fold more potent than the (+) enantiomer.

A number of pharmacological and toxicological studies have been conducted in experimental animals with atropine, although due to the route of exposure or the nature of the effects and the magnitude of dosing, these studies were deemed largely uninformative for this assessment.

The acute oral LD$_{50}$ of atropine and scopolamine in experimental animals ranges from 400 to greater than 1 000 mg/kg bw.

In contrast to atropine, the repeated dose oral toxicity of (-)-scopolamine in experimental animals has been investigated in some detail. Based on structural similarities and a common mechanism of action, (-)-hyoscyamine would be expected to exhibit a similar pharmacological/toxicological profile as (-)-scopolamine.
In short-term oral toxicity studies (up to 14 weeks), in mice and rats with scopolamine hydrobromide trihydrate, a lowest observed adverse effect level (LOAEL) of 10.4 mg/kg bw per day expressed as (-)-scopolamine free base was identified, the lowest dose tested, based on body weight decrease and pupillary dilation. In chronic oral toxicity studies of scopolamine hydrobromide trihydrate in mice and rats a LOAEL of 0.692 mg/kg bw per day expressed as (-)-scopolamine free base was identified, the lowest dose tested, based on pupillary dilation.

Results of genotoxicity testing of (-)-scopolamine indicate that it is unlikely to be genotoxic in vivo and based on the results of the chronic oral toxicity studies in mice and rats, (-)-scopolamine does not show any evidence of tumorigenic activity. Additionally, (-)-scopolamine does not induce developmental toxicity in the absence of maternal toxicity (decreased fetal body weights were observed at maternally toxic doses).

Clinical applications of atropine and (-)-scopolamine include uses as a mydriatic agent, to reduce secretion (digestive, bronchial, cutaneous, lacrimal), as an anti-spasmodic for various gastrointestinal tract conditions (intestinal and biliary colic), to reduce excess salivation caused by other medical conditions (i.e. Parkinsonism), and to treat bradycardia and motion sickness. Maximum recommended therapeutic doses of atropine for children are in the range of 0.5 mg, whereas they are 1.5 to 3.0 mg for adults. Recommended doses of (-)-scopolamine for children and adults are approximately 0.25 to 0.8 mg. Atropine is also used at higher doses as an antidote for organophosphate poisonings.

Information from the use of atropine and scopolamine during human pregnancy indicates that therapeutic doses are not associated with adverse developmental effects or significant fetotoxicity.

At low doses of atropine or (-)-scopolamine, effects observed in human studies include a transient decrease in heart rate and inhibition of salivary secretion. A LOEL of 2 µg/kg bw for a single oral dose of (-)-scopolamine was identified in human subjects, based on a reduction in heart rate. Similar heart rate effects have been observed for atropine sulphate at 7 µg/kg bw.

For the purposes of assessing the toxicity of (-)-hyoscyamine and (-)-scopolamine, human poisonings incidents due to consumption of food contaminated with tropane alkaloids were also considered. However, the information on poisonings generally lack quantitative dose-response data and usually provide only confirmation of the presence of the plant parts in the food with self-reported intake estimates.

The most informative experimental study was the randomised, double blind, placebo controlled, crossover study by Perharić et al. (2013), which investigated the combined exposure to relatively low doses of atropine and scopolamine hydrochloride added to buckwheat flour prior to cooking (doses given below are expressed as the free bases of the active enantiomers).
Body temperature, heart rate, salivary secretion, sweat secretion, and pupil size were recorded quantitatively for up to 4 hours after dosing. Although there was no consistent effect on body temperature, significant effects on heart rate, salivary secretion, sweat secretion and pupil dilation were observed. Decreased heart rate and salivary secretion were the most sensitive indicators of anticholinergic effects in this study. For example, a no observed effect level (NOEL) of 0.32 µg/kg bw was identified, based on a statistically significant decrease in heart rate at 0.97 µg/kg bw (i.e. the LOEL), a trend in decreased salivary secretion was observed, becoming statistically significant at 9.7 µg/kg bw. A benchmark dose (BMD) lower confidence limit or BMDL\textsubscript{05} of 0.38 µg/kg bw was derived for the decrease in salivary secretion. Subjective symptoms of dry mouth were not significant until doses ≥9.7 µg/kg bw. Statistically significant effects on sweat secretion and pupil dilation were not observed until 9.7 and 32.4 µg/kg bw, respectively. A statistically significant increase in heart rate was observed at 32.4 µg/kg bw. Although the magnitude of decreased heart rate and salivary secretion observed at the low doses in the Perharic\v{e} et al. (2013) are of questionable adversity to healthy individuals, the NOEL and BMDL\textsubscript{05} do represent sensitive indicators of the anticholinergic activity of (-)-hyoscyamine and (-)-scopolamine.

> Since Perharic\v{e} et al. (2013) reported loss of atropine (37 percent) and scopolamine (58 percent) during cooking, the above noted doses were adjusted accordingly. The NOEL and BMDL\textsubscript{05} from the Perharic\v{e} et al. (2013) study translate to 0.15 and 0.20 µg/kg bw, respectively, considering the adjustment factors suggested by Perharic\v{e} et al. (2013).

**Hazard characterization**

> Hyoscyamine and (-)-scopolamine are the most well studied tropane alkaloids with respect to therapeutic and adverse health effects and are typically the predominant tropane alkaloids detected in food contaminated with *D. stramonium*. Very limited data are available regarding the exposure and hazard of other tropane alkaloids in food.

> Based on the limited data available, it was considered that (-)-hyoscyamine and (-)-scopolamine are equipotent via the oral exposure route with regard to their anticholinergic effects, and a dose additivity approach was used. The anticholinergic activity of (+)-hyoscyamine is so low that it was not considered herein in the hazard characterization.

> The following points were considered for the hazard characterization for hyoscyamine and scopolamine:

> They do not bioaccumulate;

1. They have short half-lives in humans (hours);
2. Peak plasma concentrations are achieved within two hours; the effects generally occur within a short time after administration and are transient;
3. They are not genotoxic \textit{in vivo};
4. They do not cause carcinogenicity or progressive toxicity following repeated oral exposure;
5. They do not cause developmental toxicity; and
6. The effects of toxicological concern are due to the antagonism of acetylcholine binding to the peripheral and central nervous system muscarinic receptors leading to acute effects.

In light of the aforementioned points, protection from acute effects should also cover any anticipated effects following chronic oral exposure.

Adequate human data are available for assessing the acute effects of combined exposure to atropine and scopolamine. The randomised, double blind, placebo controlled, crossover study with adult male volunteers by Perharić \textit{et al.} (2013) with combined oral exposures to atropine and (-)-scopolamine was considered the most relevant for determining a point of departure. Decreases in heart rate and salivary secretion were considered the most sensitive indicators of anticholinergic effects. Both effects are commonly observed following low therapeutic doses of atropine and (-)-scopolamine.

The effects of atropine and scopolamine on heart rate are well documented, with slowing of the heart rate at low doses and increases in heart rate at higher doses. Due to the biphasic dose response for heart rate, these data were not amenable to benchmark dose modelling. Based on the transient, mild decrease in heart rate observed at 0.97 µg/kg bw (0.46 µg/kg bw adjusted for processing), the lowest dose of 0.32 µg/kg bw (0.15 µg/kg bw adjusted for processing) for the combined sum of (-)-hyoscyamine and (-)-scopolamine was considered to be NOEL. Heart rate increased to a rate similar to that in controls at 9.7 µg/kg bw (4.62 µg/kg bw adjusted for processing) while a statistically significant increase in heart rate was observed at 32.4 µg/kg bw (15.4 µg/kg bw adjusted for processing) in the same study.

The effect of atropine and scopolamine on inhibition of salivary secretion is also well documented and follows a typical monotonic dose response pattern. Benchmark dose modelling of the Perharić \textit{et al.} (2013) data yielded a model averaged BMDL₂₅ value of 0.38 µg/kg bw (0.2 µg/kg bw adjusted for processing) at the 3.5 hours observation interval. However, this represents a dose at which a minimal change in salivary secretion would occur (5 percent) and dry mouth was not apparent until 9.7 µg/kg bw (4.62 µg/kg bw adjusted for processing). It is acknowledged that in the case of a decrease in salivary secretion the default benchmark response (BMR) of 5 percent for continuous variables does not represent a level of adversity. However, it was used as a sensitive biomarker of antimuscarinic effects. At doses of ≥1.54 µg/kg bw decreases in salivary secretion were evident, becoming statistically significant in the two highest dose groups. Similarly, the magnitude of decreased heart rate induced in the Perharić \textit{et al.} (2013) study at the LOEL is not likely to cause adverse effects in healthy individuals. At higher doses, statistically significant decreased sweat secretion (≥9.7 µg/kg bw; 4.62 µg/kg bw adjusted for processing), pupil dilation
(32.4 µg/kg bw; 15.4 µg/kg bw adjusted for processing) and increased heart rate (32.4 µg/kg bw; 15.4 µg/kg bw adjusted for processing) were observed.

Based on this analysis, the expert committee determined that in healthy male adults, a dose of 1.54 µg/kg bw was considered to be a clinically significant minimal acute effect dose, based on the reduction of salivary secretion.

Hyoscyamine and scopolamine exhibit both pharmacological and toxicological properties, however determining a clear point of demarcation between transient non-adverse effects and toxicological effects, from the information available was not considered possible. Additionally, as populations typically consuming WFP products could have various underlying health conditions (e.g. malnourishment, malaria and tuberculosis) that may make them overly sensitive to tropane alkaloids toxicity, the expert meeting considered that determination of a health based guidance value (HBGV) based on results from the population studied by Perharic et al. (2013) was not possible due to these sensitivity uncertainties. However, in order to help ensure food security in the populations receiving the WFP products, the expert meeting concluded that consideration of several points of departure, ranging from a no observed effect level for anti-muscarinic, non-adverse signs to an adverse effect level, and use of a margin of exposure (MOE) approach would be most appropriate.

Dietary Exposure Assessment

Toxicological concerns due to tropane alkaloids relate to acute effects and hence to exposure over an acute timeframe (single meal or single day). Therefore, the expert meeting considered only acute dietary exposure. Acute dietary exposure techniques seek to quantify the probability of high single exposure events.

This assessment considers dietary exposure through two scenarios: the general diet of population groups in countries where WFP is active and the specific food products formulated for WFP. The specific food products are Super Cereal (SC), Super Cereal plus (SC+, for young children, 6–59 months) and lipid-based nutrient supplements (LNS). While WFP also distributes cereal grains and processed cereal and legume products (flours, meal), no monitoring data for tropane alkaloids in these products were available and these products have been considered in the context of the general diet.

For both scenarios of exposure, tropane alkaloid concentration data are available for the two most-studied alkaloids; hyoscyamine (often reported as “atropine”) and scopolamine. While a large number of other tropane alkaloids may be present in food samples, hyoscyamine and scopolamine are the dominant compounds, accounting for approximately 85 percent of tropane alkaloids from *Datura stramonium*. The analytical methods commonly used were unable to separate the enantiomers of atropine: (-)-hyoscyamine and (+)-hyoscyamine. The available evidence suggests that (-)-hyoscyamine is the predominant enantiomer in most plant material. The expert meeting concluded that there would be little opportunity for enantiomerisation during most food processing. As a result, as a default results reported as atropine have been treated as (-)-hyoscyamine.
Scopolamine and (-)-hyoscyamine appear to be approximately equipotent and dose additivity have been assumed for mixtures of the two compounds. The acute dietary exposure assessment was carried out for the sum of the concentrations reported for hyoscyamine and scopolamine (referred to here as \( t_{TA} \)). It should be noted that this nomenclature was adopted solely for the current exercise.

There is conflicting evidence on the thermal stability of hyoscyamine and scopolamine and for the current assessment the concentration of \( t_{TA} \) in foods, as consumed, has been assumed to be the same as in the foods analysed prior to further processing, including cooking.

While WFP supplies food aid to the general population, three particular nutritional risk groups have been identified: pregnant and lactating women, children (5–15 years) and young children (6–59 months). Acute dietary exposure estimates were prepared for these sub-populations. With respect to dietary habit, pregnant and lactating women were represented by women of childbearing age (15–44 years).

**General diet**

For the assessment of acute dietary \( t_{TA} \) exposure from the general diet, information on the concentrations of hyoscyamine and scopolamine were extracted from the GEMS/Food Contamination database. Records recovered were almost entirely from countries in Europe (99 percent), with a small number from the Republic of Singapore (1 percent). Data were considered only from food samples for which analysis of both hyoscyamine and scopolamine had been carried out. Hyoscyamine and scopolamine were detected in only 9 and 6 percent of food samples analysed, respectively. For foods in which neither hyoscyamine nor scopolamine were detected, the concentration of \( t_{TA} \) was taken to be zero. Food types in which hyoscyamine and/or scopolamine were never detected were excluded from the analysis. The data set contained some negatives (not detected results) with very high limits of quantitation (LOQ). Currently available LC-MS/MS methods are able to achieve LOQs of 1 μg/kg or less. Analytical results with LOQs up to 10-fold higher than this were included in the analysis but results with LOQ >10 μg/kg were considered to be insufficiently sensitive to yield useful data.

Acute dietary exposure assessment requires food consumption information for individuals for individual consumption days, rather than consumption averaged over several days. Food consumption data meeting these requirements were available from the FAO/WHO Global Individual Food consumption Tool (GIFT). GIFT includes food consumption data for seven countries in which WFP is active; People’s Republic of Bangladesh, Burkina Faso, the Plurinational State of Bolivia (Bolivia), the Lao People’s Democratic Republic (Laos), the Republic of the Philippines, the Republic of Uganda, and the Republic of Zambia. Inconsistencies with the data set for Burkina Faso meant that no acute dietary exposure estimates were prepared for this country.
For most of the individual food consumption records, the body weight of the consumer was available. Where these data were missing, they were imputed as mean cohort values. The data set for the Republic of the Philippines, including data only for women of childbearing age, did not include body weights and a uniform default body weight of 60 kg was applied.

Acute dietary \( t \)TA exposures were determined by Monte Carlo simulation using the software platform Monte Carlo Risk Assessment. The simulations were run for 100 000 iterations, with each iteration randomly assigning a \( t \)TA concentration from the concentration database to each relevant food, summing the individual exposure contributions per day for each person and dividing by body weight.

Across all iterations, 84–97 percent of \( t \)TA exposure was due to hyoscyamine, depending on the country and sub-population group.

Mean acute dietary \( t \)TA exposures across countries and subpopulations were less than 1 ng/kg bw, except for the Republic of Zambia, where the means for young children and women were 18 and 4.6 ng/kg bw, respectively. High percentile acute dietary \( t \)TA exposures (95th percentile) were in the range 2.5–3.5 ng/kg bw, except for the Republic of Zambia, where the high percentile exposures were 38 and 10 ng/kg bw, for young children and women, respectively. It should be noted that the high percentile exposure for women from the Republic of Uganda could not be defined, as only 3.4 percent of the simulated acute exposure estimates were non-zero.

The dominant foods contributing to acute dietary \( t \)TA exposure in each country were all dietary staples; rice (People’s Republic of Bangladesh, the Lao People’s Democratic Republic, the Republic of the Philippines), corn (the Plurinational State of Bolivia, the Republic of Uganda, the Republic of Zambia), and sorghum (the Republic of Zambia).

**WFP products**

Data on the concentrations of hyoscyamine and scopolamine in Super Cereal (SC), Super Cereal plus (SC+) and lipid-based nutrient supplements (LNS) were available from WFP monitoring and limited supplier monitoring. For SC and SC+, data were available on products processed before the poisoning incident (retained samples) and products processed after the incident. Measures introduced during the intervening period included supplier monitoring of ingredients for tropane alkaloids, selection of specific raw material sources with low levels of tropane alkaloids and improved grain cleaning, to remove weed seeds.

Concentration data were also available for four samples of SC known to have caused illness, with mean and maximum \( t \)TA concentrations of 13 300 and 17 390 μg/kg, respectively. While these samples were excluded from the main analysis, for a SC consumption of 100 g and an adult body weight of 60 kg, these concentrations would equate to exposure doses of 22 000 and 29 000 ng/kg bw.
(22 and 29 μg/kg bw), respectively. For young children of 15 kg consuming 100 g of product, these doses would be 89 μg/kg bw and 116 μg/kg bw.

> As a high proportion (81 percent) of samples analysed contained hyoscyamine and/or scopolamine, it was considered likely that all samples contained hyoscyamine and/or scopolamine, albeit at an undetectable level in 19 percent of samples. Consequently, all analyses were performed considering two treatments of analytical results below the limit of detection (LOD); these values were assumed to be true zero values (lower bound) or the values were assumed to be equal to the LOD (upper bound). Due to the low level of left-censorship, there was little difference between lower and upper bound estimates of concentration and dietary exposures and results reported in the following sections are upper bound estimates.

> The mean concentrations of tTA in SC and SC+ before the incident were 12.8 and 14.5 μg/kg (maxima 216 and 96 μg/kg), respectively. After the incident, the mean concentrations were 3.1 and 1.9 μg/kg (maxima 8.0 and 8.7 μg/kg), respectively.

> Based on information from WFP, a hypothetical distribution was derived for consumption of SC or SC+ using the guideline level of consumption (100 g/day) as the most likely consumption amount, the maximum expected consumption (200 g/day) and a lower bound (minimum amount consumed) of half the guideline amount (50 g/day). These inputs were used to parameterise a distribution.

> Distribution of body weights for the three identified sub-populations were derived from GIFT data and represented by normal distributions. For young children, body weight data were available from Burkina Faso, the Lao People’s Democratic Republic and the Republic of Zambia; for children, from the Plurinational State of Bolivia, the Lao People’s Democratic Republic and the Republic of Zambia; and for women of childbearing age, from People’s Republic of Bangladesh, the Plurinational State of Bolivia, Burkina Faso, the Lao People’s Democratic Republic, the Republic of Uganda and the Republic of Zambia. Mean body weights for the three subpopulations were 12.7, 25.5 and 53.8 kg for young children, children and women of childbearing age, respectively.

> Acute dietary tTA exposure was determined by Monte Carlo simulation, using the Excel add-in @Risk. Simulations were run for 100 000 iterations.

> Across all iterations, 76–84 percent of tTA exposure was due to hyoscyamine, depending on the time frame (before or after the incident), the sub-population group and the treatment of left-censored data.

> Before the incident, mean acute dietary tTA exposures for young children (SC+), children (SC) and women (SC) were 130, 45 and 26 ng/kg bw, respectively, with 95th percentile estimates of 550, 220 and 120 ng/kg bw, respectively. After the incident, implementation of additional risk management measures (monitoring, raw material source selection and improved grain cleaning), the
Mean acute dietary TA exposures for the three sub-populations were 17, 11 and 6 ng/kg bw, respectively, with 95th percentile estimates of 54, 32 and 18 ng/kg bw, respectively.

> Lipid-based nutrient supplements (LNS) was found to be rarely contaminated with tropane alkaloids and, when contaminated, only at low TA concentrations (<2 μg/kg). Based on the main target population for this product (young children), LNS may result in a maximum acute dietary TA exposure of 6 ng/kg bw. LNS is provided as an alternative to SC or SC+, but not in addition to these products.

RISK CHARACTERIZATION AND RECOMMENDATIONS

> To provide guidance for the development of operational limits for hyoscyamine and scopolamine in WFP products, an MOE approach based on pharmacological effects in humans and acute dietary exposures was used in the risk characterization.

> For the general diet, compared to a clinically significant minimal acute effect dose of 1.54 μg/kg bw, MOEs for the general population (children and women of reproductive age) ranged from 3 080 to 3 850 (mean) and 440–616 (95th percentile) for combined exposures to hyoscyamine and scopolamine. These MOEs were not considered to be of concern by the expert meeting. For doses required to produce potentially adverse effects (e.g. increased heart rate, decreased saliva, dry mouth and sweat secretion and pupil dilation at 4.62 μg/kg bw), the MOEs would be three times greater.

> During the incident, the subpopulation consuming WFP products showed severe adverse anticholinergic effects at lower doses than would be expected in the general population; this difference in sensitivity was taken into account when considering the acceptability of the MOEs. It was recognised by the expert meeting that there is currently a lack of empirical data on which to characterize the full extent of the various factors that might contribute to increased sensitivity of populations consuming WFP products. Allowing for normal individual variability of 5-fold for Cmax dependent effects (WHO, 2016) and an additional 6-fold for the increased sensitivity of the target population, the expert meeting considered an MOE of 30 or greater to be of low concern for the target population. It was also noted that the point of departure was for a non-adverse response.

> For dietary exposures related to WFP products post incident, compared to a clinically significant minimal acute effect dose of 1.54 μg/kg bw, the MOEs ranged from 91 to 241 (mean) and 29 to 86 (95th percentile). Taking into account the sensitivity and variability between individuals, these MOEs are considered to be of low concern for the target population. The concentrations of hyoscyamine and scopolamine in WFP products detected after the incident and the absence of adverse effects in those consuming them, support the inference of low concern resulting from these MOEs.
Based on the recommended intake of various WFP products of 100 g/day, a combined hyoscyamine/scopolamine concentration in dry food of less than approximately 30 μg/kg (in SC)\(^2\) or 10 μg/kg (in SC+ and LNS)\(^3\) should be health protective for adults and children respectively. These concentrations are proposed as operational limits that may be extended to other cereal and grain products when consumed in comparable quantities. If higher quantities are consumed, appropriate adjustment of the values would be necessary.

For emergency situations where food security needs to be taken into consideration it would be expected that guidance levels of 90 μg/kg (SC)\(^4\) and 30 μg/kg (SC+ and LNS)\(^5\) should still be protective against severe toxicity for adults and children respectively. These emergency guidance levels were derived from a clinically significant minimal acute effect dose (i.e. based on increasing heart rate, decreased salivation and decreased sweat secretion).

It was further considered by the expert meeting that it would be difficult to define these proposed operational limits/guidance levels based on numbers of *Datura* seeds in grain used in the production of WFP products mainly due to the large variability in tropane alkaloid concentrations in the different *Datura* species.

\(^2\) Concentration = 1.54 μg/kg bw ÷ 30 * 60 kg bw (adult body weight) ÷ 0.1 kg of dry food.
\(^3\) Concentration = 1.54 μg/kg bw ÷ 30 * 20 kg bw (child body weight) ÷ 0.1 kg of dry food.
\(^4\) Concentration = 4.62 μg/kg bw ÷ 30 * 60 kg bw (adult body weight) ÷ 0.1 kg of dry food.
\(^5\) Concentration = 4.62 μg/kg bw ÷ 30 * 20 kg bw (child body weight) ÷ 0.1 kg of dry food.
Food distribution in Mozambique by WFP.
Woman prepares porridge with Super Cereal Plus in Nigeria.
1.1 BACKGROUND

Between March and April 2019, a large number of cases of suspected food poisoning were reported by health care workers in the Karamoja region of the Republic of Uganda. Approximately 300 suspected cases were reported, with main symptoms including fever (70 percent), confusion (55 percent); headache (46 percent); dry throat (32 percent), generalised body weakness (27 percent); vomiting (21 percent); epigastric pain (14 percent), diarrhoea (11 percent) and abdominal pain (7 percent). Five suspected cases died within secondary care, two of which were unverified (Karamoja Outbreak Investigation Report, 2019).

The cases were initially suspected to be associated with consumption of a product known as Super Cereal (Corn Soy Blend) which is part of an ongoing WFP Maternal and Child Health Programme. The Karamoja region is a beneficiary of the WFP, a relationship extending back to 1968. Super Cereal consists of maize (80 percent) and soybean (20 percent), which is heated, dried, and milled, with additional vitamin and mineral fortification prior to packaging. Following the identification of Super Cereal as the likely causative agent, the WFP stopped distribution of Super Cereal in Karamoja and initiated a public recall of any remaining product.

At the request of the WHO, from 15 to 17 April 2019 a clinical toxicology investigation was initiated, in support of the Government of the Republic of Uganda. Possible causative agents based on reported symptomatology included: acute mycotoxin and/or bacterial toxin poisoning, volatile organic solvents, pyrrolizidine/tropane alkaloids, or contamination with drugs or pharmaceuticals, such excessive iron or vitamin fortification.

Acute mycotoxin poisoning, specifically aflatoxicosis, was initially investigated but, although aflatoxin B1 was detected in most SC samples, the levels were compliant with many national standards and several thousand times below levels that cause acute toxicosis. Chemical analysis did not identify high levels of lead, cadmium, mercury, copper, arsenic, iron and zinc. In addition, pesticides - organochlorine/organophosphate – were also not identified.

Tropane alkaloid (TA) exposure was initial considered but classical anticholinergic symptoms such as dilated pupils and tachycardia were not consistently reported in
affected individuals. However, additional analysis of Super Cereal samples conducted in May 2019 by Merieux Nutrisciences, Queen’s University, Belfast, on behalf of WFP, and by the FDA’s Office of Regulatory Science, Centre for Food Safety and Applied Nutrition (CFSAN) in conjunction with the CDC, identified 14 TAs at parts per million (ppm or mg/kg) concentrations, including: hyoscyamine (reported as atropine) and scopolamine. Hyoscyamine and scopolamine concentrations ranged from 6.8 to 17.2 mg/kg and 0.85 to 2.46 mg/kg, respectively, in household samples recovered from the outbreak. The presence of several different TAs in addition to hyoscyamine and scopolamine suggested a botanical source of the contamination, while chloroplast DNA analysis of household samples identified the presence of *Datura stramonium* (Jimson weed) DNA. Subsequent investigation of suppliers of corn and soy used to produce Super Cereal identified the soy component as the likely source of the *D. stramonium* seed contamination (CWA International Ltd., 2019).

While there are currently no international regulations for limits of TAs applicable to WFP products, including Super Cereal, Codex STAN 153-1985 for maize notes that products covered by the provisions of this standard shall be free from a number of toxic or noxious seeds, including Jimson weed (*Datura spp.*), in amounts which may represent a hazard to human health. No related or similar Codex standard for soy or legumes has been developed.

### 1.2 REQUEST TO FAO/WHO FOR SCIENTIFIC ADVICE

On 26 November 2019, WFP requested FAO/WHO for scientific advice to allow the development of appropriate risk management options. In particular, the requested scientific advice to FAO/WHO entailed:

1. full risk assessment/evaluation of (–)-hyoscyamine, (+)-hyoscyamine and (–)-scopolamine.
2. guidance for the development of an operational limit for atropine and scopolamine in WFP products, namely SC, SC+, and LNS, taking into consideration both the food safety for WFP’s beneficiaries, as well as food security, ensuring availability of the right foods to fulfill WFP’s mandate;
3. operational limits for applicable grains, pulses and their derived products.

Immediately following the WFP request, FAO and WHO agreed to convene an ad hoc group of experts and published a call for data on occurrence, chemical, toxicological, epidemiological and clinical data for (+)-hyoscyamine, (–)-hyoscyamine and (–)-scopolamine to serve as inputs to the development of scientific advice to be provided through an expert consultation to take place on 30 March–3 April 2020 in Rome. The meeting, while originally planned as a physical meeting at FAO headquarters in Rome, was held remotely by an electronic platform due to the various global restrictions in place during the COVID-19 pandemic.

The meeting was opened by Dr Markus Lipp, from the Food Systems and Food Safety Division of FAO on behalf of the Director-General of FAO, and by Mr Kim Petersen on behalf of the Director-General of WHO. Dr Lipp welcomed
all participants to this remote meeting and expressed gratitude on behalf of both FAO and WHO that the experts to this meeting had been willing to continue to serve in their role even under the current difficult circumstances with regard to the on-going COVID-19 pandemic. It was emphasized that FAO and WHO invited the participants to the current expert meeting in their personal capacity as leading experts in their respective areas and not as representatives of a government or organization. He further noted that FAO and WHO expected this meeting and its experts to define the best scientific approaches and results for which the group can find consensus. Dr Lipp stressed the fact that the topic of this meeting is critical for the operations of WFP and encouraged the participants to take into consideration that any practical and implementable guidance for the ongoing operations of WFP is a desirable outcome. He posed the challenge to the expert meeting to consider developing two limits applicable to WFP products; a sufficiently health-protective limit suitable for checking compliance of raw and finished products and a upper threshold that would trigger the need for suitable follow-up actions if found that materials in the markets were to exceed such a limit. He continued by emphasizing that the deliberations of the meeting are of keen interest to other stakeholders as well, and that FAO and WHO intended to present the outcome of this expert meeting to the Codex Alimentarius Committee on Contaminants in Foods (CCCF) for consideration of developing a global standard. In closing, Dr Lipp urged the participants to focus on developing all possible conclusions using a holistic view of the available data, to provide the risk assessment with the highest utility for WFP and the Codex Alimentarius.
Figure on top: Datura stramonium plant showing flower and seedpods. Figure on bottom: D. stramonium seeds.
2.1 CHEMISTRY

Tropane alkaloids (TAs) are a group of alkaloids that contain a tropane skeleton, a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbon atoms. The TA-group comprises more than 200 compounds that are formed in plants from the esterification of tropine with a variety of acids, such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid and atropic acid.

(-)-Hyoscyamine and (-)-scopolamine are, respectively, esters of tropane-3α-ol and 6-7 epoxide of tropane-3α-ol with tropic acid. The asymmetric α-carbon of tropic acid allows the formation of two stereoisomers. Atropine is the racemic mixture of (±)-hyoscyamine. The structures of (-)-hyoscyamine (+)-hyoscyamine and (-)-scopolamine are presented on Table 1.

TAs are distributed in the Solanaceae family with numerous species of the genera Mandragora, Brugmansia, Duboisia, Hyoscyamus, Datura, Atropa, and Scopolia. The TA content differs according to the plant species and varies in the range of 0.01–3 percent (Yamada and Tabata, 1997; Georgiev et al., 2013). Many species from genera Datura, Brugmansia, Hyoscyamus, Scopolia, Atropa, and Duboisia accumulate the pharmacologically active (-)-hyoscyamine and scopolamine as the main compounds in their alkaloid profiles.

Hyoscyamine has similar modes of action and effects to scopolamine. The pharmacological action of TAs is stereoselective, due to the differing affinity and binding of the stereoisomers to muscarinic receptors. The S-(−) isomer of hyoscyamine is estimated to be 30 to 300-fold more potent than the R-(+) isomer (Kohnen-Johannsen et al., 2019).

Hyoscyamine is synthesized in the plant as the optically active S-(−) form and may undergo enantiomerisation over time. Therefore, both enantiomers of hyoscyamine are found in plants in varying ratios (Eich, 2008).
<table>
<thead>
<tr>
<th>NAME, IUPAC NAME, CAS AND SYNONYMS</th>
<th>CHEMICAL STRUCTURE</th>
<th>PHYSICOCHEMICAL PROPERTIES</th>
</tr>
</thead>
</table>
| Name: (-)-Hyoscyamine\(^a\) | ![Chemical Structure](image1) | MW: 289.37 g/mol  
Molecular formula: C\(_{17}\)H\(_{23}\)NO\(_3\)  
Solubility: slightly soluble in water; very soluble in alcohol, ether, chloroform and benzene  
Specific optical rotation, \([\alpha]_D^{20}\): -21.0 ° (in alcohol) |
| IUPAC name: (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (2S)-3-hydroxy-2-phenylpropanoate  
CAS: 101-31-5  
Synonyms: (L)-hyoscyamine, daturine |
| Name: (+)-Hyoscyamine\(^b\) | ![Chemical Structure](image2) | MW: 289.37 g/mol  
Molecular formula: C\(_{17}\)H\(_{23}\)NO\(_3\)  
Solubility: slightly soluble in water; very soluble in alcohol, ether, chloroform and benzene  
Specific optical rotation, \([\alpha]_D^{20}\): +31.3 ° |
| IUPAC name: (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (2R)-3-hydroxy-2-phenylpropanoate  
CAS: 101-31-5  
Synonyms: (D)-hyoscyamine, (R)-atropine, (+)-atropine |
| Name: Atropine (Racemic mixture of (+)-hyoscyamine and (-)-hyoscyamine) | ![Chemical Structure](image3) | MW: 289.37 g/mol  
Molecular formula: C\(_{17}\)H\(_{23}\)NO\(_3\)  
Solubility: slightly soluble in water; very soluble in alcohol, ether, chloroform and benzene  
Optically inactive. |
| IUPAC name: (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl 3-hydroxy-2-phenylpropanoate  
CAS: 51-55-8  
Synonyms: DL-hyoscyamine, tropine tropate |
| Name: (-)-Scopolamine\(^d\) | ![Chemical Structure](image4) | MW: 303.35 g/mol  
Molecular formula: C\(_{17}\)H\(_{21}\)N\(_O_4\)  
Solubility: slightly soluble in hot water; very soluble in alcohol, ether, chloroform and acetone  
Log P 0.98  
Specific optical rotation, \([\alpha]_D^{20}\): -28 ° |
| IUPAC name: (1R,2R,4S,5S)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0\(^2,4\)]nonan-7-yl (2S)-3-hydroxy-2-phenylpropanoate  
CAS: 51-34-3  
Synonyms: hyoscine, (-)-hyoscine, scopoline (-)-tropate |

2.2 ENANTIOMERISATION AND DEGRADATION

There are a small number of studies that report experimental data on the enantiomerisation of TAs. Much of the published research on enantiomerisation is in the context of the stability of pharmaceutical products containing hyoscyamine or scopolamine. Few of these studies appear to be relevant for the scenario of grain-based food preparation, as the experimental designs do not use grain matrices, or the experimental conditions are extremely alkaline, and unlikely to be encountered during preparation of grain-based foods.

The studies using pharmaceutical products showed that enantiomerisation and hydrolysis of tropic acid alkaloids depends upon the pH and temperature (Blaschke et al., 1993). These degradation reactions are proposed to occur through an enol intermediate, with the extent of hydrolysis dependent upon the base used (Rosenblum et al., 1995). Enantiomerisation of scopolamine-N-butyl bromide, as well as degradative hydrolysis, occurred in an alkaline buffer solution at pH 9.7 and 50 ºC (Bunke et al., 1996). The authors suggested racemisation could occur through formation of an enol catalyzed by hydroxide ions. It was verified that in aqueous solution the inversion of the (S)-(−) enantiomer to the (R)-(+) enantiomer and hydrolysis to tropic acid and corresponding tropane derivatives of (S)-(−)-hyoscyamine and (S)-(−)-scopolamine occurs. The enantiomerisation and hydrolysis rates increase with increasing pH (5 to 7) and temperature (41 ºC to 60 ºC) (Blaschke et al., 1993). However, the half-lives for hydrolysis and enantiomerisation of hyoscyamine and scopolamine enantiomers under these conditions ranged from 16 to 33 days, suggesting that, while relevant for pharmaceuticals, these degradation reactions will not be relevant for food products.

Mateus et al. (2000) examined the effect of extraction procedure on enantiomerisation of (−)-hyoscyamine in *Hyoscyamus albus* root extracts. They used liquid-solid extraction with sonication, liquid-solid extraction according to the Swiss Pharmacopeia, and supercritical fluid extraction. They found that only 2–8 percent of hyoscyamine was present as the (+) enantiomer after triplicate extractions of root by the three different extraction methods. The variability amongst triplicate extractions was very low (%RSD for all three extractions ranged from 2.50–2.59 percent) indicating that heterogeneity of root was not a confounding factor. Cieri (2005) also reported a mean enantiomeric fraction of 0.800 (n = 2) for (−)-hyoscyamine in commercially available belladonna extracts subject to unnamed processing. These results suggest that preparation of plant materials for food will not cause significant enantiomerisation of the biologically active (−)-hyoscyamine into the inactive (+) enantiomer.

Marin-Sáez et al. (2016) assessed the enantiomerisation of (−)-hyoscyamine in *D. stramonium* seeds at various temperatures and pH. Racemisation (i.e., the equilibration of a 1:1 mixture of enantiomers) only occurred at high pH (9) and temperature (80 ºC) after 3 days. Only 20 percent enantiomerisation of (−)-hyoscyamine to the (+) isomer was observed at pH 5 after 4 days. In addition, enantiomerisation of (−)-hyoscyamine observed was minimal (0.6–1.6 percent)
during their modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample extraction with 1 percent acetic acid in acetonitrile and clean-up with primary secondary amine and graphitized carbon black.

The published work most relevant to preparation of grain-based food products is that of Marin-Sáez et al. (2016) and Mateus et al. (2000), as they examined naturally occurring hyoscyamine in plant or seed extracts. The results from these papers suggest that enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine is unlikely to occur during preparation of grain-based foods, aside from perhaps during nixtamalization of maize, because of the extreme alkaline conditions required to promote enantiomerisation.

2.3 TROPANE ALKALOID BIOSYNTHESIS IN PLANTS

TAs are secondary metabolites occurring in Solanaceae and Erythroxylaceae and other plant families. Important TAs are hyoscyamine, (+)-hyoscyamine and scopolamine, which occur naturally. Naturally occurring TAs can be found in plants of the tribe **Datureae**, which contains the greatest range of TAs. *Datura* and *Brugmansia* are two genera that are recognised. *D. stramonium* (*D. tatula*) is an herbaceous species widely distributed in the warm regions of the world (Robins et al., 1997; Griffin and Lin, 2000). Other *Datura* species include *D. ferox* (the People’s Republic of China), *D. leichhardtii* (central Australia), *D. discolor* (W. Indies), *D. metel* (Asia), *D. wrightii* (the United States of America), and *D. quercifolia*, *D. pruinosa* and *D. innoxia* (the United Mexican States) (Griffin and Lin, 2000).
**D. stramonium, D. ferox and D. innoxia** are known to have high levels of TAs in most parts of the plants. According to Hashimoto et al. (1991), TAs are synthesized in young root cells and translocated to the aerial parts of the plant.

Studies on the biosynthesis of TAs have generally encompassed plants belonging to the Solanaceae family and, to a lesser degree, on cultivated species of Erythroxylaceae.

In the biosynthesis of TAs, enzymatic processes give rise to the tropane moiety, which involves phytochemical precursors. Precursors found in Solanaceae plants include ornithine (Sneader, 2005), N-methyl ornithine (Holmes and Manske, 1961) and 1,4-butadiamine (Saxton, 1965). The intermediate N-methylpyrrolinium cation of TAs is bio-synthesized by three enzymes: ornithine decarboxylase (Docimo et al., 2012), putrescine N-methyltransferase (Hashimoto et al., 1989, Biastoff et al., 2009) and N-methylputrescine oxidase (Katoh et al., 2007).

Biosynthesis of the first of the two rings of TAs (tropane moiety) starts from polyamine putrescine, which is derived from ornithine or arginine. Thereafter, putrescine is N-methylated via the action of putrescine methyltransferase. This is an essential step in TA production (Hashimoto et al., 1994) and the compound formed is oxidized to give rise to 4-(methyl-1-amino) butanal which is believed to re-arrange into the five-membered–ring compound N-methyl-1-pyrrolinium (Jirschitzka et al., 2012; Kohnen-Johannsen et al., 2019) which is converted to tropinone and finally tropine (Zhang et al., 2007).

Li et al. (2006) have also described two different biosynthetic routes giving rise to (-)-hyoscyamine from (R)-littorine. Esterification of tropine with (R)-phenyllactate (from phenylalanine) gives rise to (R)-littorine. Yun et al. (1992) and Zhang et al. (2007) noted that synthesis of (-)-scopolamine from (-)-hyoscyamine involves hyoscyamine 6-β–hydroxylase (6H6). The steps involved and described by Kohnen-Johannsen et al. (2019) in the biosynthesis of TAs are shown in Figure 1.

The formation of the second TA ring of is not well understood according to Jirschitzka et al. (2012). However, in the biosynthetic pathway of TAs, tropinone (Kitamura et al., 1992) is reduced by reductase to tropine (Jirschitzka et al., 2012). With respect to tropinone biosynthesis, Bedewitz et al. (2018) found that the key intermediate 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoic acid (Humphrey et al., 2001) results from an atypical type III polyketide synthase AbPYKS-catalysed condensation between N-methylpyrrolinium cation and malonyl-CoA, while tropinone is formed by a P450-enzyme AbCYP82M3-mediated oxidation and cyclization of racemic 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoic acid. Tropinone synthesis according to Bedewitz et al. (2018) is via a polyketide synthase and P450-mediated cyclization.
FIGURE 1. SCOPOLAMINE BIOSYNTHESIS, STARTING WITH THE $N$-METHYLPYRROLINIUM CATION.

$N$-Methylpyrrolinium cation $\rightarrow$ PYKS $\rightarrow$ 4-(1-Methyl-2-pyrrolidinyl)-3-oxo-butenoic acid $\rightarrow$ CYP82M3 $\rightarrow$ Tropinone. TR-I/II $\rightarrow$ TR-I/II $\rightarrow$ Littorine synthase $\rightarrow$ Littorine $\rightarrow$ Littorine mutase $\rightarrow$ Hyoscyamine aldehyde $\rightarrow$ H6H $\rightarrow$ Hyoscyamine. H6H $\rightarrow$ Scopolamine.

PYKS = polyketide synthase. CYP82M3 = cytochrome P450 enzyme. TR-I/II = tropinone reductase I/II. Littorine synthase (sequence not known). H6H = hyoscyamine 6β-hydroxylase.

Source: Kohnen-Johannsen et al., 2019.
2.3.1 DISTRIBUTION AND OCCURRENCE OF TROPANE ALKALOIDS IN PLANTS

TAs have been measured in a variety of genera belonging to the Solanaceae family, including *Brugmansia*, *Datura*, and *Hyoscyamus*. Tables 2 and 3 list reports of scopolamine and hyoscyamine concentrations quantified in seeds and other plant tissues from various species in the Solanaceae family. The breadth of source locations in North America, Europe, the Middle East, Asia, and Africa highlights the global occurrence of TAs in plants. For example, *D. stramonium* is widely distributed in temperate and tropical regions of the world and the seeds have been reported to have contaminated linseed, soybean, millet, sunflower and buckwheat (EFSA, 2013).

Reported concentrations range from 12 mg/kg (Aga and Geyid, 1992; Miraldi et al., 2001; Onen et al., 2002) to 7 093 mg/kg (Zayed et al., 2006) scopolamine and less than the detection limit (Naude et al., 2005) to 4 300 mg/kg (List et al., 1979) hyoscyamine in seeds from various *Brugmansia*, *Datura* and *Hyoscyamus* species (Table 2). Concentrations of these TAs were also measured in other plant tissues including leaves, seedlings, flowers, stems, pericarp and roots (Table 3). The range of reported concentrations is similar to the range of concentrations reported in seeds; scopolamine ranged from less than detection limit (Miraldi et al., 2001) to 7 000 mg/kg (Hiraoka et al., 1996) and hyoscyamine ranged from less than detection limit (Hiraoka et al., 1996) to 5 910 mg/kg (Jakabová et al., 2012). Many publications also qualitatively note the presence of TAs in various species and plant tissues. For example, Hall et al. (1977) and Erhardt et al. (2008) reported the presence of (-)-hyoscyamine and (-)-scopolamine in the flowers of *Datura suaveolans*.

Many factors have been reported to affect concentrations and proportions of TAs in seeds and other plant tissues, including growing location, plant maturity, plant tissue, as well as species and variety (Adamse et al., 2010; EFSA, 2013). Proportions of TAs differed in *D. innoxia* grown in the Arab Republic of Egypt and the Republic of Bulgaria (Philipov and Berkov, 2002; Berkova and Zayed, 2004; Zhang et al., 2007). Concentrations of scopolamine and hyoscyamine varied in *D. stramonium* seeds obtained from seven different locations in the United States of America (Friedman and Levin, 1989). The coefficient of variation of mean scopolamine and hyoscyamine concentrations across the different locations were 24 percent and 16 percent, respectively.

The noted differences amongst samples from different geographical locations is likely due to a combination of factors related to growing conditions. *D. stramonium* grown in areas with less precipitation in the United Mexican States were found to contain lower amounts of hyoscyamine (Miranda-Pérez et al., 2016). Baricevic et al. (1999) also reported that hyoscyamine and scopolamine concentrations in deadly nightshade (*Atropa belladonna*) decreased in dry environments.
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>SCOPOLAMINE</th>
<th>HYOSCYAMINE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SOURCE OF PLANT</th>
<th>ANALYTICAL TECHNIQUES</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brugmansia arborea</td>
<td>-</td>
<td>(+) 18, (-) 327</td>
<td>Spain</td>
<td>LC-MS/MS</td>
<td>Marín-Sáez et al., 2016</td>
</tr>
<tr>
<td>Datura ferox</td>
<td>700–1 200</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>South Africa</td>
<td>LC-UV</td>
<td>Naude et al., 2005</td>
</tr>
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<td>840–1 250</td>
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<td>Berkov, 2001</td>
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<td>Mroczek et al., 2006</td>
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<td>3 522</td>
<td>Egypt</td>
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<td>500–1 000</td>
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<td>Dugan et al., 1989</td>
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<td>Datura stramonium</td>
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<td>1 900–4 300</td>
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</table>

<sup>a</sup> Publication did not differentiate between hyoscyamine stereoisomers, referred to analyte as “hyoscyamine”, or referred to analyte as “atropine” without further definition.

<sup>b</sup> Not determined.

<sup>c</sup> Not reported.
<table>
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<th>SPECIES</th>
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<th>ANALYTICAL TECHNIQUE</th>
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<td>30</td>
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<tr>
<td><em>Datura innoxia</em></td>
<td>flowers</td>
<td>214</td>
<td>395</td>
<td>Egypt</td>
<td>GC-MS</td>
<td>Zayed et al., 2006</td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>stems</td>
<td>1 950</td>
<td>400</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>stems</td>
<td>356</td>
<td>177</td>
<td>Egypt</td>
<td>GC-MS</td>
<td>Zayed et al., 2006</td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>leaves</td>
<td>940–4 530</td>
<td>20–60</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura innoxia</em></td>
<td>leaves</td>
<td>70–3 244</td>
<td>ndc</td>
<td>Egypt</td>
<td>GC-MS</td>
<td>Zayed et al., 2006</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>flowers</td>
<td>2 000–7 000</td>
<td>nd–1 000</td>
<td>Japan</td>
<td>GC-FID</td>
<td>Hiraoka et al., 1996</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>flowers</td>
<td>450</td>
<td>2450</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>flowers</td>
<td>83–216</td>
<td>103–199</td>
<td>India</td>
<td>TLC</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>stem</td>
<td>10–3420</td>
<td>190–510</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>leaves</td>
<td>840.4</td>
<td>69.87</td>
<td>Poland</td>
<td>GC-MS</td>
<td>Ciechomska et al., 2016</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>leaves</td>
<td>460–2 530</td>
<td>nd–400</td>
<td>Japan</td>
<td>GC-FID</td>
<td>Hiraoka et al., 1996</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>leaves</td>
<td>10–280</td>
<td>70–1 430</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>leaves</td>
<td>1 110</td>
<td>904</td>
<td>Poland</td>
<td>HPLC-DAD</td>
<td>Mroczek et al., 2006</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>fruit + seeds</td>
<td>3 440</td>
<td>530</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura metel</em></td>
<td>roots</td>
<td>111–160</td>
<td>337–577</td>
<td>India</td>
<td>TLC</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>flowers</td>
<td>1 360</td>
<td>1 690</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura stramonium</em>  var. <em>tatula</em></td>
<td>flowers</td>
<td>2 740</td>
<td>3 970</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>flowers</td>
<td>106</td>
<td>299</td>
<td>Italy</td>
<td>GC-MS</td>
<td>Miraldi et al., 2001</td>
</tr>
<tr>
<td><em>Datura stramonium</em>  var. <em>tatula</em></td>
<td>stem</td>
<td>20–3 320</td>
<td>360–5 510</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura stramonium</em>  var. <em>tatula</em></td>
<td>stem</td>
<td>2 130–2 510</td>
<td>4 730–5 910</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td>SPECIES</td>
<td>MATRIX</td>
<td>SCOPOLAMINE (mg/kg)</td>
<td>HYOSCYAMINEA (mg/kg)</td>
<td>SOURCE OF PLANT</td>
<td>ANALYTICAL TECHNIQUE</td>
<td>REFERENCE</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>-----------------</td>
<td>-----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>stem</td>
<td>129</td>
<td>915</td>
<td>Italy</td>
<td>GC-MS</td>
<td>Miraldi et al., 2001</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>leaves</td>
<td>130–1 160</td>
<td>1 450–1 910</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura stramonium var. tatula</em></td>
<td>leaves</td>
<td>230–1 790</td>
<td>1 070–4 710</td>
<td>Hungary</td>
<td>UHPLC-MS</td>
<td>Jakabová et al., 2012</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>leaves</td>
<td>35–73</td>
<td>156–831</td>
<td>Italy</td>
<td>GC-MS</td>
<td>Miraldi et al., 2001</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>leaves</td>
<td>96–4 100</td>
<td>28–3 030</td>
<td>Mexico</td>
<td>HPLC-DAD</td>
<td>Miranda-Pérez et al., 2016</td>
</tr>
<tr>
<td><em>Datura stramonium var. tatula</em></td>
<td>leaves</td>
<td>713</td>
<td>658</td>
<td>Poland</td>
<td>HPLC-DAD</td>
<td>Mroczek et al., 2006</td>
</tr>
<tr>
<td><em>Datura stramonium var. stramonium</em></td>
<td>leaves</td>
<td>713</td>
<td>658</td>
<td>Poland</td>
<td>HPLC-DAD</td>
<td>Mroczek et al., 2006</td>
</tr>
<tr>
<td><em>Datura stramonium var. godronii</em></td>
<td>leaves</td>
<td>571</td>
<td>427</td>
<td>Poland</td>
<td>HPLC-DAD</td>
<td>Mroczek et al., 2006</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>leaves</td>
<td>229</td>
<td>1 654</td>
<td>Poland</td>
<td>HPLC-DAD</td>
<td>Mroczek et al., 2006</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>pericarp</td>
<td>nd</td>
<td>1</td>
<td>Italy</td>
<td>GC-MS</td>
<td>Miraldi et al., 2001</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>roots</td>
<td>14</td>
<td>121</td>
<td>Italy</td>
<td>GC-MS</td>
<td>Miraldi et al., 2001</td>
</tr>
</tbody>
</table>

* Publication did not differentiate between hyoscyamine stereoisomers, referred to analyte as “hyoscyamine”, or referred to analyte as “atropine” without further definition.

b not analysed;

c not detected.
In addition to localized differences in TA concentrations within plants, TAs are differentially distributed in plants. Berkov et al. (2005) reported that the roots of *D. stramonium* contained a more complex mix of alkaloids as compared to the leaves, stems and fruits. Phillipson and Handa, (1975), found that in *D. stramonium* and other plant species, N-oxides of hyoscyamine were present in several parts of the plant. Many studies have observed changes in TA proportions and concentrations during plant development. Proportions of different alkaloid structural families varied in tissues from newly emerged to mature *D. stramonium* (Berkov et al., 2005; Iranbakhsh et al., 2006). TAs in *D. stramonium* reached their maximum concentration 10 weeks after germination, then decreased (Iranbakhsh et al., 2006). Jakabová et al. (2012) reported that concentrations of scopolamine and hyoscyamine were higher in leaves and stems collected from *D. innoxia*, *D. metel* and *D. stramonium* in the summer as compared to the fall. Differences in concentrations ranged from two to ten times. Higher concentrations of scopolamine and hyoscyamine were also noted in leaves from younger *D. stramonium* plants by Miraldi et al. (2001).

TA occurrence and concentrations have also been noted to vary amongst species and variety within a species. Hyoscyamine was most abundant in *D. stramonium* var. *tatula*, and the least in *D. innoxia*, grown in the same location in Hungary (Iranbakhsh et al., 2006). Others have reported differences in TA concentrations amongst varieties, but growing location and conditions are likely confounding factors (Sharma et al., 2009).

### 2.4 Contamination of Grains with Solanaceae Seeds

TA contamination of foods and feed can occur when plant tissues containing TAs are accidentally mixed with edible plants during harvest or processing. Numerous examples have been reported, for example the mixing of *Datura* flower buds with canned beans in France and the contamination of buckwheat meant for human consumption (Adamse et al., 2010).

However, as opposed to other tissues, seeds from *Brugmansia, Datura* and *Hyoscyamus* species, are the likeliest materials to contaminate grains (and subsequently grain-based foods) because their density, size, and shape are similar to those of grains (Table 4). The relatively high concentrations of TAs reported for seeds (Hiraoka et al., 1996; Miraldi et al., 2001; Sharma et al., 2009) also reinforce the importance of this plant material as a major source of TA contamination of foods. There are some online resources providing morphological information and pictures of many TA-containing plants and their tissues, including the Centre for Agriculture and Biosciences International’s “Invasive Species Compendium”, the Canadian Food Inspection Agency’s “Seed Identification” and the International Seed Testing Association’s Purity Committee “Universal List of Species”.

---

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>COMMON NAME</th>
<th>SEED LENGTH × WIDTH (mm)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TA-containing seeds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>jimsonweed</td>
<td>2.5–4.0 × 2.5–3.0</td>
<td><a href="https://www.inspection.gc.ca/plant-health/seeds/seed-testing-and-grading/seeds-identification/datura-stramonium/eng/1476290557484/1476290557859">https://www.inspection.gc.ca/plant-health/seeds/seed-testing-and-grading/seeds-identification/datura-stramonium/eng/1476290557484/1476290557859</a></td>
</tr>
<tr>
<td><em>Brugmansia suaveolens</em></td>
<td>white angel’s trumpet</td>
<td>4 × 6</td>
<td><a href="https://www.cabi.org/isc/datasheet/107903">https://www.cabi.org/isc/datasheet/107903</a></td>
</tr>
<tr>
<td><em>Hyoscyamus niger</em></td>
<td>black henbane</td>
<td>1.5 (length)</td>
<td><a href="https://www.cabi.org/isc/datasheet/28251">https://www.cabi.org/isc/datasheet/28251</a></td>
</tr>
<tr>
<td><strong>Food grains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>soybean</td>
<td>6–11 × × 5–8</td>
<td><a href="http://www.seedtest.org/upload/cms/user/UniversalListofSpecies20142">http://www.seedtest.org/upload/cms/user/UniversalListofSpecies20142</a></td>
</tr>
<tr>
<td><em>Fagopyrum esculentum</em></td>
<td>buckwheat</td>
<td>2–4 × 4–6</td>
<td><a href="https://hort.purdue.edu/newcrop/Crops/Buckwheat.html">https://hort.purdue.edu/newcrop/Crops/Buckwheat.html</a></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>wheat</td>
<td>5–9 × 1.8–4.5</td>
<td><a href="https://www.seedtest.org/upload/cms/user/Triticumaestivum.pdf">https://www.seedtest.org/upload/cms/user/Triticumaestivum.pdf</a></td>
</tr>
</tbody>
</table>
Legislation in the Republic of South Africa has stipulated allowable limits of *Datura* seed in grain for human consumption (Naude *et al.*, 2005). Seeds of *Datura* species appear morphologically indistinguishable from each other (Friedman and Levin, 1989), therefore the authors have proposed that a genus name rather than species should be used when characterizing the presence of weed seeds containing TAs. The South African legislation limited the presence of *Datura* seeds to 1 seed in 10 kg of maize, 3 seeds in 400 g ground nuts, and 5 seeds in 400 g soybeans. It is not clear if this legislation is still in force.

There have been numerous reports of seeds from TA-producing species contaminating food grains. Jimsonweed seeds (*D. stramonium*) were identified in soybean samples from the 1984 harvest in the United States of America (Dugan *et al.*, 1989). An earlier survey in the country reported the frequent occurrence of jimsonweed seeds in soybeans collected from across the nation (List *et al.*, 1979). Seeds separated from soybeans by passing samples through a series of slotted sieves (3/4”, 10/64”, 9/64”, 8/64”) were identified by comparison to authentic jimsonweed seeds. Of the 274 truck, rail car, or vessel bulk soybeans shipments sampled and tested, 61 percent were found to contain jimsonweed seeds. List *et al.* (1979) report that 50 percent of samples from truck and rail cars contained jimsonweed seeds, followed by 66 percent of vessel export samples, and 68 percent of samples taken from other unspecified sources. On average, the soybean samples inspected contained 28.4 seeds/kg or 0.022 percent by mass, with 22.6 seeds/kg noted for export samples as compared to 30.7 seeds/kg for other soybean samples. The average seed contamination was similar to results reported for an earlier study (List and Spencer, 1976). The authors did not determine if the apparent differences in occurrence of jimsonweed seeds amongst the different conveyances were significant or not.

Over the past decade, bulk exports of various food grains from Canada have been inspected for the presence of various weed seeds. *D. stramonium* was only observed twice out of over 7 100 samples analysed (Blaine Timlick, Canadian Grain Commission, personal communication, 2020).

### 2.5 DISTRIBUTION AND OCCURRENCE OF TROPANE ALKALOIDS IN FOODS

The concentrations of hyoscyamine and (-)-scopolamine in some food products of plant origin are shown in Table 5. The concentration of hyoscyamine in the analysed food samples was expressed as “atropine”. Atropine is defined as the racemic mixture (1:1) of (-)-hyoscyamine and (+)-hyoscyamine. However, the analytical methods used did not measure each enantiomer of hyoscyamine in the samples separately. Therefore, taking into account the potential variability of the enantiomeric fraction, the expert meeting considered it more accurate to express the results as the sum of (-)-hyoscyamine and (+)-hyoscyamine, instead of as “atropine”.

In a study reported by Mulder *et al.* (2016), 1 709 samples of plant-derived food products, mainly produced in Europe and collected in nine European countries,
<table>
<thead>
<tr>
<th>FOOD CATEGORY</th>
<th>SAMPLES COLLECTED AND ANALYSED</th>
<th>ALL TAs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HYOSCYAMINE + SCOPOLAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PERCENT OF SAMPLES &gt; LOD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MEAN CONC. (µg/kg)</td>
<td>MAX CONC. (µg/kg)</td>
</tr>
<tr>
<td>Single component flours</td>
<td>268</td>
<td>21.3%</td>
<td>3.11</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>113</td>
<td>9.7%</td>
<td>2.69</td>
</tr>
<tr>
<td>Millet and sorghum</td>
<td>102</td>
<td>23.5%</td>
<td>5.15</td>
</tr>
<tr>
<td>Corn and others</td>
<td>53</td>
<td>20.8%</td>
<td>0.07</td>
</tr>
<tr>
<td>Cereals available at retail stores</td>
<td>838</td>
<td>14.0%</td>
<td>0.30</td>
</tr>
<tr>
<td>Bread and pasta</td>
<td>195</td>
<td>9.2%</td>
<td>0.06</td>
</tr>
<tr>
<td>Bread</td>
<td>114</td>
<td>15.8%</td>
<td>0.10</td>
</tr>
<tr>
<td>Pasta</td>
<td>81</td>
<td>0.0%</td>
<td>0.00</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>219</td>
<td>6.8%</td>
<td>0.63</td>
</tr>
<tr>
<td>Biscuits and pastry</td>
<td>164</td>
<td>14.6%</td>
<td>0.14</td>
</tr>
<tr>
<td>Biscuits</td>
<td>150</td>
<td>14.7%</td>
<td>0.14</td>
</tr>
<tr>
<td>Pastry</td>
<td>14</td>
<td>14.3%</td>
<td>0.15</td>
</tr>
<tr>
<td>Cereal-based foods for children</td>
<td>260</td>
<td>20.0%</td>
<td>9.49</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>135</td>
<td>13.3%</td>
<td>0.16</td>
</tr>
<tr>
<td>Cookies</td>
<td>107</td>
<td>13.1%</td>
<td>0.85</td>
</tr>
<tr>
<td>Pasta and cereal-based meals</td>
<td>18</td>
<td>55.6%</td>
<td>130.7</td>
</tr>
<tr>
<td>Other products available at retail store</td>
<td>199</td>
<td>52.3%</td>
<td>73.35</td>
</tr>
<tr>
<td>Dry (herbal) teas</td>
<td>121</td>
<td>70.2%</td>
<td>71.38</td>
</tr>
<tr>
<td>Legumes, stir-fry mixes, oil, seeds</td>
<td>78</td>
<td>24.4%</td>
<td>76.42</td>
</tr>
<tr>
<td>Legumes, stir-fry mixes</td>
<td>65</td>
<td>26.2%</td>
<td>91.70</td>
</tr>
<tr>
<td>Oil seeds</td>
<td>13</td>
<td>15.4%</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>1 305</td>
<td>22.5%</td>
<td>12.90</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analysis of 24 TAs, including hyoscyamine and scopolamine.

<sup>b</sup> Products containing at least one TA above the LOD. LOD differs between components, matrices and laboratories.

Source Mulder et al., 2016.
were analysed for TAs. Food samples comprised 268 single component flours from buckwheat, millet and corn, 260 cereal-based foods for young children age 6–36 months (breakfast cereals, biscuits and other cereal-based foods), 219 breakfast cereals, 164 biscuits and pastry, 114 bread, 81 pasta, 121 dry (herbal) teas, 65 legumes and stir-fry mixes. One or more TAs were detected in 21.3 percent of single component flours, 20 percent of cereal-based food for young children age 6–36 months, 15.8 percent of bread, 26.2 percent of legumes and stir-fry mixes, and 14.6 percent of biscuits. Due to the large number of samples and broad scope of sampled food matrices, this is the most significant study currently available on TA levels in food, even though samples were obtained only from markets in European countries. The EU (2016) has set maximum limits for the presence of TAs of 1.0 µg/kg for atropine and 1.0 µg/kg for scopolamine, respectively, in certain cereal-based foods.

Marín-Sáez et al. (2019a) analysed 18 cereal-based baby food samples, with one sample found to contain hyoscyamine (11.5 µg/kg), scopolamine (2.8 µg/kg) and apotropine (7.5 µg/kg).

2.6 Sampling for Tropane Alkaloid Analysis

The contamination of grains and foods by TAs occurs due to the presence of TA-containing plant materials; in particular, by the presence of TA-containing seeds, as described above. This mode of contamination leads to discrete points of high TA concentrations, rather than an even distribution of contamination in the bulk commodity or food. The heterogeneity from point sources of contamination can be amplified by the variability of TA concentrations in plant material from different sources. The heterogeneity of plant materials with respect to TA concentration is illustrated by the coefficients of variation (CVs) ranging from 40–115 percent for scopolamine in D. metel leaves from multiple plants of the same variety grown in the same location (Hiraoka et al., 1996). In seeds, the CVs for scopolamine ranged from 15 to 43 percent. Similar high variation in TA concentrations amongst similar plant materials has been reported in literature (List et al., 1979; Sharma et al., 2009; Miranda-Perez et al., 2016).

This heterogeneity is comparable to that observed for contamination by certain mycotoxins (e.g. ergot alkaloids, aflatoxins, ochratoxin A), which leads to challenges when sampling for contaminant analysis (Whitaker 1977, Grusie et al., 2017). To mitigate the effect of heterogeneity on the variance of TA test results, the same principles used in mycotoxin analysis can be applied to the analysis of TAs.

Firstly, the use of sampling and dividing equipment that gives every component of the bulk sample an equiprobable chance of being selected, without discrimination due to size, density, location in bulk sample, etc., will avoid bias and help ensure the sample taken for analysis is representative of the bulk. The International Association for Cereal Science and Technology sampling method for cereal grains (ICC, 2018), Canadian Grain Commission Sampling Systems Handbook and Approval Guide (CGC, 2015), United States of America Department of Agriculture Grain Inspection Handbook (FGIS, 2006) and the Equipment Handbook (FGIS, 2016),
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and the International Seed Testing Association Rules for Seed Testing and Sampling Handbook describe proper sampling and dividing equipment, as well as their proper use, for grains.

After the selection of proper equipment, there are a number of sampling and sample preparation strategies to reduce the variance of test results due to sample heterogeneity. The first involves taking multiple, equally sized samples from the bulk commodity and combining them into a composite sample. Another strategy is to comminute the entire composite sample prior to any further sub-sampling and analysis. An additional strategy is to increase composite sample and test portion (the portion of sample extracted and analysed for TAs) size. Since the sampling, sample preparation, and analytical variance has not been characterized for the analysis of TAs in grains or foods, laboratories will have to determine the sample sizes and degree of comminution that best suit their needs, balancing the resources required to handle and process larger samples with the reduction in test result variance and uncertainty.

2.7 ANALYTICAL METHODS FOR TROPANE ALKALOIDS IN FOOD – HYOSCYAMINE AND SCOPOLAMINE

Various analytical methods have been reported for the determination of TAs in a variety of samples, including foods, pharmaceuticals, plants and biological fluids. Gas chromatography-mass spectrometry, liquid-chromatography with different detection systems (UV, mass spectrometry, tandem mass spectrometry), capillary electrophoresis, enzyme-linked immunosorbent assay (Friedman and Levin, 1989) and thin layer chromatography (Jaremicz et al., 2014) have been widely used. However, only a few methods are adequate for the determination and monitoring of residues of TAs in food due to the complex matrix and the typically low concentrations of the analytes. In addition, most methods cannot distinguish between enantiomers of TAs. In general, for dietary exposure assessment, multi-residue methods have been employed.

Hyoscyamine and scopolamine are the Datura-type TAs most frequently detected in food at the highest concentrations, in particular in cereals and cereal-based products (Marin-Sáez et al., 2019b), herbs and herbal teas (Romera-Torres et al., 2018), dietary supplements and honey (Martinello et al., 2017). Other Datura-type TAs included in multi-residue methods reported in the literature are O-acetylscopolamine, anisodamine, anisodine, apotropine, aposcopolamine, homatropine, 2a-hydroxymethyl atropine, littorine, noratropine, norscopolamine and phenylacetoxytropane. Besides these analytes, low molecular weight TAs and Convolvulaceae-type TAs are also included in multi-residue methods.

Considering the physicochemical properties of hyoscyamine and scopolamine and the sample matrix, liquid chromatography coupled to tandem or high-resolution mass

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spectrometry is nowadays the state-of-art analytical technique. For the separation of the enantiomers of hyoscyamine, chiral columns are required, and there are limited methods available for the determination of these enantiomers in food.

Sample matrix effects may cause difficulties meeting relative intensity tolerances for identity confirmation using mass spectrometry. Therefore, clean-up steps in the sample preparation procedures need to be included, and quantitation should be carried out using a matrix-matched calibration curve or isotopically labelled standards.

2.7.1 SAMPLE PREPARATION

Diverse sample preparation procedures for the extraction of TAs from food and plant matrices and clean-up procedures are described in the literature. Grain and seed are complex matrices, rich in sugars, fat, proteins and pigments, which may interfere with the quantitation of TAs (Jandric et al., 2011) and therefore, should be removed during the sample preparation stage.

TAs contain a tertiary amine in their structure, which is protonated in acidic medium. The protonated species of TAs are more water-soluble in comparison to their free bases and this property allows a selective extraction of the analytes from the matrix with the exclusion of lipophilic compounds. Therefore, solid-liquid extraction with mixtures of water and polar organic solvents has enabled selective extraction of the alkaloids in the presence of lipophilic compounds. The addition of formic acid to the solvent enhances the extraction efficiency, whereas in alkaline media, hydrolysis of the ester alkaloids occurs (Dräger, 2002).

Jandric et al. (2011) extracted TAs and glycoalkaloids from ground (≤1 mm) wheat using dispersive solid-phase extraction. A mixture of 0.5 percent formic acid in acetonitrile:water 75:25 v/v with a salt mixture of 2 g of magnesium sulfate, 0.5 g of sodium chloride, 0.5 g of disodium hydrogen citrate sesquihydrate and 0.25 g of trisodium citrate dihydrate are added to the samples. The salts enhance phase separation, resulting in a larger extract volume. The mixtures are shaken and centrifuged (2 600 g, 4 ºC, 10 minutes). Aliquots (8 mL) of the extract are evaporated under a stream of nitrogen at 45 ºC. Extraction efficiencies were in the range of 78 to 106 percent. No further clean-up step is required for wheat, rye and maize. However, for soybean and linseed, the extracts were fatty, and it was necessary to include a further clean-up step. The addition of 0.05 g/mL silica based C18 sorbents allowed a rapid purification of the extracts.

Hyoscyamine and scopolamine were extracted from D. stramonium, D. metel and D. innoxia using a mixture of water:methanol 40:60 v/v with efficiencies in the range of 95 to 101 percent (Jakabova et al., 2012). Temerdashev et al. (2012) verified that the most complete extraction of TAs in D. metel is achieved using a of 0.1 mol/L hydrochloric acid: 70 percent ethanol (1:1, v/v) and ultrasonication.

TAs were extracted from cereals and cereal-based products with 0.4 percent aqueous formic acid:methanol 40:60 v/v reaching extraction efficiencies from 86 to 91 percent (Mulder et al., 2015). A 4 g test portion is weighed and extracted with 40 mL
of solvent. The suspension is shaken for 30 minutes and then centrifuged at 3 500 g for 15 min. An aliquot of 10 mL of the extract is purified by solid-phase extraction (OASIS or Strata-X cartridges).

A non-aqueous solid-phase extraction method using silica based strong cation exchange has been successfully employed for the enrichment of TAs and for the determination of hyoscyamine and scopolamine in extracts from *Scopolia tanguitica* by HPLC-DAD and LC-MS (Long *et al.*, 2012).

A microwave-assisted extraction followed by QuEChERS dispersive solid-phase extraction was used for the determination of hyoscyamine and scopolamine in *Datura* genera (leaves and seeds) by GC-MS (Ciechomska *et al.*, 2016). The sample preparation procedure was optimized by the Doehlert uniform shell design and the response surface methodology.

Alberts *et al.* (2018) extracted TAs from African *Erythroxylum* trees using pressurized liquid extraction (PLE) and quantified the analytes by GC-MS. Atropine was identified in the leaves of *E. emarginatum* for the first time.

### 2.7.2 Quantitation of Hyoscyamine and Scopolamine

**Gas chromatography-mass spectrometry**

Methods reported in the literature using gas-chromatography focus mainly on the identification of TAs in plants (Philipov and Berkov, 2002, El Bazaoui *et al.*, 2011, El Bazaoui *et al.*, 2012, Sramska *et al.*, 2017), and their determination in biological matrices (Namera *et al.*, 2002). Gas-chromatography coupled to mass spectrometry (GC-MS) is a powerful technique when a fast and reliable identification of a wide range of possible compounds is necessary in intoxications, however it is not the technique of choice for the determination of TAs in food.

Although the determination of TAs can be carried out without derivatization, since hyoscyamine and scopolamine may dehydrate at high temperatures (as used in the injection port) forming apoatropine and aposcopolamine, derivatization reactions are commonly employed in GC analysis (Namera *et al.*, 2002; Aehle and Drager, 2010). Silylation of the target analytes with hexamethyldisilazane, bis(trimethylsilyl)-trifluoroacetamide or with 10 percent N,O-bis(trimethylsilyl) trifluoroacetamide/trimethylcholorosilane mixture in acetonitrile have been used (Caligiani *et al.*, 2011; Temerdashev *et al.*, 2012; Ciechomska *et al.*, 2016).

A comparative GC-MS investigation of the alkaloid patterns of three varieties of *D. stramonium* var. *stramonium*, *tatula* and *godronii*, grown in the Republic of Bulgaria, was reported by Berkov *et al.* (2006). Twenty-five TAs were identified in the plant organs. Alkaloid patterns of roots, leaves and seeds of the varieties grown at similar conditions were very similar. In contrast, the alkaloid pattern of *D. stramonium* var. *stramonium*, grown in the Arab Republic of Egypt, showed significant differences indicating that it is influenced more strongly by the environmental factors than genetic ones (Berkov *et al.*, 2006).
Sixty-seven TAs were identified by GC-MS in different plant organs (roots, stems, leaves, flowers and seeds) from *D. stramonium* cultivated in the Kingdom of Morocco. Analyte separation was carried out on a capillary HP-5MS column. The identities of the TAs were confirmed by comparing the measured mass spectral data with those obtained from literature and the National Institute of Standards and Technology (NIST) database. Hyoscyamine and scopolamine were the major TAs detected in the analysed plant organs. In seeds, hyoscyamine and scopolamine accounted for 66 percent and 20 percent (percentage estimated using total ion current) of the total TA content, respectively (El Bazaoui et al., 2011).

Ciechomska *et al.* (2016) described a GC-MS method for the determination of hyoscyamine and scopolamine in plant samples from *D. metel* (leaves and seeds) and *Brugmansia pittieri* (leaves). Microwave-assisted extraction followed by QuEChERS dispersive solid-phase extraction was used for extraction and clean-up. A 30 m HP-5MS capillary column was used for the separation. N,O-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane in acetonitrile were used for the derivatization of hyoscyamine and scopolamine. Atropine-d₃ was used as the internal standard. The limits of quantification (LOQ) of the method was about 10 µg/g for hyoscyamine and scopolamine in leaves and seeds. The mean concentrations (±SD) of hyoscyamine and scopolamine determined in *Datura* seeds were 2 788 ± 100 µg/g and 2 020 ± 70 µg/g, respectively. In leaves, the concentration of scopolamine was always higher than for hyoscyamine, ranging from 65 to 70 µg/g for hyoscyamine and 448 to 840 µg/g for scopolamine.

Caligiani *et al.* (2011) developed and validated a method for the determination of hyoscyamine and scopolamine in buckwheat (*Fagopyron esculentum* L.) fruits, flours and foods using GC-MS. For the extraction of the TAs, 5 g of buckwheat is defatted twice with 60 mL of hexane in an automatic Soxhlet apparatus. An aliquot of 1 g of the defatted sample (plant or food) is added to 1 mL of internal standard (nicotine, 1 mg/mL) and the mixture alkalinized with potassium hydroxide (5 percent in methanol). Then, extraction with dichloromethane was performed, the solvent evaporated, and the residue washed with hexane and dissolved dichloromethane. The solvent was evaporated, and hexamethyldisilazane was added for derivatization. The reaction was kept at 80 °C for 15 minutes and analysed by GC-MS. The separation of hyoscyamine and scopolamine was achieved using a DB-5MS-UI 30 m × 0.25 mm × 0.25 µm capillary column. Quantitation was carried out in single ion monitoring mode (hyoscyamine, m/z 124 and 361 and scopolamine m/z 138 and 375). The linearity of the method was in the range of 1 to 1 000 µg/kg. The limits of detection and quantitation for hyoscyamine were 0.3 and 1.0 µg/kg and for scopolamine 1.0 and 6 µg/kg, respectively.

(b) Capillary electrophoresis

Ye *et al.* (2013) reported a method for the simultaneous determination of hyoscyamine, scopolamine and anisodamine in plant extracts (*Flos daturae*) by capillary electrophoresis (CE) using a graphene oxide coated capillary. The sample preparation consisted of assisted ultrasound extraction of 0.2 g pulverized samples with 2 mL of methanol. The mixture is centrifuged, and the supernatant diluted with water 1:1 v/v.
The CE separation is performed in a 38 cm effective length capillary using 40 mM phosphate buffer, pH 7.0 containing 20 percent v/v methanol and 30 percent v/v acetonitrile. The applied voltage is 18 kV and the detection wavelength 196 nm. The developed method was linear in the concentration range of 0.5–200 µg/mL for hyoscyamine and scopolamine and the limit of detection (LOD) was 0.5 µg/mL for both analytes.

(c) Liquid chromatography-mass spectrometry

Liquid chromatography-coupled to mass spectrometry is the most used and appropriate technique for the determination of hyoscyamine and scopolamine in food and biological matrices. Different mass analysers have been used; the triple quadrupole (QqQ) is the most recommended for the determination of TAs in food (Jandric et al., 2011, Jakabova et al., 2012, Chen et al., 2017). When operated in the selected reaction monitoring (SRM) mode, the quadrupole analyser achieves better sensitivities than high-resolution mass spectrometry using ion trap technology, such as the Exactive Orbitrap, which in turn is a powerful analyser for identification of unknown substances (non-target analysis) and widely used in profiling studies.

Reversed-phase chromatography on C18 stationary phases and mobile phases containing methanol or acetonitrile and ammonium salts (formate, acetate) or formic acid have been used for the separation of TAs, mainly without enantiomeric separation of (+)- and (-)-hyoscyamine. Jandric et al. (2011) reported that the use of a Chirobiotic V column, even though not achieving enantiomeric separation, improved resolution and signal-to-noise ratio, as well as long-term stability as compared to other reversed-phase C18 columns. Marin-Sáez et al. (2017) tested different columns for the separation of TAs - Hypersil Gold aQ (100 × 2.1 mm, 1.9 µm) (Thermo Fisher Scientific, Bremen, Germany), Zorbax Eclipse Plus C8 (100 × 2.1 mm, 1.8 µm) (Agilent Technologies), Hypersil Gold Phenyl (100 × 2.1 mm, 1.9 µm) (Thermo Fisher Scientific, Bremen, Germany), Zorbax Hilic plus (100 × 2.1 mm, 3.5 µm) (Agilent Technologies) and Zorbax Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm) (Agilent Technologies) – and observed that these columns are adequate for the separation of high molecular weight TAs. However, TAs of low molecular weight (tropine, pseudotropine, cushohygrine, physoperuvine and tropinone) eluted before 1 minute and experienced large matrix effects. Therefore, a serial column approach was used, coupling a HILIC stationary phase (first column) with a C18 (second column).

Marin-Sáez et al. (2019a) reported an automated method for screening TAs in cereal-based baby foods, using an on-line solid-phase extraction coupled to mass-spectrometry (without a chromatographic step). The performance of two mass analysers was evaluated, a triple quadrupole and an Orbitrap. Sample preparation briefly consisted of mixing 1 g of sample with 10 mL of a solution of methanol:water 2:1 v/v 0.5 percent acetic acid in a vortex for 1 min. The mixture was agitated for 30 min in a rotatory agitator and centrifuged. The supernatant was filtered (0.22 µm), diluted tenfold with methanol:water (2:1 v/v) containing 1 percent of acetic acid and 8 mL loaded in the on-line SPE sorbent (Strata XC Polymeric Strong Cation Exchange column, 20 × 2 mm, 25 µm). For both analysers, signal suppression was observed for hyoscyamine and scopolamine and matrix effects were, respectively, -67 percent and -33 percent for
Exactive-Orbitrap and -62 percent and -37 percent when triple quadrupole was used. The LOQs achieved for hyoscyamine (0.5 μg/kg) and scopolamine (2.5 μg/kg) with the triple quadrupole were half of the values determined with the Orbitrap analyser.

In another study, Marin-Sáez et al. (2017) described a method for performing multi-analyte analysis of TAs (hyoscyamine, scopolamine, anisodamine, tropine, tropine, litorine, homatropine, apoatropine, aposcopolamine, scopoline, tropinone, physoperuvine, pseudotropine and cuscohygrine) in cereals. One g of sample was mixed with 10 mL of 0.5 percent acetic acid:methanol (1:2, v/v). The mixture was agitated and centrifuged. The supernatant was cleaned up on a strong cationic exchange SPE cartridge. The analytes were eluted with methanol containing 3 percent of ammonium hydroxide (25 percent). The extract was evaporated under nitrogen flow and the residue reconstituted in 0.5 mL of methanol:water 0.1 percent formic acid (50:50 v/v). The chromatographic separation was carried out using two columns in series (HILIC stationary phase (first column) with a C18 (second column)). The mobile phase was a mixture of acetonitrile and an aqueous solution of 0.1 percent formic acid. The quantitation was carried out with a single-stage Exactive-Orbitrap. The LOQ was 1 μg/kg for hyoscyamine and 2 μg/kg for scopolamine estimated in buckwheat, millet, soybean and linseed.

A rapid and sensitive method using LC-MS/MS for the simultaneous determination of TAs (tropine, hyoscyamine, scopolamine, homatropine, anisodamine) as well as glycoalkaloids (α-solanine and α-chaconine) in crops (wheat, rye, maize, soybean and linseed) was reported by Jandric et al. (2011). The samples were ground to a particle size of ≤1 mm and the sample preparation procedure consisted of dispersive solid phase extraction (DSPE), which had 5 g of sample with 0.5 percent formic acid in acetonitrile:water (75:25 v/v) (20 mL) and a mixture of magnesium sulfate (2 g), sodium chloride (0.5 g), sodium citrate dihydrate (0.5 g) and sodium hydrogen citratesesquihydrate (0.25 g). After agitation and centrifugation, an aliquot of 8 mL was evaporated to dryness under nitrogen flow at 45 ºC. For soybean and linseed, an additional clean-up step was included using matrix solid-phase dispersion (addition of 0.05 g of C18 per mL). The resuspended residue was filtered and injected into the LC-MS/MS. Chromatographic separation was achieved on a Chirobiotic V column using a mobile phase of water and acetonitrile or methanol and ammonium formate as an ionization additive. The electrospray ionization source was operated in the positive mode and quantitation performed in the SRM mode. Scopolamine-d₃ was used as internal standard. The transitions monitored for hyoscyamine were (m/z): 290.2→92.9 (collision energy: 32 eV) and 290.2→124.0 (collision energy: 23 eV) for quantitation and identification, respectively. For scopolamine the transitions monitored were (m/z): 304→137.9 (collision energy: 26 eV) and 304→156.0 (collision energy: 17 eV) for quantitation and identification, respectively. The LOD and LOQ were estimated by injecting decreasing concentrations of matrix-matched calibrators and measuring the response at a signal-to-noise ratio of equal to or greater than (≥) 3 and 10, respectively. The LOD of the TAs ranged from 0.7 to 0.8 ng/g and the LOQ from 2.2 to 2.5 ng/g in all grain and seed extracts, except for tropine in soybean and linseed (LOD of 1.6 ng/g and LOQ of 4.9 ng/g).
2.7.3 STEREOSELECTIVE SEPARATION OF \((-\)-HYOSCYAMINE AND \((+\)-HYOSCYAMINE

Stereoselective separation of the enantiomers of hyoscyamine has been achieved by capillary electrophoresis (Mateus et al., 2000) and liquid chromatography with chiral columns without any derivatization procedure (Breton et al., 2005). A prepacked α1-acid glycoprotein coated on silica column (Chiral-AGP) allowed the separation of the optical isomers of hyoscyamine (Breton et al., 2005). Also, aminopropylsila-bonded bovine serum albumin s-triazine (Zhang et al., 2000) and heptakis(6-azido-6-deoxy-2,3-di-O-phenylcarbamoylated)-β-cyclodextrin immobilized on the surface of aminized silica gel (Chen et al., 2002) were successfully employed for the enantioseparation of hyoscyamine in test solutions.

Although several methods for the enantioselective determination of \((-\)-hyoscyamine and \((+\)-hyoscyamine have been published, they were used mainly to determine the purity of \((-\)-hyoscyamine in plant extracts, pharmaceuticals or human plasma (Siluk et al., 2007) and are not appropriate for the analysis of food.

Marin-Sáez et al. (2016) developed an LC-MS/MS method for the enantioselective separation and determination of \((-\)-hyoscyamine and \((+\)-hyoscyamine in Solanaceae seeds and contaminated buckwheat. The separation was achieved using a Chiralpal-AY3 column (150 × 4.6 mm, 3 µm) and ethanol with 0.1 percent of diethanolamime as the mobile phase. The extraction procedure was based on a modified QuEChERS approach. Briefly, 5 g of sample were added to 10 mL of water and 10 mL of acetonitrile containing 1 percent v/v of formic acid. The mixture was vortexed and anhydrous sodium sulfate and ammonium acetate were added. The mixture was centrifuged, and the supernatant was cleaned with primary secondary amine (PSA) and graphitized black carbon (GBC). Finally, the supernatant was filtered and diluted with ethanol (50/50, v/v) prior to chromatographic analysis. \((-\)-Hyoscyamine and \((+\)-hyoscyamine were ionized in positive ESI mode and detected using selected reaction monitoring (SRM) mode. The LOQ for both enantiomers was 1 µg/kg. No enantiomerisation was observed using the described conditions. pH values up to 9 and temperatures up to 80 °C had no effect on enantiomerisation during extraction while increasing extraction time from 10 minutes to 1 hour had only a marginal effect on enantiomerisation. However, the combination of a high pH (9) and a high temperature (80 °C) resulted in appreciable enantiomerisation, which approached 50 percent after 2 days. After prolonged periods under these condition (7 days), the compounds degraded and were not detectable. At lower pH (about 5) and high temperature (80 °C), enantiomerisation was slower and less substantial, reaching 20 percent after 4 days, after which there was very little further change. In conclusion, enantiomerisation occurs only when high temperatures occur over long periods of time.

A summary of analytical methods for the determination of TA in food and feed are presented in Table 6.
<table>
<thead>
<tr>
<th>COMMODITY</th>
<th>TREPANE ALKALOIDS (TAs)</th>
<th>SAMPLE PREPARATION</th>
<th>TECHNIQUE</th>
<th>LINEARITY (µg/kg)</th>
<th>LOQ (µg/kg)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flours and cereal based products</td>
<td>24 TAs, including hyoscyamine and scopolamine</td>
<td>SLE + SPE</td>
<td>UHPLC-MS/MS</td>
<td>0–50</td>
<td>0.5</td>
<td>Mulder et al., 2016</td>
</tr>
<tr>
<td>Herbal tea and infusions, extracts and tablets</td>
<td>Hyoscyamine, scopolamine, homatropine, anisodamine</td>
<td>SLE</td>
<td>UHPLC-MS/MS</td>
<td>0.5–5</td>
<td>0.5</td>
<td>Cirlini et al., 2019</td>
</tr>
<tr>
<td>Oats and wheat (whole grain)</td>
<td>Hyoscyamine, scopolamine</td>
<td>SLE</td>
<td>2D-LC-MS/MS</td>
<td>0.2–50</td>
<td>0.16–0.4</td>
<td>Urban et al., 2019</td>
</tr>
<tr>
<td>Porcine muscle, eggs and milk</td>
<td>Scopolamine, (-)-hyoscyamine, (+) sparteine, (-) sparteine</td>
<td>QuEChERS</td>
<td>LC-MS/MS</td>
<td>Scopolamine: 5-30</td>
<td></td>
<td>Zheng et al., 2019</td>
</tr>
<tr>
<td>Baby food (containing buckwheat, millet or combination of cereals)</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>SLE</td>
<td>SPE-LC-MS/MS (Orbitrap)</td>
<td>0.5–50</td>
<td>2.5</td>
<td>Marin-Sáez et al., 2019a</td>
</tr>
<tr>
<td>Cereal based baby foods</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>SLE</td>
<td>SPE-MS/MS (QqQ or Orbitrap)</td>
<td>0.5–50</td>
<td>0.3 (LOD)</td>
<td>Marin-Sáez et al., 2019a</td>
</tr>
<tr>
<td>Wheat, corn</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>SLE</td>
<td>2D-LC-MS/MS</td>
<td>5.0</td>
<td></td>
<td>Kresse et al., 2019</td>
</tr>
<tr>
<td>Cereal-based products for infants and young children</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>SLE</td>
<td>LC-MS/MS</td>
<td>0–50</td>
<td>0.3 (LOD)</td>
<td>Mulder et al., 2015</td>
</tr>
<tr>
<td>Buckwheat, wheat, soy, buckwheat flour, buckwheat noodle, amaranth grain, chia seeds, and peeled millet</td>
<td>Hyoscyamine, scopolamine + 23 transformation products</td>
<td>QuEChERS, GBC</td>
<td>UHPLC-MS/MS and high resolution Orbitrap</td>
<td>0.1–100</td>
<td>Atropine: 0.4</td>
<td>Chen et al., 2017</td>
</tr>
<tr>
<td>COMMODITY</td>
<td>TROPANE ALKALOIDS (TAs)</td>
<td>SAMPLE PREPARATION</td>
<td>TECHNIQUE</td>
<td>LINEARITY (µg/kg)</td>
<td>LOQ (µg/kg)</td>
<td>REFERENCE</td>
</tr>
<tr>
<td>----------------------------------------------------</td>
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<td>----------------------------</td>
</tr>
<tr>
<td>Solanaceae seeds and contaminated buckwheat</td>
<td>(+)-Hyoscyamine and (-)-hyoscyamine</td>
<td>QuEChERS, GBC</td>
<td>LC-MS/MS</td>
<td>1–250</td>
<td>1</td>
<td>Marin-Sáez et al., 2016</td>
</tr>
<tr>
<td>Grains and seeds (wheat, rye, maize, soybean, linseed)</td>
<td>Hyoscyamine, scopalamine + 3 TAs</td>
<td>DSPE + MSPD</td>
<td>LC-MS/MS</td>
<td>5–80</td>
<td>TAs: 2.2–2.5</td>
<td>Jandric et al., 2011</td>
</tr>
<tr>
<td>Soy, linseed and millet, seeds and flour, and buckwheat seeds, flour and pasta</td>
<td>Hyoscyamine, scopalamine + 11 TAs</td>
<td>SPE</td>
<td>LC-Orbitrap</td>
<td>0.5–100</td>
<td>Atropine: 1 scopalamine: 2</td>
<td>Marin-Sáez et al., 2017</td>
</tr>
<tr>
<td>Buckwheat fruits, flours, crackers, flakes, porridge and pasta</td>
<td>Hyoscyamine, scopalamine</td>
<td>Soxlet extraction, LLE + derivatization</td>
<td>GC-MS</td>
<td>1–1 000</td>
<td>Atropine: 1 scopalamine: 6</td>
<td>Caligiani et al., 2011</td>
</tr>
</tbody>
</table>

GBC: graphitized black carbon; LLE: liquid-liquid extraction; SLE: solid-liquid extraction; SPE: solid phase extraction; DSPE: dispersive solid-phase extraction; MSPD: matrix solid-phase dispersion. QuEChERS: quick, easy, cheap, effective, rugged and safe; LC: liquid chromatography; UHPLC: ultra-high performance liquid chromatography; MS/MS: tandem mass spectrometry; GC: gas chromatography.
2.7.4 LIVE:.CID. INTERLABORATORY METHODS FOR THE MONITORING OF TROPANE ALKALOIDS IN FOOD

Interlaboratory studies were conducted in the project GP/EFSA/CONTAM/2014/01, which aimed to evaluate the occurrence of TAs in food for human consumption, from different regions in Europe to serve as supporting information to the EFSA CONTAM panel for future exposure assessments for TAs (Arcella et al., 2018).

For the quantitation of TAs in flours and cereal-based products, validated state-of-the-art analytical methods were available. An interlaboratory study was conducted on the determination of 24 TAs, including hyoscyamine and scopolamine. Four laboratories participated in the proficiency testing: Wageningen University (RIKILT), the Netherlands; Institute for Research and Technology in Food and Agriculture (IRTA), the Kingdom of Spain; FERA Science, the United Kingdom of Great Britain and Northern Ireland, and University of Chemistry and Technology (UCT), the Czech Republic (Mulder et al., 2016). The final method proposed for the determination of TAs is presented in Table 7. The SANCO/1275/2013 guidance document was followed for analytical quality control and validation procedures (SANCO, 2013).

### TABLE 7 RIKILT– METHOD FOR THE DETERMINATION OF HYOSCYAMINE AND SCOPOLAMINE IN GRAINS AND IN ANIMAL FEED
(continue)

<table>
<thead>
<tr>
<th>ANALYTICAL STEP</th>
<th>PROCEDURE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Preparation</strong></td>
<td></td>
</tr>
<tr>
<td>Aggregate sample amount</td>
<td>1 kg</td>
</tr>
<tr>
<td>Sub-samples of buckwheat, millet, breakfast cereals and biscuits</td>
<td>40 g after grinding and mixing the aggregate sample.</td>
</tr>
<tr>
<td>Sample storage temperature</td>
<td>-20 °C for frozen products; at room temperature, in a dark and dry place for products with an extended shelf-life and at cooled conditions for ready-to-eat products.</td>
</tr>
<tr>
<td>Sample</td>
<td>Ground to 0.5 mm and homogenized.</td>
</tr>
<tr>
<td>Sample preparation procedure</td>
<td>4 g of sample + 40 µL of the internal standards + 40 mL of methanol: water: formic acid 60:40:0.4 v/v/v. Extraction: 30 min in a rotary tumbler. Centrifugation for 15 minutes at 3 300 g. 10 mL of the extract is cleaned-up over an Oasis MCX 150 mg/6 mL cartridge (Waters) or a StrataX 200 mg/6 mL cartridge (Phenomenex). The cartridges were conditioned with 6 mL of methanol and equilibrated with 6 mL of methanol: water: formic acid 75:25:1 v/v/v. The cartridges are loaded with 10 mL of the extract, washed with 6 mL of methanol: water: formic acid 75:25:1 v/v/v and dried under vacuum for 5–10 minutes. The analytes were eluted with 6 mL of methanol containing 0.5 percent ammonia (dry or added from 25 percent concentrated ammonia solution). The eluates were evaporated under a nitrogen stream in a water bath at 50 °C and the residue reconstituted in 500 µL water: methanol 90:10 v/v. The final solutions were filtered (0.45 µm) and analysed.</td>
</tr>
</tbody>
</table>
### TABLE 7 RIKILT– METHOD FOR THE DETERMINATION OF HYOSCYAMINE AND SCOPOLAMINE IN GRAINS AND IN ANIMAL FEED (continued)

<table>
<thead>
<tr>
<th>ANALYTICAL STEP</th>
<th>PROCEDURE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UHPLC</strong></td>
<td></td>
</tr>
<tr>
<td>Analytical column</td>
<td>BEH C_{18} (150 × 2.1 mm, 1.7 µm) Waters, temperature: 50 °C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Solvent A: water containing 10 mmol/L of ammonium carbonate buffer, pH 10.0 (pH adjusted with ammonia). Solvent B: acetonitrile</td>
</tr>
<tr>
<td><strong>UHPLC Cont.</strong></td>
<td></td>
</tr>
<tr>
<td>Elution gradient</td>
<td>0–2 minutes: 100:0 A:B v/v, 2–12 minutes linear gradient to 60:40 A:B v/v, 12.0–12.2 linear gradient to A:B 100:0 v/v and 12.2–15.0 minutes, isocratic A:B 100:0 v/v.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.40 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>2 µL</td>
</tr>
<tr>
<td><strong>MS/MS</strong></td>
<td></td>
</tr>
<tr>
<td>ESI</td>
<td>positive</td>
</tr>
<tr>
<td>Capillary voltage</td>
<td>3 kV</td>
</tr>
<tr>
<td>Desolvation temperature</td>
<td>600 °C</td>
</tr>
<tr>
<td>Desolvation gas flow</td>
<td>800 L/h</td>
</tr>
<tr>
<td>Source temperature</td>
<td>150 °C</td>
</tr>
<tr>
<td>Cone gas (argon) flow rate</td>
<td>100 L/h</td>
</tr>
<tr>
<td>Collision gas flow rate</td>
<td>0.18 mL/min</td>
</tr>
<tr>
<td><strong>MRM conditions</strong></td>
<td><strong>Hyoscyamine</strong></td>
</tr>
<tr>
<td></td>
<td>Precursor ion (m/z): 290.2</td>
</tr>
<tr>
<td></td>
<td>Transitions (m/z): 290.2 → 124.0 (collision energy: 20 eV); 290.2 → 93.0 (collision energy: 25 eV) and 290.2 → 91.0 (collision energy: 35 eV)</td>
</tr>
<tr>
<td></td>
<td><strong>Scopolamine</strong></td>
</tr>
<tr>
<td></td>
<td>Precursor ion (m/z): 304.2</td>
</tr>
<tr>
<td></td>
<td>Transitions (m/z): 304.2 → 156.0 (collision energy: 25 eV); 304.2 → 138.0 (collision energy: 20 eV) and 304.2 → 103.0 (collision energy: 35 eV)</td>
</tr>
<tr>
<td></td>
<td><strong>Atropine-d_{3}</strong></td>
</tr>
<tr>
<td></td>
<td>Precursor ion (m/z): 293.2</td>
</tr>
<tr>
<td></td>
<td>Transitions (m/z): 293.2 → 127.0 (collision energy: 20 eV); 293.2 → 93.0 (collision energy: 25 eV) and 293.2 → 91.0 (collision energy: 35 eV)</td>
</tr>
<tr>
<td></td>
<td><strong>Scopolamine-d_{3}</strong></td>
</tr>
<tr>
<td></td>
<td>Precursor ion (m/z): 307.2</td>
</tr>
<tr>
<td></td>
<td>Transitions (m/z): 307.2 → 156.0 (collision energy: 25 eV); 307.2 → 141.0 (collision energy: 20 eV) and 307.2 → 103.0 (collision energy: 35 eV)</td>
</tr>
<tr>
<td><strong>Internal standards</strong></td>
<td>40 µL of atropine-d_{3} and scopolamine-d_{3} prepared in methanol at a concentration of 1000 ng/mL. Added before the extraction procedure.</td>
</tr>
</tbody>
</table>

Source: Mulder et al., 2016.
The in-house validation parameters for the determination of hyoscyamine and scopolamine in single flours and cereal-based products in the four participating laboratories are shown in Table 8 and 9. The LOD and LOQ were calculated as 3 and 10 times, respectively, the signal-noise ratio from lowest matrix or extract spiked sample.

**TABLE 8  VALIDATION PARAMETERS OF THE UHPLC-MS/MS METHOD FOR THE DETERMINATION OF HYOSCYAMINE IN SINGLE FLOURS AND CEREAL-BASED PRODUCTS**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>UNIT</th>
<th>RIKILT</th>
<th>IRTA</th>
<th>FERA</th>
<th>UCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range</td>
<td>µg/kg</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
</tr>
<tr>
<td>Recovery (fortification of 10 µg/kg)</td>
<td>%</td>
<td>8 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intraday precision (CV)</td>
<td>%</td>
<td>10 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LOD</td>
<td>µg/kg</td>
<td>0.05</td>
<td>0.2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>LOQ</td>
<td>µg/kg</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 6.
<sup>b</sup> n = 4.
RIKILT: Wageningen University, the Kingdom of Netherlands.
IRTA: Institute for Research and Technology in Food and Agriculture, the Kingdom of Spain.
FERA: Science, United Kingdom of Great Britain and Northern Ireland.
UCT: University of Chemistry and Technology, the Czech Republic.

**TABLE 9  VALIDATION PARAMETERS OF THE UHPLC-MS/MS METHOD FOR THE DETERMINATION OF SCOPOLAMINE IN SINGLE FLOURS AND CEREAL-BASED PRODUCTS**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>UNIT</th>
<th>RIKILT</th>
<th>IRTA</th>
<th>FERA</th>
<th>UCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range</td>
<td>µg/kg</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
<td>0-50</td>
</tr>
<tr>
<td>Recovery (fortification of 10 µg/kg)</td>
<td>%</td>
<td>103 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intraday precision (CV)</td>
<td>%</td>
<td>13 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (1 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 (5 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (25 µg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LOD</td>
<td>µg/kg</td>
<td>0.05</td>
<td>0.2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>LOQ</td>
<td>µg/kg</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 6.
<sup>b</sup> n = 4.
RIKILT: Wageningen University, the Kingdom of Netherlands.
IRTA: Institute for Research and Technology in Food and Agriculture, the Kingdom of Spain.
FERA: Science, United Kingdom of Great Britain and Northern Ireland.
UCT: University of Chemistry and Technology, the Czech Republic.

The LODs and LOQs differed among laboratories which is attributed to the different LC-MS/MS equipment used. The LODs for hyoscyamine and scopolamine were in the range of 0.05 to 0.2 µg/kg and the LOQs were in the range of 0.5 to 1.0 µg/kg.
2.8 STABILITY OF TROPANE ALKALOIDS IN FOOD SAMPLES

Stability tests for sample storage were carried out at room temperature, 4 °C, -20 °C and -80 °C. Long-term stability (12 months) and mid-term stability (4 months) were evaluated for buckwheat, breakfast cereals, biscuits and bread. All samples were spiked with TAs at 10 µg/kg. It was shown that TAs were stable in the fortified food matrices at all storage conditions and over the entire study period. Recoveries higher than 90 percent were achieved (Mulder et al., 2016).

2.8.1 STABILITY OF TROPANE ALKALOIDS DURING FOOD PROCESSING

A summary of relevant studies examining the effects of food processing on hyoscyamine and scopolamine is provided below. Studies that involved preparation of grain-based ingredients and foods were considered. The experimental design of studies, including correction for changes in moisture in food products during preparation, considerations for heterogeneity, use of concentrations relevant to the current WFP contamination scenario, as well as the analytical methodology used in the studies, were all assessed in order to determine whether or not processing during preparation of WFP products was likely to affect the exposure of consumers to hyoscyamine and scopolamine.

Marín-Sáez et al. (2019b) investigated the effect of bread making on TA levels using intentionally contaminated raw materials (buckwheat and millet flour contaminated with *D. stramonium* and *Brugmansia arborea*). Concentrations of TAs in test materials were around 5 mg/kg and are relevant for the reported concentrations in WFP products. It is not clear if the flour was fortified with whole or ground seeds, or in case when whole seeds were used whether fortified flour was comminuted after fortification. The relatively low relative standard deviations (2–13 percent) of TA concentrations for replicate (n = 4) test portions of unprocessed dough, proofed dough, and baked dough suggest that comminution was performed, and that heterogeneity of test materials is not a confounding factor in this study.

A total of 17 TAs, including hyoscyamine and scopolamine, were monitored. The samples were subjected to proofing (37 °C) and baking (190 °C) processes. For the determination of TAs, a simple solid-liquid extraction with methanol:water 2:1 (v/v) containing 0.5 percent acetic acid was used, whereas a chromatographic method employing a Zorbax C18 column coupled to an Extractive-Orbitrap analyser was used for their determination. The analytical method used appears appropriate and robust enough for the analysis of TAs. Method validation and quality control information was provided by the authors. Concentrations of TAs were presented on a dry weight basis, and therefore take into account changes in moisture content during dough preparation, fermentation, and baking.

The results indicate that concentrations of TAs decrease under proofing conditions (degradation between 13 and 95 percent), while they were almost completely degraded under baking conditions (degradation between 94 and 100 percent).
Overall, 65–70 percent and 70–80 percent of hyoscyamine and scopolamine were lost (degraded) during proofing and baking, respectively. An in vitro experiment in the same study using pure TAs showed >94 percent loss of all compounds. Since matrix appears to play a role in mitigating degradation, the precise amount of degradation in the in vitro experiment is not relevant, but it does support the loss of these TAs that was observed in this study during baking experiments. Some degradation pathways have been proposed, indicating that most of the compounds degrade into tropane and tropine, and into tropine and tropinone under proofing and baking conditions respectively.

Friedman and Levin (1989) added ground D. stramonium (jimsonweed) seed to wheat flour that was baked into bread to evaluate the stability of TAs during the high temperature of bread-baking (215 ºC). The flour was fortified with 12 percent seeds by mass in final dry weight of dough mix. This amount is much higher than used by Marin-Sáez et al. (2019b) and leads to very high concentrations of TAs (g/kg) that are orders of magnitude above those reported in the WFP products from the Karamoja Outbreak. Samples were freeze dried prior to chemical analysis, thereby removing the confounding factor of variable moisture contents amongst matrices. The details provided for the GC-MS and LC-MS/MS analytical methods suggest they were adequate for analysis of the D. stramonium seed TAs. However, method evaluation was performed on a different matrix (ground D. stramonium seeds) than dough and bread. Therefore, performance of the analytical method on these food matrices is unknown.

There were also some aspects of the experimental design that would limit confidence in the results from the Friedman and Levin (1989) study. The baking experiment design is not as strong as that used by Marin-Sáez et al. (2019b). It appears that one sample of unbaked flour was compared to one sample of freeze-dried crumb (i.e., inside of bread loaf) and one sample of freeze-dried crust (it was not specified how crumb and crust were differentiated). The “starting material” of unbaked flour is not necessarily linked to the bread as in Marin-Sáez et al. (2019b) and therefore the comparison between baked and unbaked concentrations could be between two different “production batches”. The lack of replicate bakings and test portions analysed for TAs is a drawback in this study as well. There is also no mention of correcting concentrations for the presence of non-flour dry ingredients. This is not straightforward to do as yeast and other minor ingredients will be consumed during proofing. However the overall the effect on mass from these non-flour components should be less than 10 percent.

Friedman and Levin (1989) reported losses of 18–25 percent and 13–28 percent during proofing and baking for hyoscyamine and scopolamine, respectively. These losses are two to three times lower than reported by Marin-Sáez et al. (2019b).

Perharič et al. (2013) fortified buckwheat flour with commercially available pure atropine and scopolamine. Solutions of these tropane alkaloids were added to buckwheat flour prior to cooking together with table salt and water to make a porridge-like, traditional Slovenian dish. This preparation method would result in
the food matrix most relevant to the WFP food products, which are also prepared as a porridge. Concentrations of the TAs in the finished food (75 to 7,500 µg/kg) are very relevant to the WFP food products from the Karamoja Outbreak. Replicate test portions of food (n=2) were analysed per dose. The low variability of hyoscyamine and scopolamine concentrations observed suggest that heterogeneity of food product was not a confounding factor when assessing loss during cooking. The quality of the analytical method is not clear, particularly regarding extraction efficiency and accuracy for analysis of the two different matrices of cooked food and fortified flour, as data on these methodological aspects were not provided in the publication. However, personal communication with the author provided some indication that the laboratory analyses of food products involved quality control to monitor extraction efficiency.

More importantly, the fortified flour was not analysed prior to cooking. Therefore the “loss” of TAs was determined using the nominal concentrations of compounds estimated based on the volume of standard solution used to fortify the flour as the starting point. Differences in matrix effects (including extraction efficiency and MS-based effects during instrumental analysis) between flour and cooked food would affect estimation of “loss” performed in this way. It is also not clear if the authors accounted for the different moisture contents of starting and end food matrices, or the loss of moisture during cooking, when calculating the losses due to cooking.

Losses during cooking were reported as 63 percent for hyoscyamine and 42 percent for scopolamine. These apparent losses are consistent with those reported by Marín-Sáez et al. (2019b), particularly for hyoscyamine.

In another study, the stability of hyoscyamine and scopolamine was evaluated during normal baking conditions for cookies (Tong et al., 2017). One kg of dough was prepared by mixing 540 g of flour, 200 g of margarine, 200 mL of water, 150 g of sugar, 0.1 g sodium carbonate, 0.7 g ammonium carbonate, 1 mg of atropine and 0.5 mg of scopolamine. Concentrations used (about 1.2 mg/kg total atropine and scopolamine) are relevant for the reported concentrations in WFP products from the Karamoja Outbreak. Half of the dough was submitted to baking (220 ºC, 30 min) and the other half was used as reference control. It does not appear that replicate test portions were analysed for dough pre-baking nor for cookies after baking.

At a first glance the concentration of hyoscyamine and scopolamine seemed to decrease under baking conditions. However, after the loss of water due to the baking process was taken into account (19.3 percent), the concentration of hyoscyamine in unbaked dough (0.87 mg/kg) is comparable to that of baked bread (0.89 mg/kg). The same was shown for scopolamine (unbaked dough 0.38 mg/kg vs. baked bread 0.36 mg/kg). These data suggest that hyoscyamine and scopolamine are thermally stable (Tong et al., 2017) and they are not consistent with the results reported by Perharić et al. (2013), Friedman and Levin (1989), and Marín-Sáez et al. (2019b).
The final processing study considered dealt with the processing of soybeans (List and Spencer, 1976). For the first experiment, the authors used *D. stramonium* seeds. Seeds were “finely ground” and added to soybeans dehulled and flaked using pilot plant equipment. The mixture of comminuted *D. stramonium* and flaked soybeans was extracted with petroleum ether and separated into crude oil and defatted meal. Crude oil and defatted meal were analysed for hyoscyamine and scopolamine.

The second experiment used pure atropine from a commercial laboratory to fortify a sample of crude oil extracted from dehulled and flaked soybeans. The concentration of hyoscyamine in the fortified crude oil used (2,000 mg/kg) is very high as compared to that in the WFP food products from the Karamoja Outbreak. A portion of the fortified crude oil was analysed for hyoscyamine. Another portion of the crude oil was refined and washed using an alkaline procedure. The refined and washed oil was analysed for hyoscyamine. Hyoscyamine and scopolamine were analysed using solvent extraction and derivatization (silylation) to render analytes amenable to gas chromatography.

As with some of the other studies considered, there were methodological and experimental design aspects that led to uncertainty in the value of the study results. No replicate analyses were performed for the crude oil or defatted meal in the first experiment; no replicate analyses were performed on the refined and washed oil in the second experiment either. In addition, the quality of the analytical method could not be fully assessed. Mean extraction efficiency was determined to be 106 percent for atropine in fortified crude oil (n = 3); no other information on the performance of the analytical method is provided.

List and Spencer (1976) reported that hyoscyamine and scopolamine were found mainly in defatted meal as opposed to crude oil. For hyoscyamine, 90 percent of mass observed in *D. stramonium* seeds was found in the defatted meal and only 0.13 percent was found in the unrefined oil. For scopolamine, 84 percent of the mass observed in *D. stramonium* seeds was found in defatted meal and only “trace” amounts were found in the unrefined oil. The apparent 10 percent and 16 percent loss of hyoscyamine and scopolamine, respectively, from *D. stramonium* seeds to processed soy may be due to natural variance of TAs in *D. stramonium* seeds, degradation of these TAs, and/or matrix effects in the analytical method. Unfortunately, the source of the apparent loss cannot be determined in this study.

During further processing of the unrefined oil, 93 percent of the small amount of hyoscyamine present was lost during alkali refining and washing. Based on similar physicochemical properties, it would be expected that scopolamine would be similarly lost during the refinement process.

The authors noted some limitations of their study. The *D. stramonium* seeds used were neither flaked nor dehulled, as would occur during commercial processing of contaminated soybeans. In this study the seeds were comminuted, which likely resulted in a greater surface area and therefore higher accessibility and extraction efficiency of TAs. Therefore, the comminution may have masked additional loss in excess of the apparent loss of 10–16 percent of TAs during processing.
The Soxhlet extraction used in the first experiment also did not entirely simulate the extraction of soybean flakes at a commercial level in oil processing plants. It is not clear if the experiment would have under- or overestimated extraction or loss of TAs as compared to modern extraction methods.

Overall, there are few published studies on the fate of TAs during preparation of grain-based foods. There are inconsistencies in the losses or stability reported in the few relevant publications. Along with variation in experimental design and methods of analysis of the TAs, the variable conclusions on the fate of hyoscine and scopoline likely also reflect the variable impact of food matrix and processing conditions.

### 2.9 OCCURRENCE

TAs can be found in plant raw material, as well as in foods that have been contaminated with plants that contains these substances. Such incidents are well known and recorded (EFSA, 2013). Following the request by the European Commission (EC) the CONTAM Panel of EFSA published a scientific opinion on TAs in food and feed in 2013 but had to accept that only limited occurrence data were available. Therefore, the Panel suggested additional data should be obtained and recommended occurrence data for various TAs, including atropine and scopoline, should be collected. Hence, EFSA published a call for a European wide survey for TA in food. Therefore, most of the available data derives from these surveys between 2014 and 2017. Occurrence data are summarized in Table 10.

### 2.10 PREVENTION AND CONTROL

In most temperate climate regions, annuals *D. stramonium*, *D. innoxia*, and *D. ferox* can thrive as invasive weeds in a large number of crops, and wherever weed management, post-harvest handling or controls are not adequately performed, some seeds may go undetected to subsequent stages of the food chain (EFSA, 2008; Caligiani *et al.*, 2011). While the whole plant is known to contain toxic alkaloids, seeds are usually considered to be the most likely cause of contamination due to their small size and to the simultaneous maturation of *Datura* and *Atropa* species with crops such as cereals, legumes and pseudocereals sharing the same cultural cycle. As described above, seeds of *D. ferox* and *D. stramonium* are frequently found in linseed and soybean, although other species such as *D. metel*, *D. meteloides* and *D. innoxia* have also been identified.

The possibility for contamination of food with TAs is global, as *Datura* spp. exist in a number of countries in tropical and warm temperate climates [Centre for Agriculture and Biosciences International’s “Invasive Species Compendium”10].

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10 https://www.cabi.org/ISC.
<table>
<thead>
<tr>
<th>COMMODITY</th>
<th>NUMBER OF SAMPLES ANALYSED</th>
<th>YEAR</th>
<th>COUNTRY</th>
<th>ANALYTES</th>
<th>TECHNIQUE</th>
<th>POSITIVE SAMPLES</th>
<th>MAXIMUM CONCENTRATION</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbal tea and infusions, extracts and tablets</td>
<td>60</td>
<td>ni</td>
<td>Italy</td>
<td>Hyoscyamine, scopolamine, homatropine, anisodamine</td>
<td>UHPLC-MS/MS (non enantioselective)</td>
<td>5</td>
<td>Hyoscyamine: 69 µg/kg</td>
<td>Scopolamine: 50 µg/kg</td>
</tr>
<tr>
<td>Porcine muscle, eggs and milk</td>
<td>30</td>
<td>ni</td>
<td>South Korea</td>
<td>Scopolamine, (-)-hyoscyamine, (+) sparteine, (-) sparteine</td>
<td>LC-MS/MS</td>
<td>none</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Baby food (pap, biscuits, crackers, snack and grissines)</td>
<td>18</td>
<td>ni</td>
<td>Spain, France and Italy</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>SPE-LC-MS/MS</td>
<td>1</td>
<td>Scopolamine: 2.8 µg/kg</td>
<td>Hyoscyamine: 11.5 µg/kg</td>
</tr>
<tr>
<td>Cereal-based products for infants and young children</td>
<td>226</td>
<td>2010–2014</td>
<td>Netherlands</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
<td>Scopolamine: 18</td>
<td>Scopolamine: 15.2 µg/kg</td>
<td>Hyoscyamine: 65.6 µg/kg</td>
</tr>
<tr>
<td>Buckwheat, wheat, soy, buckwheat flour, buckwheat noodle, amaranth grain, chia seeds, and peeled millet</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>Hyoscyamine, scopolamine</td>
<td>UHPLC-MS/MS</td>
<td>none</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Buckwheat fruits, flours, crackers, flakes, porridge and pasta</td>
<td>16</td>
<td>ni</td>
<td>Italy</td>
<td>Hyoscyamine, scopolamine</td>
<td>GC-MS</td>
<td>none</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cereal bars</td>
<td>30</td>
<td>2015</td>
<td>Switzerland</td>
<td>Hyoscyamine, scopolamine</td>
<td>LC-MS/MS</td>
<td>1</td>
<td>Hyoscyamine: 5.0 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Buckwheat, wheat, corn (from organic agriculture)</td>
<td>346</td>
<td>2015–2017</td>
<td>Switzerland (country of origin of samples: China, Germany, Austria, Hungary, Czechia, Switzerland)</td>
<td>Hyoscyamine, scopolamine</td>
<td>LC-MS/MS</td>
<td>49</td>
<td>only sum of TA available &gt; 10.0 µg/kg</td>
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### OCCURRENCE DATA (continue)

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<tr>
<td>Herbal teas</td>
<td>20</td>
<td>2015–2016</td>
<td>United Kingdom of Great Britain and Northern Ireland (for EFSA survey)</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
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<td>Hyoscyamine: 129 µg/kg Scopolamine: 34.1 µg/kg</td>
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<td>Green beans and stir fry vegetables</td>
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<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
<td>3</td>
<td>Tropine: 571 µg/kg Pseudotropine: 43.6 µg/kg Fillalbine: 11.6 µg/kg</td>
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<td>LC-MS/MS</td>
<td>14</td>
<td>Hyoscyamine: 3.73 µg/kg Scopolamine: 0.51 µg/kg</td>
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<td>LC-MS/MS</td>
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<td>Hyoscyamine: 0.67 µg/kg Scopolamine: 0.38 µg/kg</td>
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<td>2015–2016</td>
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<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
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<td>Hyoscyamine: 0.63 µg/kg Scopolamine: 0.16 µg/kg</td>
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<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
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<td>Hyoscyamine: 9.8 µg/kg Scopolamine: 12.9 µg/kg</td>
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<td>20</td>
<td>2015–2016</td>
<td>United Kingdom of Great Britain and Northern Ireland, new data</td>
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<td>LC-MS/MS</td>
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<td>Hyoscyamine: 3.73 μg/kg</td>
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<td>Single grain flours and maize products</td>
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<td>2015–2016</td>
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<td>Hyoscyamine: 9.8 μg/kg Scopolamine: 12.9 μg/kg</td>
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<tr>
<td>Black and green teas</td>
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<td>2015–2016</td>
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<td>Hyoscyamine, scopolamine, and others</td>
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<td>Scopolamine: 0.15 μg/Kg</td>
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<td>Single grain flours sampled directly at mills</td>
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<td>2015–2016</td>
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<td>&lt; LOD</td>
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<td>2017</td>
<td>Germany</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
<td>7</td>
<td>Hyoscyamine: 72.0 μg/kg Scopolamine: 14.0 μg/kg</td>
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<td>Herbal teas</td>
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<td>2017</td>
<td>Germany</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
<td>8</td>
<td>Sum of hyoscyamine and scopolamine: 37.0 μg/kg</td>
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<td>Fennel tea</td>
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<td>LC-MS/MS</td>
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<td>Green and black tea</td>
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<td>2017</td>
<td>Germany</td>
<td>Hyoscyamine, scopolamine, and others</td>
<td>LC-MS/MS</td>
<td>2</td>
<td>Sum of hyoscyamine and scopolamine: 8.0 µg/kg</td>
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<td>35</td>
<td>2017–2019</td>
<td>Singapore</td>
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<td>LC-MS/MS</td>
<td>3</td>
<td>Hyoscyamine: 4.81 µg/kg Scopolamine: 2.46 µg/kg</td>
<td>Singapore (via FAO call for data)</td>
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<td>Super cereal and Super cereal Plus</td>
<td>510</td>
<td>60 in 2019; rest unknown</td>
<td>WFP, different locations</td>
<td>Hyoscyamine, scopolamine</td>
<td>LC-MS/MS</td>
<td>380</td>
<td>Hyoscyamine: 15 528 µg/kg Scopolamine: 1 863 µg/kg</td>
<td>WFP internal testing</td>
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</table>

* Not Indicated.
The incidence of *D. stramonium* contamination is higher in the warm temperate zone of southern Europe and South America, whereas *D. ferox* predominates in South America (Piva *et al.*, 1995).

As with other weed seeds and undesirable material, physical cleaning can remove TA-containing seeds from grain. Sorting based on density, size and colour can be used to clean grain (Wenblom, 1973; Tkachuk *et al.*, 1991; Hurburgh *et al.*, 1996; Schaarschmidt and Fauhl-Hassek, 2018). While these processes will improve the quality and safety of the cleaned grain, caution must be exercised regarding the use of rejected grain and grain screenings as feed components, since this material will have elevated TA concentrations if the TA-containing seeds are concentrated within this material (Naude *et al.*, 2005).

### 2.11 SUMMARY

- *Datura* and *Hyoscyamus* species are the likeliest materials to contaminate grains (and subsequently grain-based foods) because their density, size, and shape are similar to those of grains. Seeds of *D. stramonium* have been reported in linseed/flaxseed, soybean, millet, sunflower and buckwheat.

- The concentration of hyoscyamine in the analysed food samples has usually been expressed as “atropine”. Atropine is defined as the racemic mixture (1:1) of (-)-hyoscyamine and (+)-hyoscyamine. However, most of the analytical methods used to generate occurrence data did not measure each enantiomer of hyoscyamine in the samples separately. Therefore, considering the potential variability of the enantiomeric fraction, the expert meeting considered it more accurate to express the results as the sum of (-)-hyoscyamine and (+)-hyoscyamine, instead of as “atropine”.

- In the study reported by Mulder *et al.* (2016), 1709 samples of plant-derived food products, mainly produced in Europe and collected in nine European countries, were analysed for TAs. One or more TAs were detected in 21.3 percent of single component flours, 20 percent of cereal-based food for young children age 6–36 months, 15.8 percent of bread, 26.2 percent of legumes and stir-fry mixes, and 14.6 percent of biscuits. Due to the large number of samples and broad scope of sampled food matrices this is the most significant study currently available on TA levels in food, even though samples were obtained only from markets in European countries.

- Caution must be taken when interpreting analytical results of samples exposed to highly alkaline aqueous or alcoholic conditions since hyoscyamine may hydrolyse into tropane and tropic acid. Enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine may also occur under these conditions.

- A validated analytical method using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of the sum of (-)-hyoscyamine and (+)-hyoscyamine, and scopolamine in grain-based foods and grains is available and may be used for monitoring purposes.
The limits of quantitation of this method for the determination of the sum of (-)-hyoscyamine and (+)-hyoscyamine, and scopolamine ranged from 0.5 to 1.0 μg/kg. Limits of detection of this method ranged from 0.05 to 0.2 μg/kg.

The number of studies reporting data on the fate of TAs during food processing is limited. Even among the few studies that are available, most do not consider effects from sample heterogeneity and changes in moisture content, during food processing or do not provide complete analytical method descriptions. Hence, this did not allow the evaluation of data quality and increases the uncertainty in assessing the fate of hyoscyamine and scopolamine during food processing.

Two studies reported in the literature and relevant to WFP products suggest that hyoscyamine and scopolamine diminish in concentration during cooking processes. Decreases reported were 63–70 percent of hyoscyamine and 42–80 percent of scopolamine. However, food products were fortified and the impact of matrix effects on the results was not assessed and nor was the measurement uncertainty reported. These studies also did not identify and quantify all hyoscyamine and scopolamine degradation products, therefore the risk from potential degradation products cannot be assessed.

The small number of cooking studies considered relevant, the uncertainty surrounding results from these studies, the absence of information on degradation products in these studies, and the lack of information on how food matrix and specific food processing techniques will impact the degree of hyoscyamine and scopolamine loss, prevented the expert meeting from estimating degradation factors for use in dietary exposure assessments. The expert meeting agreed as a default to assume that there was no loss of hyoscyamine and scopolamine due to food processing for the purpose of the dietary exposure assessments in order to maximize protection of consumers.

Enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine is possible, but unlikely under most food processing conditions.

There is a small number of studies that report enantiomerisation of (-)-hyoscyamine to (+)-hyoscyamine in pharmaceuticals and plant extracts. Few of these studies are relevant for grain-based food processing, as experimental conditions are extremely alkaline and unlikely to be encountered during processing of grain-based foods, aside from nixtamalisation of corn/maize.
Woman feeding her child porridge made from Super Cereal Plus in Nigeria.
CHAPTER 3
BIOLOGICAL DATA

3.1 BIOCHEMICAL ASPECTS

In general, most TAs, including hyoscyamine and scopolamine, appear to be effectively absorbed from the gastrointestinal (GI) tract, rapidly distributed to tissues and excreted primarily via the renal system (Beermann et al., 1971; Murrin, 1973; Ali-Melkkilä et al., 1993; Renner et al., 2005; EFSA, 2013; Alvarez-Jimenez et al., 2016).

Clinical and experimental studies typically use commercial atropine which is the 1:1 racemic mixture of (-) and (+)-hyoscyamine. The anticholinergic activity of atropine is associated with the (-) enantiomer. The true enantiomeric fraction was not assessed in the studies of this section; however, where atropine was administered and a non-enantiomer specific method was used for analysis, the results were expressed as atropine.

3.1.1 HYOSCYAMINE/ATROPINE

Hyoscyamine (active (-) enantiomer) is reported to be well absorbed from the GI tract with peak pharmacological activity occurring 30–60 minutes after an oral dose, with a half-life of 3.5 hours. Hyoscyamine is distributed throughout the body, crosses the blood-brain barrier and is bound approximately 50 percent to plasma proteins. It is metabolised in the liver to tropic acid, tropine and hyoscyamine glucuronide. Hyoscyamine is excreted primarily unchanged in the urine (Myers et al., 1997). Similar findings can be seen with racemic atropine; following i.v. administration, a distribution half-life of approximately 1–2 minutes in adults is seen, along with an apparent volume of distribution of 1.7 L/kg (average for adults 28–71 years old) (Kanto and Klotz, 1988). Rapid tissue distribution is consistent with the time of onset of various pharmacological effects associated with atropine such as changes in heart rate and inhibition of salivary secretion.

Food in the GI tract has been reported not to affect absorption of TAs (Muhtadi, 1994). The number of human poisoning cases documented worldwide after accidental or deliberate ingestion of TA plant parts also provides evidence that the absorption of TAs from the digestive tract takes place (Gaire and Subedi, 2013; Chan, 2017; Adibah and Azzreena, 2019; Kohnen-Johannsen and Kayser, 2019).
(a) Absorption

Six male Sprague–Dawley rats (200 ± 20 g) were administered a single oral dose (presume by gavage) of *Hyoscyamus niger* L. extract, equivalent to doses of 1.06 mg/kg bw anisodamine, 16.19 mg/kg bw scopolamine and 13.46 mg/kg bw atropine. Blood samples were collected for analysis of TAs by LC–MS/MS beginning 5 minutes after dosing and at various times up to 10 hours (Zhang *et al.*, 2014). The $C_{\text{max}}$ (peak serum concentration) for atropine was reported as 31.4 ng/ml with the $T_{\text{max}}$ (time to achieve peak serum concentration) of 15 minutes and a half–life of 2.2 hours.

Atropine sulfate (50 mg/kg bw) was administered by gavage to fasted male Sprague–Dawley rats (n = 4; 180–220 g) and serial blood samples collected for analysis by LC/MS-MS five minutes after dosing, and then at various time points up to 12 hours following dosing (Tian *et al.*, 2015). $C_{\text{max}}$ (228 ng/mL) was reached after approximately 30 minutes and was similar to the $C_{\text{max}}$ for an i.v. dose of 1.5 mg atropine/kg bw. Half-life was calculated as 6.7 hours with an estimated bioavailability of 21.8 percent.

Atropine, in tablet form, was administered to fasted New Zealand white rabbits (1.5 ± 0.2 kg; n = 3/dose) by gavage at single doses of 0.021 or 0.084 mg and blood samples collected 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dosing for atropine analysis by HPLC (Sangetha and Samanta, 2016). $C_{\text{max}}$ was similar for both dose groups (7.51 and 8.46 µg/mL, respectively) with $T_{\text{max}}$ occurring at 2 hours after dosing. Half-life for both dose groups was also similar (3.08 and 3.33 hours) as well as the AUC(0–24 hours) (27.50 and 31.48 µg/hr/mL, respectively).

The absorption of an atropine/scopolamine mixture was studied in human volunteers who had ingested a single combined dose of 0.12–12.10 µg/kg bw atropine/0.10–9.50 µg/kg bw scopolamine in a buckwheat flour meal. Blood samples were collected 30, 60, 120 and 240 minutes after dosing and analysed for the TAs by LC-MS/MS (Kozelj *et al.*, 2014). Atropine was detected above the lower LOQ (0.1 ng/mL) only for the three highest combined doses (1.22 µg of atropine/0.95 µg of scopolamine/kg bw, 3.58 µg of atropine/2.81 µg of scopolamine/kg bw and 12.10 µg of atropine/9.50 µg of scopolamine/kg bw). $C_{\text{max}}$ was reached by the 120 minute sample collection and was quantitatively related to the dose (0.25 ng/mL-1.22 µg of atropine/0.95 µg of scopolamine/kg bw; 0.5 ng/ml-3.58 µg of atropine/2.81 µg of scopolamine/kg bw; 1.65 ng/mL-12.10 µg of atropine/9.50 µg of scopolamine/kg bw (estimated from graph for single volunteer)).

Following ingestion by human volunteers (see Perharić *et al.*, 2013 for details) of cooked buckwheat flour spiked with an atropine/scopolamine mixture designed to provide doses of 0.12–12.10 µg/kg bw atropine/0.10–9.50 µg/kg bw scopolamine, the maximum plasma concentrations of alkaloids were reached 120 minutes after dosing, with a $C_{\text{max}}$ for atropine of 2.52 ± 0.19 µg/L (0.49 µg/L for scopolamine) at the highest dose (12.10 µg/kg bw of atropine/9.50 µg of scopolamine/kg bw) (Perharić *et al.*, 2012).
Children, average age of 4.8–5.5 years, were administered doses of either 0.03 mg/kg bw (oral) or 0.02 mg/kg bw (i.m.) atropine sulphate (n = 10 per dose) prior to elective surgery. Average total doses of atropine for the two groups were 0.56 mg and 0.41 mg, respectively. Heart rate, blood pressure and rectal temperature immediately prior to dosing and then 30 minutes, 1, 2, 4, 6 and 8 hours afterwards were monitored along with analysis of atropine in blood samples by radioimmunoassay (Saarnivaara et al., 1985). $C_{\text{max}}$ for the oral dose averaged 3 nmol/L (0.88 µg/L) and was reached after 2 hours ($T_{\text{max}}$) while the $C_{\text{max}}$ for the i.m. dose was 5.7 nmol/L (1.6 µg/L) with a $T_{\text{max}}$ of 30 minutes. Average serum half-life was 4.4 hours for both dose routes. In two children, accidentally provided an oral dose of atropine ten-fold greater (0.3 mg/kg bw), serum atropine measured 2.5 hours after dosing was 100 nmol/L and 54 nmol/L. In the main study, all children were also treated with 70 mg/kg bw triclofos (chloral hydrate) in combination with the atropine doses.

In human male adult subjects (n = 10; 46–62 years), the absorption of orally administered $^3$H-atropine (2 mg in an aqueous solution) was shown to occur mainly in the duodenum and in the jejunum, with no absorption in the stomach (Beermann et al., 1971). Based on radioactivity measurements, 90 percent of the $^3$H-atropine had been absorbed within 1 hour after dosing.

The peak plasma levels of atropine depend on the route of administration, ranging from 13 minutes for i.m., up to 60 minutes for oral and 1.5–4 hours for aerosol administration (Lakstygal et al., 2019).

Blood levels of atropine associated with typical clinical use range from 0.002–0.035 mg/L, with levels in the range 0.03–0.1 mg/L associated with toxicity and 0.2 mg/L comatose or fatality (Schulz et al., 2012).

(b) Distribution

In male Sprague–Dawley rats administered atropine sulphate (50 mg/kg bw) by gavage, the volume of distribution was 485 L/kg bw (Tian et al., 2015). Pregnant Sprague–Dawley rats were administered a single s.c. dose of 0.5 mg/kg bw [N-methyl-$^3$H]atropine (87.0 Ci/mmol) on gestation day 19 and tissues/whole blood of dams and fetuses were collected 1, 2 and 4 hours after dosing for radioassay to determine placental and blood-brain-barrier transfer (Watanabe et al., 1990). Radioactivity was detected in fetal plasma samples at approximately 50 percent the level of maternal plasma, indicating placental transfer. Blood-brain-barrier transfer of atropine label was also detected in both dams and fetuses.

In healthy human male volunteers (n = 3) administered (i.v.) 1.35 and 2.15 mg of atropine sulphate, the steady-state volume of distribution was 210 L, implying extensive tissue distribution (Hinderling et al., 1985). Volume of distribution was similar (1.6–2.2 L/kg) in human volunteers of various age groups who had been provided a single i.v. dose of atropine sulphate of 0.02 mg/kg bw (Virtanen et al., 1982). Pregnant women (n = 25) undergoing Caesarean section surgery were administered a single i.v. dose of $^3$H-atropine (0.5 µg/kg bw or 0.7 µCi/kg bw) at 1, 3, 5, 10 or 30 minutes prior to clamping of the umbilical cord, and blood samples collected (maternal and umbilical cord)
at delivery (Kivalo and Saarikoski, 1977). Rapid transfer of label was evident within 1 minute after maternal dosing with umbilical vein values 12 percent of the maternal levels, increasing to approximately 50 percent by 10 minutes after maternal dosing. Similar results have been observed in another group of pregnant women (n = 22; 38–41 weeks gestation) administered a single dose of atropine sulphate (0.01 mg/kg bw i.v.); atropine was detected in fetal plasma samples (by radioimmunoassay) within 1–2 minutes after maternal dosing and increased to concentrations similar to maternal values within 6 minutes after dosing (Kanto et al., 1981).

(c) Metabolism and excretion

In the systemic circulation, up to 50 percent of atropine is bound to plasma proteins (primarily to alpha-1-acid glycoprotein) (Buck, 2014) and the plasma half-life for atropine in humans has been estimated at 2.5 hours (Beermann et al., 1971). The main route of excretion is in the urine. During the first 8 hours after dosing (i.m.) human volunteers (n = 4) with atropine (2 mg; 5 μCi/mg), up to 80 percent of a labelled dose (14C) of atropine could be detected in the urine, with up to 94 percent of the dose detected during the period of one day (24 hours). A small amount was recovered in the faeces and a small amount of label (up to 3 percent), supporting N-demethylation, was recovered as carbon dioxide (Kalser, 1971). Approximately 30–50 percent of an oral dose is excreted through the urine as non-metabolised atropine (Kanto and Klotz, 1988).

Certain species of animals, in particular rabbits, have been shown to tolerate relatively large doses of atropine. This was determined to be due to the genetically determined presence in some rabbits of a plasma carboxylesterase with high affinity for atropine (atropinesterase; EC 3.1.1.10) which metabolises atropine to tropic acid and tropine (Liebenberg and Linn, 1980). Rabbit plasma positive for atropinesterase incubated with atropine induces a rapid decline in detectable atropine for the first 10 minutes and then a plateau is reached after disappearance of approximately 50 percent of the atropine (Harrison et al., 2006). A similar time course in the appearance of tropic acid is seen. A possible explanation is that atropinesterase preferentially hydrolyses one enantiomer of atropine, and the slower rate could be a combination of hydrolysis of the alternative enantiomer and re-racemisation of the remaining atropine (Harrison et al., 2006). No appreciable atropinesterase was detected in plasma samples tested from dog, goat, guinea pig, rhesus and humans.

SPF Wistar rats (sex not indicated) were fasted for 24 hours and then administered an oral dose of atropine (25 mg/kg bw). Urine samples were collected at various time points up to 110 hours after dosing for analysis of atropine and its metabolites by LC-MS (Chen et al., 2006). Only atropine and phase 1 metabolites, which are N-demethyltropine, tropine, p-hydroxyatropine, p-hydroxyatropine N-oxide, and N-demethylatropine were detected (not quantified) in urine samples collected within the first hour while phase 2 metabolites, glucuronide conjugates, sulfate conjugates of N-demethylatropine, atropine and p-hydroxyatropine, were detected at various times up to 106 hours after dosing. Both atropine and p-hydroxyatropine could still be detected in urine samples from the final collection period.
Wistar rats (180 ± 5 g; n = 5) were fasted for 24 hours and then administered by oral gavage a single dose of 25 mg/kg bw atropine. Blood, urine and fecal samples were collected up to 24 hours after dosing and analysed for atropine metabolites by LC-MS (Chen et al., 2007). Eleven metabolites (nortropine, tropine, noratropine, hydroxyatropine, hydroxyatropine N-oxide, and glucuronide and sulfate conjugates of noratropine, hydroxyatropine, and atropine) were identified in urine samples while atropine, nortropine, tropine, tropic acid, apoatropine and hydroxyatropine were identified in rat plasma samples. Atropine, tropine, tropic acid and hydroxyatropine were present in plasma samples collected between 0.25 and 24 hours after dosing while nortropine appeared in plasma only between 0.75 and 6 hours. Apoatropine was detected only in plasma samples from between 1–12 hours after dosing. Similar metabolites to those found in plasma samples were detected in faeces, with the addition of N-oxide hydroxyatropine and hydroxymethoxy atropine. No sulfate or glucuronide conjugates of atropine or its metabolites were detected in rat faeces. Atropine was also incubated in vitro with rat liver homogenates and only apoatropine and noratropine were detected as metabolites.

In the study by Beermann et al. (1971), total radioactivity detected in urine following the single gavage dose of ³H-atropine (2 mg, 50 µCi) ranged from 68.9 to 85.7 percent of the dose with 1.5 percent of the dose recovered in the faeces. Analysis of urine sample by electrophoresis suggested 51–68 percent of the atropine dose had been metabolised, with some of the unidentified metabolites including glucuronide conjugates.

Following administration (i.v.) of 2 mg of atropine sulphate and 100 µCi of [³H]atropine sulphate to a single human volunteer, the metabolism of atropine was investigated using HPLC analysis of urinary radioactivity as compared to reference standards of postulated metabolites (Van der Meer et al., 1986). Fifty-seven percent of the administered radioactivity was recovered in urine as (+)-hyoscyamine, 24 percent as noratropine, 15 percent as atropine-N-oxide, 3 percent as tropic acid and 2 percent as tropine. The recovery of only (+)-hyoscyamine was interpreted as possible stereoselective metabolism of the active (-) isomer. Approximately 50 percent of total radioactivity detected in the urine was present by 6 hours after dosing, which increased to over 80 percent by 22 hours. After incubation of the urine samples with glucuronidase/arylsulfatase, no metabolite-related conjugates were identified.

In human male volunteers (n = 3; 21–24 years old), after i.v. administration of 1.35 or 2.15 mg atropine, blood and urine samples were collected and analysed for atropine and tropine by GC-MS at regular intervals up to 10 hours (blood) and 22 hours (urine) after dosing (Hinderling et al., 1985). Excretion of parent compound in urine was reported as 56.8 percent of the dose (average) with 28.7 percent as tropine. Pre-operative human volunteers (n = 8–13 per age group) were administered a single i.v. dose of 0.02 mg/kg bw atropine with blood collections starting 2 minutes after dosing and continuing at regular intervals for up to 24 hours. Concentrations of atropine in serum were determined by radioimmunoassay (Virtanen et al., 1982).
Half-life for atropine was not significantly different between children (4.8 hours; 0.08–10 years) and adults (3.0 hours; 16–58 years) but was increased in older adults (10.0 hours; 65–75 years old) compared to the 2 other age groups. While volume of distribution in the three groups was similar (2.2 L/kg-children; 1.6 L/kg-adults; 1.8L/kg-older adults), total serum clearance of atropine for the older age group (2.9 mL/min/kg) was approximately half that of the two younger age groups (6.4–6.8 mL/min/kg). Children less than 2 years of age from the first group were analysed separately and compared to children greater than 2 years of age. Both atropine half-life and volume of distribution were greater in the younger children ($T_{1/2}$ 6.9 hours vs. 2.5 hours; $V_B$ 3.2 vs. 1.3 l/kg). When heart rate (up to 15 minutes post dose) was compared between adults 16–41 years of age and the older adult group, an increase in heart rate (maximum of approximately 30 bpm) was observed only in the younger group.

### 3.1.2 SCOPOLAMINE

(-)-Scopolamine is rapidly absorbed from the human GI tract including via the buccal exposure route. Most likely, due to extensive first pass metabolism in the gut and liver, the reported oral bioavailability of (-)-scopolamine following oral exposure is generally considered low (Renner et al., 2005; EFSA, 2013). Although limited information is available regarding the distribution of (-)-scopolamine following oral exposure, it is expected that (-)-scopolamine is extensively distributed in human tissues (Renner et al., 2005). The main route of excretion of (-)-scopolamine appears to be via the urine. The half-life ($t_{1/2}$) is relatively short and the clearance of scopolamine may be age-dependent with clearance being lower in older adults than younger adults. Limited evidence is available suggesting that small amounts of (-)-scopolamine are excreted into human milk.

#### (a) Absorption

Serum (-)-scopolamine concentrations were measured in male and female F344/N rats following daily gavage exposure to 1 000, 5 000 or 25 000 µg/kg bw per day (-)-scopolamine hydrobromide trihydrate (equivalent to 692, 3 460, 17 302 µg/kg bw per day (-)-scopolamine) for 15 months (NTP, 1997). One hour after the last dose, female rats receiving 3 460 µg/kg bw (-)-scopolamine, showed serum concentrations of 6 ng/mL. The serum (-)-scopolamine concentrations in male rats receiving 692 and 3 460 µg/kg bw (-)-scopolamine, were below the minimum detection limit (4 ng/mL) of the analysis method (GC/MS). The serum (-)-scopolamine concentration of female rats in the low dose groups were not determined due to a flooding incident that killed 16 females in this group. The serum (-)-scopolamine concentrations were 12 and 28 ng/mL for male and female rats receiving 17 302 µg/kg bw (-)-scopolamine, respectively.

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11 Dose (-)-scopolamine = dose (-)-scopolamine hydrobromide trihydrate × (303.35 ÷ 438.31 g/mol).
Tian et al. (2015) reported a $T_{\text{max}}$ of approximately 30 minutes and a corresponding $C_{\text{max}}$ of $62.8 \pm 14.5$ ng/mL in male Sprague-Dawley rats exposed via gavage exposure to 27 500 µg/kg bw (−)-scopolamine (material specification not provided). The absolute oral bioavailability ($\text{AUC}_{\text{oral}}/\text{AUC}_{\text{IV}}$) in this study was approximately 37 percent. Plasma concentrations of scopolamine were determined using LC-MS/MS.

Ebert et al. (2000) reported a $T_{\text{max}}$ of 23.5 ± 8.2 minutes and a corresponding $C_{\text{max}}$ of $0.54 \pm 0.01$ ng/mL in healthy volunteers (7 men and 7 women; mean age 23 year; weight range 53 to 100 kg bw) exposed orally to 0.5 mg (−)-scopolamine hydrobromide in solution with 150 ml of water (equivalent to approximately 4–7.5 µg/kg bw (−)-scopolamine$^{12}$). Ebert et al. (2000) calculated an absolute oral bioavailability of 13 ± 1 percent (range 6 to 37 percent) based on the ratio of $\text{AUC}_{\text{oral}}/\text{AUC}_{\text{IV}}$. Serum samples were analysed using GC-ion trap tandem MS.

Putcha et al. (1989) reported a mean $T_{\text{max}}$ of 47 minutes (reported range: ~20–120 minutes) and a mean peak plasma concentration of $2.9 \pm 0.24$ ng/ml in six healthy male subjects (ages 25 to 45) exposed orally to 0.4 mg tablets of (−)-scopolamine hydrobromide (approximate dose of $4.5 \mu g/kg$ bw (−)-scopolamine; assuming 70 kg bw). Based on the ratio of $\text{AUC}_{\text{oral}}/\text{AUC}_{\text{IV}}$ Putcha et al. (1989) calculated a mean absolute oral bioavailability of 26.8 ± 5.1 percent with values ranging between 11.7 and 48.2 percent. Plasma samples were analysed using a radioreceptor binding assay. Putcha et al. (1989) and Renner et al. (2005) suggested that the pharmacological effects of (−)-scopolamine on intestinal motility and gastric secretion might affect its oral bioavailability.

Pihlajamäki et al. (1986) reported a median $T_{\text{max}}$ of 53 ± 8 minutes and a corresponding $C_{\text{max}}$ of $6.38 \pm 5.84$ ng/mL in six young Caesarean section patients (ages 24 to 34; 68 to 103 kg bw) following mouth only exposure to a dose of 35 µg/kg bw (−)-scopolamine (material specification not provided). Pihlajamäki et al. (1986) administered (−)-scopolamine to the mouths of intubated patients for one hour before removing it via suction. Serum concentrations of (−)-scopolamine were determined using an enzyme immunoassay. Golding et al. (1991) reported a $T_{\text{max}}$ of approximately 47.9 ± 16.7 minutes and a corresponding $C_{\text{max}}$ of $1.38 \pm 0.29$ ng/mL in ten healthy men (mean age 33.9; mean weight 79.4 kg bw) following mouth only exposure to 0.6 mg standard tablets of (−)-scopolamine hydrobromide (allowed to dissolve in mouth but not swallowed). A $T_{\text{max}}$ of approximately 60 ± 36.1 minutes and a corresponding $C_{\text{max}}$ of $1.42 \pm 0.41$ ng/mL following oral exposure to 0.6 mg standard tablets of (−)-scopolamine hydrobromide (equivalent to approximately 6 µg/kg bw (−)-scopolamine) were also reported. Plasma concentrations of (−)-scopolamine were determined using a radioreceptor binding assay. According to the Golding et al. (1991) data, mouth only exposure produced similar peak plasma concentrations and similar $T_{\text{max}}$ values as did the ingestion of standard tablets.

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$^{12}$ Dose (−)-scopolamine = 0.5 mg (−)-scopolamine hydrobromide × (303.35 ÷ 384.27 g/mol) ÷ bodyweight.
(b) Distribution

According to Putcha et al. (1989) the volume of distribution in healthy male volunteers was 1.36 ± 0.28 L/kg bw following i.v. administration of 0.4 mg (-)-scopolamine hydrobromide. Guay et al. (2003) reported a mean central volume of distribution of 1.18 ± 0.48 L/kg and a mean steady-state volume of distribution of 5.02 ± 0.84 L/kg in healthy men receiving an i.v. bolus dose of 5 µg/kg bw (-)-scopolamine (summary data; no additional details).

(-)-Scopolamine administered during parturition can cross the placental barrier and, to a more limited extent, the blood cerebrospinal fluid (CSF) barrier. For example, Kanto et al. (1989) administered 5 µg/kg bw (-)-scopolamine hydrobromide (equivalent to 4 µg/kg bw (-)-scopolamine) i.m. to seven healthy parturients (ages 26 to 36 years; weight from 57 to 83 kg bw). Maternal venous blood concentrations of (-)-scopolamine, taken approximately 41 minutes after administration, were not statistically different from umbilical venous blood and umbilical arterial blood concentrations, which were taken approximately 60 min after administration. Kanto et al. (1989) also reported that a small proportion (20 percent) of parturients showed measurable but significantly lower concentrations of (-)-scopolamine in the lumbar CSF. Samples were analysed using a radioreceptor binding assay.

(c) Metabolism and excretion

According to Renner et al. (2005), only 2.6 percent of (-)-scopolamine following oral exposure is excreted in the urine as its ‘pharmacologically active form.’ Given the very low urinary excretion of unchanged (-)-scopolamine, it is expected that this compound undergoes extensive metabolism in humans following oral administration, likely due to oxidative demethylation by CYP3A (Putcha et al., 1989; Ebert et al., 2000; Renner et al., 2005; EFSA, 2013). A CYP3A metabolic pathway is supported by the work of Ebert et al. (2000) who showed that co-administration of (-)-scopolamine hydrobromide with grapefruit juice (a known CYP3A inhibitor) induced an elongation of T_{max} (by 36.4 minutes) and increased AUC (142 percent), compared to co-administration with water.

Kentala et al. (1990) analysed the urine of seven healthy parturients following i.m. administration of 5 µg/kg bw (-)-scopolamine (material specification not provided), using a radioreceptor binding assay. Following incubation of the urine with β-glucuronidase and sulfatase, the concentrations of (-)-scopolamine increased on average by a factor of seven, suggesting that β-glucuronide and sulfate conjugation are important pathways for (-)-scopolamine metabolism. As previously mentioned, Ebert et al. (2000) showed that approximately 145 ± 12.7 µg and 154.4 ± 34.7 µg of (-)-scopolamine and (-)-scopolamine glucuronide were eliminated by the kidneys of males and females respectively, following oral exposure to 0.5 mg (-)-scopolamine hydrobromide with 150 ml of water (dose range 4 to 7.5 µg/kg bw (-)-scopolamine). The detection of phase II metabolites in the urine of both men and women, suggest that the glucuronidation and/or sulfation pathways are involved in (-)-scopolamine metabolism.
The evidence for sulfation is weaker than that for glucuronidation (Renner et al., 2005). Additionally, according to the monograph for TRANSDERM SCOP (transdermal patch), the plasma concentration of free (-)-scopolamine represents only approximately 25 percent of the total plasma concentration, e.g. the average plasma concentration for total (-)-scopolamine (free + conjugates) is 354 pg/mL with only 87 pg/mL (0.28 nM) representing free (-)-scopolamine.

Ebert et al. (2000) found that following administration of 500 µg of (-)-scopolamine hydrobromide via i.v. or oral routes, the amount of unchanged (-)-scopolamine plus (-)-scopolamine glucuronide recovered in the urine 24 hours after administration were similar, i.e. 125.4 ± 30.5 µg (~25 percent) was recovered following i.v. administration and 154.4 ± 34.7 µg (~30 percent) was recovered post oral administration.

Marked differences in the metabolism of (-)-scopolamine have been reported in experimental mammals. For example, Wada et al. (1991) observed aryl hydroxylation of the tropic acid moiety in rats; hydrolysis of the tropic ester in rabbits; tropic ester hydrolysis, dehydroxylation and N-demethylation in guinea pigs; and Phase II conjugation and N-demethylation in mice following s.c. administration of 200 000 µg/kg bw (-)-scopolamine hydrobromide. Furthermore, Wada et al. (1991) showed that New Zealand White rabbits and Hartley guinea pigs excrete mostly tropic acid, while ddY mice excrete aposcopolamine (6.5 ± 3.1 percent); aponorscopolamine (3.6 ±1.5 percent); (-)-scopolamine (free form: 12.2 ± 3.3 percent; conjugate: 25.4 ± 7.3 percent); norscopolamine (free form: 2.5 ± 2.5 percent; conjugate: 18.6 ± 3.5 percent); p-hydroxyscopolamine (conjugate: 5.7 ± 2.2 percent); and tropic acid (2.6 ± 0.7 percent). In these studies, male animals received 200 mg/kg (-)-scopolamine hydrobromide (157 884 µg/kg bw (-)-scopolamine) by s.c. injection and urine was collected for 24 hours.

Chen et al. (2005 and 2008) have identified the following metabolites in rat excreta following oral gavage exposure to 55 000 µg/kg bw (-)-scopolamine hydrobromide (43 418 µg/kg bw (-)-scopolamine):

> norscopine, scopine, tropic acid, aponorscopolamine, aposcopolamine, norscopolamine, hydroxyscopolamine, hydroxyscopolamine N-oxide, p-hydroxy-m-methoxyscopolamine, trihydroxyscopolamine, dihydroxy-methoxyscopolamine, hydroxyl-dimethoxyscopolamine, glucuronide conjugates and sulfate conjugates of norscopolamine, hydroxyscopolamine.

Wada et al. (1991) detected the following metabolites in the urine of male Wistar rats, over 24 hours after s.c. administration of 200 000 µg/kg bw (-)-scopolamine hydrobromide (157 884 µg/kg bw (-)-scopolamine):

> aposcopolamine (2.1 ± 0.5 percent); aporscopolamine (1.3 ± 0.3 percent); (-)-scopolamine (free form: 6.5 ± 1.0 percent; conjugate: 0.7 ± 0.2 percent); norscopolamine (free form: 0.9 ± 0.2 percent; conjugate: 1.0 ± 0.4 percent); p-hydroxy-m-methoxyscopolamine (free form: 3.0 ± 1.1 percent; conjugate:

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13 https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/017874s038lbl.pdf.
p-hydroxyscopolamine (free form: 7.0 ± 1.8 percent; conjugate: 0.8 ± 0.5 percent); and m-hydroxyscopolamine (free form: 4.3 ± 1.2 percent; conjugate: 0.4 ± 0.3 percent).

While no studies in which the toxicity of (-)-scopolamine metabolites was evaluated have been identified, aposcopolamine showed high affinity for muscarinic receptors in vitro in membranes prepared with porcine brains (i.e. IC$_{50}$ = 0.0192 µM); the affinity of scopine for muscarinic receptors in vitro was lower (i.e. IC$_{50}$ = 3.0 µM) (Schmeller et al., 1995). The significantly greater proportion of tropic acid excreted in rabbits and guinea pigs is due to differences in esterase activity. According to EFSA (2013), rabbits and poultry species are more resistant to TA toxicity, due to the varied expression of hydrolytic enzymes that can cleave and inactivate the majority of TAs. These hydrolytic enzymes have been detected in various tissues including the serum and liver of guinea pigs and rabbits (NTP, 1997).

Renner et al. (2005) suggest that contraceptives may inhibit the metabolism of (-)-scopolamine by altering drug metabolizing systems and the concentration of plasma proteins.

Peeples and Dalvi (1982) showed that (-)-scopolamine undergoes oxidative demethylation by microsomal enzymes in vitro. Additionally, Peeples and Dalvi, (1982) showed that (-)-scopolamine binds effectively to microsomal P450 enzymes from the liver of phenobarbital-induced rats but not to those from the liver of 3-methylcholanthrene-induced rats, suggesting the involvement of CYP2B and/or CYP3A enzymes, but not CYP1A enzyme.

According to the TRANSDERM SCOP monograph, in vitro studies using human hepatocytes suggest that (-)-scopolamine does not induce CYP1A2 and CYP3A4 enzymes at concentrations up to 10 nM. Additionally, (-)-scopolamine did not inhibit CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4, in human liver microsomes at concentrations up to 1 micromolar. No in vivo drug-drug interaction studies have been conducted.

Ebert et al. (2000) reported a t$_{1/2}$ of 63.7 ± 1.3 minutes for (-)-scopolamine and that approximately 145 ± 12.7 µg (-)-scopolamine and 154.4 ± 34.7 µg of (-)-scopolamine glucuronide were eliminated by the kidneys of males and females respectively, following oral exposure to 0.5 mg (-)-scopolamine hydrobromide with 150 ml of water (dose range of 4 to 7.5 µg/kg bw (-)-scopolamine). Serum and urine samples were analysed using GC-ion trap MS/MS. Comparatively, Putcha et al. (1989) reported a mean t$_{1/2}$ of 5.0 ± 1.4 hours in healthy male volunteers consuming a 0.4 mg tablet of (-)-scopolamine hydrobromide (approximate dose of 4.5 µg/kg bw (-)-scopolamine); and a mean systemic clearance of 65.3 ± 5.17 L/hr following i.v. administration of 0.4 mg (-)-scopolamine hydrobromide. Plasma and urine samples were analysed using a radioreceptor binding assay.

14 https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/017874s038lbl.pdf.
In an analysis of four separate studies, Alvarez-Jimenez et al. (2016) reported a clearance of 1.09 ± 0.096 L/min (~65 L/hr) following i.v. administration of 0.5 or 0.3 mg (-)-scopolamine hydrobromide in 135 healthy subjects (age range 18 to 78 years). Plasma samples were analysed using a LC-MS/MS method. Based on the available data, Alvarez-Jimenez et al. (2016) suggested that older adults are more sensitive to the effects (i.e. cognitive function tests) of (-)-scopolamine because clearance of (-)-scopolamine is lower in older adults than younger adults, rather than to any difference in pharmacodynamics.

There is scant information on (-)-scopolamine levels in breast milk. O’Brien (1974) indicated that although (-)-scopolamine is excreted into human milk, the concentrations in the milk are ‘not significant in therapeutic doses to affect child.’ No indication of the concentration of (-)-scopolamine in human milk was provided, nor was any additional information found following a search of the scientific literature. However, EFSA (2013) indicated that precautions are recommended for therapeutic use by breast feeding women, since (-)-scopolamine may inhibit lactation (e.g. reduce milk production or milk let down) by affecting growth hormone, oxytocin and/or prolactin levels during nursing (Drugs and Lactation Database, 2006).

In experimental animals, there is evidence that (-)-scopolamine is excreted in the urine and faeces. For example, Chen et al. (2005 and 2008) identified metabolites of (-)-scopolamine in the urine and faeces of rats 24 hours following oral gavage exposure to 55 000 µg/kg bw (-)-scopolamine hydrobromide (equivalent to 43 418 µg/kg bw (-)-scopolamine). Samples were analysed using LC-MS methods and were identified but not quantified. Wada et al. (1991) detected metabolites in the urine of rats, mice, rabbits and guinea pigs following s.c. injection of 200 000 µg/kg bw (-)-scopolamine hydrobromide (equivalent to 157 884 µg/kg bw (-)-scopolamine). According to Wada et al. (1991) greater than 70 percent of the administered dose was detected in the urine of rabbits, guinea pigs and mice 24 hours after administration, while only approximately 30 percent was detected in the urine of rats. Samples were analysed by gas liquid chromatography and determined using mass and nuclear magnetic resonance spectra.

Renner et al. (2005) reported that in mammals, radiolabeled 14C02 was detected in expired air following administration of L-(−)-[9-14C]scopolamine or injection with L-(−)-[9-14C]scopolamine-9’-glucuronide.
3.2 TOXICOLOGICAL STUDIES

3.2.1 ACUTE TOXICITY

(a) Hyoscyamine/atropine

The acute lethal doses of atropine are summarized in Table 11.

<table>
<thead>
<tr>
<th>SPECIES (Strain)</th>
<th>SEX</th>
<th>ROUTE</th>
<th>LD₅₀ (mg/kg bw)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice (Swiss)</td>
<td>Male</td>
<td>Oral</td>
<td>400</td>
<td>Cahen and Tvede, 1952</td>
</tr>
<tr>
<td>Rat (Wistar)</td>
<td>Male</td>
<td>Oral</td>
<td>750</td>
<td>Cahen and Tvede, 1952</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Male</td>
<td>Oral</td>
<td>1 100</td>
<td>Cahen and Tvede, 1952</td>
</tr>
<tr>
<td>Human</td>
<td>NS</td>
<td>Oral</td>
<td>5–50</td>
<td>Munro et al., 1990</td>
</tr>
</tbody>
</table>

bw: body weight; CI: confidence interval; LD₅₀: median lethal dose; M: male; NS: not specified

Atropine LD₅₀ values for other routes of exposure (i.v.) in the same experimental animal species are on average less than 100 mg/kg (EFSA, 2013). Also, it has been reported (Buckett and Haining, 1965) that stereoisomerism of hysoscyamine does not impact acute lethality in mice. The LD₅₀ value for (+)-hyoscyamine sulphate following a single i.v. injection was 81 mg/kg bw compared to a value of 95 mg/kg bw for (-)-hyoscyamine hydrobromide. ED₅₀ values to induce convulsions in the same test species were also similar (102 mg/kg bw and 110 mg/kg bw, respectively). However, marked differences in potency were observed between stereoisomers at lower doses.
(b) Scopolamine

The acute lethal doses of (-)-scopolamine are summarized in Table 12.

<table>
<thead>
<tr>
<th>SPECIES (Strain)</th>
<th>SEX</th>
<th>ROUTE</th>
<th>LD₅₀ (95 percent CI), (mg/kg bw)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>NS</td>
<td>Oral</td>
<td>1 270⁵</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Rat</td>
<td>NS</td>
<td>Intraduodenal</td>
<td>670⁵</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Rat</td>
<td>NS</td>
<td>Subcutaneous</td>
<td>296⁵</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Oral</td>
<td>1 880⁵</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Oral</td>
<td>1 275⁵</td>
<td>Frommel et al., 1961¹</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Intraperitoneal</td>
<td>650⁵</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Mouse</td>
<td>M</td>
<td>Intraperitoneal</td>
<td>400⁴ (approximate LD₅₀)</td>
<td>Morpurgo, 1971</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Intravenous</td>
<td>163³ (150 – 176)</td>
<td>Buckett and Haining, 1965</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Intravenous</td>
<td>203⁶</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Intravenous</td>
<td>100⁴ (32 – 316)</td>
<td>Atkinson et al., 1983</td>
</tr>
<tr>
<td>Mouse</td>
<td>NS</td>
<td>Subcutaneous</td>
<td>1650⁴</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>NS</td>
<td>Subcutaneous</td>
<td>850⁴</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Cat</td>
<td>NS</td>
<td>Intravenous</td>
<td>LD₅₀ = 80⁴</td>
<td>ChemIDplus¹</td>
</tr>
<tr>
<td>Rabbit</td>
<td>NS</td>
<td>Intravenous</td>
<td>LD₅₀ = 100⁴</td>
<td>ChemIDplus¹</td>
</tr>
</tbody>
</table>

bw: body weight; CI: confidence interval; LD₅₀: median lethal dose; M: male; NS: not specified.

¹ (-)-scopolamine hydrobromide.

² (-)-scopolamine (free base).


⁴ Study details as reported in EFSA 2013 Table 11.
3.2.2 SHORT-TERM STUDIES OF TOXICITY

(a) Hyoscyamine/atropine

No short-term, repeated-dose toxicity studies with hyoscyamine or atropine were identified.

(b) Scopolamine

Mouse

In a National Toxicology Program (NTP) study, six-week-old male and female B6C3F1 mice (n = 5 per group per sex) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage for 16 days at doses of 0, 150, 250, 450, 900 or 1800 mg/kg bw per day (equivalent to 103.8, 173.0, 311.4, 622.9, 1245.8 mg/kg bw per day (-)-scopolamine\(^{15}\)). At the highest dose, 1/5 males and 2/5 females died and at the lowest dose, 1/5 females died (cause of death not reported). No changes in body weight were observed in male or female mice at any of the tested doses. A significant increase in relative liver weight was reported in male mice at the highest dose compared to control (approximately 14 percent increase) and in female mice at all tested doses compared to controls (approximately 4 to 14 percent increase), with no dose-response relationship, except possibly at the highest dose. Effects on liver weight were not confirmed in the 14-week study (see below). There was no evidence of treatment-related gross or microscopic lesions. Clinical findings related to (-)-scopolamine hydrobromide trihydrate, which included bilateral pupillary dilation and squinting, were reported in both sexes at all tested doses (NTP, 1997).

In another short-term toxicity study conducted by the NTP, six- to seven-week old male and female B6C3F1 mice (n = 10 per group per sex) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage for 14 weeks at doses of 0, 15, 45, 135, 400 or 1200 mg/kg bw per day (equivalent to 10.4, 31.1, 93.4, 276.8 or 830.5 mg/kg bw per day (-)-scopolamine). At the intermediate dose of 93.4 mg/kg bw per day (-)-scopolamine, 1/10 males died and at the highest dose 2/10 males and 1/10 females died (cause of death not reported). Clinical observations included bilateral pupillary dilation, squinting, hypoactivity and hyperactivity (doses associated with clinical findings were not specified). Reduced body weights were reported in both male and female mice at all tested doses (approximately 10 to 12 percent decrease compared to control in males and approximately 5 to 9 percent decrease compared to control in females). There were no significant differences in organ weights and no incidences of treatment-related gross or microscopic lesions. Mild mature neutrophilia was observed in male mice after 14 weeks at doses ≥ 31.1 mg/kg bw per day (-)-scopolamine compared to controls; however, there was no microscopic evidence of inflammation that could account for the neutrophilia. A significant increase in the length of the estrous cycle.

\(^{15}\) Dose (-)-scopolamine = (dose of (-)-scopolamine hydrobromide trihydrate) × (303.35 ÷ 438.31g/mol).
was reported in female mice at the highest dose compared to controls, but no changes in the concentration, motility or morphology of sperm in male mice at any of the tested doses (NTP, 1997). The alteration in estrous cycle occurred only at the highest dose where significant decreases in bodyweight occurred, potentially reflecting an indirect effect of decreased bodyweight.

**Rat**

In a NTP study, six-week-old male and female F344/N rats (n = 5 per group per sex) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage for 16 days at doses of 0, 75, 150, 300, 600 or 1 200 mg/kg bw per day (equivalent to 51.9, 103.8, 207.6, 415.3 or 830.5 mg/kg bw per day (-)-scopolamine). No deaths were reported in male or female rats at any dose. Reductions in body weight were reported in male rats at doses ≥ 415.3 mg/kg bw per day (-)-scopolamine compared to controls (approximately 11 percent decrease compared to controls). Statistically significant reductions in net body weight gains were also reported in male rats at doses ≥ 207.6 mg/kg bw per day (-)-scopolamine compared to controls. Clinical observations included bilateral pupillary dilation in all dosed rats and red eyelids in male and female rats exposed to the highest dose. There were no significant differences in organ weights and no treatment-related gross or microscopic lesions (NTP, 1997).

In another short-term toxicity study conducted by the NTP, six- to seven-week-old male and female F344/N rats (n = 10 per group per sex) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage for 14 weeks at doses of 0, 15, 45, 135, 400 or 1200 mg/kg bw per day (equivalent to 10.4, 31.1, 93.4, 276.8 or 830.5 mg/kg bw per day (-)-scopolamine). In males, 2/10 exposed to 93.4 mg/kg bw per day (-)-scopolamine died, 6/10 exposed to 276.8 mg/kg bw per day (-)-scopolamine died, and 8/10 exposed to 830.5 mg/kg bw per day (-)-scopolamine died. In females, 1/10 exposed to 31.1 mg/kg bw per day (-)-scopolamine died, 1/10 exposed to 93.4 mg/kg bw per day (-)-scopolamine died, 1/10 exposed to 276.8 mg/kg bw per day (-)-scopolamine died, and 7/10 exposed to 830.5 mg/kg bw per day (-)-scopolamine died. Some of the males (0/10, 0/10, 0/10, 2/10, 3/10, 4/10) and females (0/10, 0/10, 1/10, 1/10, 0/10, 3/10) died from tracheal and esophageal obstructions of feed and bedding material in the oropharyngeal region and posterior pharynx. The obstructions were considered to be secondary to the inhibitory effects of (-)-scopolamine hydrobromide trihydrate on salivary gland secretion and esophageal smooth muscle (NTP, 1997).

In both sexes, reductions in body weight and net body weight gains were reported in all sexes at all tested doses compared to controls (males: approximately 8 to 19 percent decrease and females: approximately 3 to 7 percent decrease compared to respective controls). The relative liver weights were increased by approximately (9 and 22 percent in female rats exposed to 276.8 and 830.5 mg/kg bw per day (-)-scopolamine, respectively, compared to controls. The relative thymus weights were decreased in female rats at all doses compared to controls (approximately 11 to 33 percent decrease in thymus weight compared to controls).
decrease compared to controls). In males, the relative thymus weights were reduced at all tested doses compared to controls (approximately 13 to 30 percent decrease compared to controls). There were no other significant treatment-related gross or microscopic lesions (NTP, 1997).

In the 14-week studies, a slight increase in hematocrit, hemoglobin concentration and/or erythrocyte count was reported in male and female rats at doses ≥ 31.1 mg/kg bw per day (-)-scopolamine compared to controls, which was consistent with dehydration. Mild mature neutrophilia was observed in male mice at doses ≥ 31.1 mg/kg bw per day (-)-scopolamine compared to controls; however, there was no microscopic evidence of inflammation that could account for the neutrophilia. There were no other treatment-related hematological differences. Clinical observations of bilateral pupillary dilation were reported in both sexes at all tested doses. Hyperactivity was visually observed in some males (0/10, 0/10, 1/10, 1/10, 0/10, 1/10) and females (0/10, 2/10, 5/10, 3/10, 1/10, 1/10). Hypoactivity was also visually observed in some males (0/10, 0/10, 0/10, 0/10, 3/10, 3/10) and females (0/10, 0/10, 0/10, 1/10, 1/10, 5/10).

Based on the results of the 16 day and 14-week toxicity studies in rats, the NTP suggested that male rats were less tolerant to (-)-scopolamine hydrobromide trihydrate than female rats. In the 16-day study, a reduction in body weight was observed in male rats, but not in female rats. Similarly, in the 14-week study, the final mean body weights of dosed male rats were approximately 8 to 19 percent lower than the male controls whereas in dosed female rats they were only 3 to 7 percent lower than the female controls. In addition, the survival rate of the female rats was higher than that of the male rats in the 14-week study.

(c) Combined exposure to tropane alkaloids

Rat

Albino-Wistar rats (male; 200 to 250 g) were given an i.p. dose of 100 mg/kg total alkaloids extracted from 100 g of *D. stramonium* seeds reported to contain 4 mg atropine and 2 mg scopolamine. No toxic symptoms such as paralysis, ataxia, lacrimation, laboured breathing or death were observed up to 5 days after dosing (Bouzidi et al., 2011).

Dried seeds from *D. stramonium* were added to standard rodent diets at 0.5, 1.58 and 5.0 percent seed by weight to provide diet concentrations of 13.6, 42.8 and 135.5 mg atropine and 3.3, 10.4 and 33.0 mg scopolamine/kg diet, in the low-, medium- and high-dose diets. The seeds were determined by HPLC analysis to contain 2.71 mg atropine and 0.66 mg scopolamine per gram. Weanling Sprague-Dawley rats (n = 20 per sex) were assigned to each diet group and exposed for 90 days with access to food ad libitum (Dugan et al., 1989). A consistent effect noted in all dose groups was a dose-dependent decrease in body weight gain, which was not associated with feed intake (17 percent and 23 percent at the highest dose group for males and females, respectively). Changes in various clinical chemistry measurements were also seen related to dose with a consistent effect being a dose-dependent increase in
serum alkaline phosphatase in both sexes. Relative testes weight in males and relative brain and liver weights in both male and female rats were significantly increased but no histological changes were noted in any organs. It was considered that significant effects were caused by the lowest dose (0.5 percent w/w seeds) which provided atropine/scopolamine doses of 0.81–1.45 mg/kg bw/day (males) and 1.08–1.63 mg/kg bw/day (females) (approximately 80 percent of dose would be atropine).

Groups of Wistar rats (n = 10) in the study of Bouzidi et al. (2011) were also provided combined daily i.p. doses of 5.2 mg/kg bw per day of atropine sulphate plus 2.6 mg/kg bw per day of scopolamine bromide for 28 days and 4.5 mg/kg bw atropine sulfate and 2.25 mg/kg bw scopolamine bromide for 120 days. Symptoms of diarrhea and hypoactivity were observed in the animals but no significant change in body weight, while relative liver weight was significantly decreased by approximately 10 percent. Histological changes in the liver of the treated group were described as centrilobular necrotic areas, hyperplasia of hepatocytes, as well as cytoplasmic dystrophy characterized by vacuoles tightly packed in the cytoplasm. Serum glutamic-oxaloacetic transaminase (GOT or AST) and glutamic-pyruvic transaminase (GPT or ALT) were also significantly increased compared to the control group (Mahdeb et al., 2012).

(d) Summary

No NOAEL for oral exposure to (-)-scopolamine could be identified in either mice or rats, as effects were observed at all doses. The LOAEL for the short-term toxicity studies was 10.4 mg/kg bw per day (-)-scopolamine, the lowest dose tested, based on bodyweight decrease and pupillary dilation.

Similarly, no NOAEL for the combined oral exposure to TAs was identified in rats. The LOAEL for the short-term toxicity studies for atropine/scopolamine was 0.81 mg/kg bw/day (males) and 1.08 mg/kg bw/day (females), the lowest dose tested, based on decreased body weight gain.

3.2.3 LONG-TERM STUDIES OF TOXICITY AND CARCINOGENICITY

(a) Hyoscyamine/atropine

Rat

Female Sprague-Dawley rats (n = 70), 39 days of age, were injected i.p. with 2.5 mg/kg bw atropine twice a day for 5 days and then observed for 28 months, with weekly palpations to detect formation of mammary tumours (Cabello et al., 2001). At termination, none of the animals treated with atropine had mammary tumours.

Sprague-Dawley rats (30 males and 31 females) were administered atropine (i.p., 6 mg/kg bw per week) over the course of their lifetime and observed for tumour formation (Schmähl and Habs, 1976). No effects were noted on either tumour frequency or mean lifespan compared to a control group.
Male Wistar rats (5 weeks old; n = 20) were provided drinking water containing 50 µg/mL N-methyl-N-nitro-N-nitrosoguanidine (MNNG) for 25 weeks and, at week 26, given 0.5 mg/kg bw atropine per day (dosing route not indicated), up to week 52 (Tatsuta et al., 1989). Atropine had no effect on the overall incidence of gastric cancer compared to the MNNG-olive oil control dose group (79 percent vs. 83 percent, respectively), but it was reported to significantly increased the number of gastric tumours per animal (control 1.2 ± 0.2 gastric tumours/rat, atropine-treated 2.7 ± 0.4 gastric tumours/rat, p < 0.001). Animals in the MNNG-atropine dose group also showed significant reductions in gastric acid secretion compared to the controls.

In a similar study, Wistar rats (6 weeks old male; n = 19) were provided drinking water containing 50 µg/ml MNNG for 25 weeks and then dosed s.c. with 0.5 mg/kg bw atropine every other day until the end of the experiment (week 52) (Tatsuta et al., 1996). Atropine had no effect on overall body weight gain, incidence rate of gastric tumours or number of tumours per rat compared to MNNG plus saline controls. Histological types and depth of involvement of gastric cancers in the atropine treated group also did not differ from controls.

(b) Scopolamine

Mouse

In a two-year chronic toxicity study conducted by the NTP, six-week-old male and female B6C3F1 mice (n = 70 per sex per treatment) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage at doses of 0, 1, 5 or 25 mg/kg bw per day (equivalent to 692, 3 460 and 17 302 µg/kg bw per day (-)-scopolamine). Ten male and female mice from each group received ophthalmic examinations prior to the start of dosing and again at 15 months. These mice were removed from the study following the 15-month examination without necropsy. An additional 10 male and female mice from each group were evaluated at 15 months for alterations in hematology, histopathology and organ weights (NTP, 1997). Survival rates for treated male and female mice were similar to the controls. A significant reduction in mean body weight was reported in male and female mice exposed to 17 302 µg/kg bw per day (-)-scopolamine from week 13 onwards, with final mean body weights 19 percent (males) and 16 percent (females) lower than the respective control group. A slight decrease in body weight was reported in male and female mice administered 3 460 µg/kg bw per day (-)-scopolamine after week 13. The body weights of male and female mice treated with 692 µg/kg bw per day (-)-scopolamine were similar to controls. Bilateral pupillary dilation was observed in both sexes at all tested doses; although ophthalmic examinations at 15 months revealed no significant findings (NTP, 1997).

At the 15-month interim evaluation, hematocrit, hemoglobin concentration, and erythrocyte count were slightly lower in female mice treated with 17 302 µg/kg bw per day (-)-scopolamine compared to controls, which was considered to be consistent with anemia related to the lower body weights and decreased
nutritional status exhibited by these mice. There were no treatment-related increases in the incidence of neoplastic or non-neoplastic lesions in male or female mice. A decreased incidence of hepatocellular neoplasms and some spontaneously occurring lesions, including pancreatic islet hyperplasia (males), alveolar epithelial hyperplasia (males), kidney nephropathy, pituitary gland pars distalis hyperplasia (females), bone marrow myelofibrosis (females), uterine cystic hyperplasia and hematopoietic cell proliferation in the spleen (females), were reported in dosed male and female mice compared to controls. This was considered to be related to the lower body weights of the dosed mice (NTP, 1997).

On the basis of the results summarized above, a LOAEL of 692 µg/kg bw per day (-)-scopolamine can be identified based on pupillary effects observed at the lowest dose tested in male and female B6C3F1 mice.

**Rat**

In a two-year chronic toxicity study conducted by the NTP, six-week-old male and female F344/N rats (n=60 per sex per treatment) were exposed to (-)-scopolamine hydrobromide trihydrate (adjusted for 89 percent purity) by oral gavage at doses of 0, 1, 5 or 25 mg/kg bw per day (equivalent to 692, 3 460 and 17 302 µg/kg bw per day (-)-scopolamine). Ten male and female rats from each group received ophthalmic examinations prior to the start of dosing and again at 15 months. These same rats were evaluated at 15 months for alterations in hematology, histopathology, and organ weights. Ten male and female rats from each group were administered neurobehavioural tests (including motor activity grip strength, thermal sensitivity, startle responsiveness, and passive avoidance) prior to the start of the study, on the first day of the study and after 3, 6, 9, 12, and 24 months of exposure (NTP, 1997).

Survival rates were significantly lower in female rats administered 692 and 17 302 µg/kg bw per day, although the reduced survival rate in female rats treated with 692 µg/kg bw was primarily attributed to a cage flooding accident. There were no treatment-related differences in survival in dosed male rats compared to controls. The mean body weights of female rats exposed to 17 302 µg/kg bw per day were reduced from week 25 onwards, with a final mean body weight 19 percent lower than that of the controls. The mean body weights of male rats treated with 17 302 µg/kg bw per day were slightly lower than controls on weeks 25 to 97 but had similar final body weights to those of controls. No differences in mean body weight were observed in male or female rats dosed with 692 or 3 460 mg/kg bw per day. Bilateral pupillary dilation was observed in both sexes at all tested doses; although ophthalmic examinations at 15 months revealed no significant findings (NTP, 1997).

Hematocrit levels were slightly higher in male rats treated with 17 302 µg/kg bw per day compared to controls, which was considered to be consistent with dehydration. Reticulocyte count was slightly lower in females exposed to 17 302 µg/kg bw per day, which was considered to be consistent with lower body weights and decreased nutritional status exhibited by these rats. Neurobehavioural changes were also reported in dosed female rats compared to controls, including increased horizontal motor activity at the highest dose on day 90, 180 and 360 and reduced
startle responses at the middle and highest doses on day 90. In male rats, exposure to 17 302 µg/kg bw per day also impaired passive avoidance behaviour on day 180 compared to controls. There were no treatment-related increases in the incidence of neoplastic or non-neoplastic lesions in male or female rats. A dose-dependent decrease in the incidence of pituitary gland adenoma and mononuclear cell leukemia was reported in male and female rats, which was considered partially related to the lower body weights in the dosed rats (NTP, 1997).

(c) Summary

Based the results summarized above, neither atropine nor (-)-scopolamine were carcinogenic in mice and/or rats. No NOAEL for (-)-scopolamine could be identified following chronic exposure regarding non-neoplastic effects, as effects were observed at all doses. The LOAEL for the long-term toxicity studies was 692 µg/kg bw per day (-)-scopolamine, the lowest dose tested, based on pupillary dilation. Given the lack of data on the long-term toxicity of atropine, a NOAEL and LOAEL for non-neoplastic effects could not be identified for this compound.

3.2.4 GENOTOXICITY

(a) Hyoscyamine/atropine

Atropine sulfate was tested at concentrations up to 5 000 nmol/plate in the Ames assay, using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 in the presence of rat liver S9 (Aroclor 1254 induced). No significant increase in revertants was noted at any dose (McCann et al., 1975).

(b) Scopolamine

In vitro, (-)-scopolamine was non-mutagenic in bacterial reverse mutation assays with two bacterial strains, Salmonella typhimurium TA98 and TA100, at concentrations up to 1 mg (-)-scopolamine/plate with or without rat liver homogenate (Waskell, 1978). Likewise, in an NTP study, (-)-scopolamine hydrobromide trihydrate did not induce mutations in Salmonella typhimurium TA97, TA98, TA100, TA1535 or TA1537 at concentrations of 100 to 10 000 µg/plate in the presence and absence of liver S9 from Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters (NTP, 1997).

In cytogenetic tests conducted by the NTP, there was no evidence of increased sister chromatid exchanges (SCE) in cultured Chinese hamster ovary cells at concentrations up to 500 µg (-)-scopolamine hydrobromide trihydrate/mL without metabolic activation or concentrations up to 5 000 µg (-)-scopolamine hydrobromide trihydrate with metabolic activation (liver S9 from Aroclor 1254-induced male Sprague-Dawley rats). While an increase in SCE was noted in the presence of metabolic activation, these findings were attributed to a pH shift in the culture medium produced by high concentrations of (-)-scopolamine hydrobromide trihydrate (≥ 2 000 µg/mL). In the presence of N-(2-hydroxyethyl) piperazine-
N’-(2-ethanesulfonic acid) (HEPES) buffer to maintain physiological pH no induction of SCE was observed. There was also no induction of chromosomal aberrations in cultured Chinese hamster ovary cells by (-)-scopolamine hydrobromide trihydrate without metabolic activation. However, with metabolic activation, (-)-scopolamine hydrobromide trihydrate increased chromosomal aberrations at the highest test concentration of 5 000 µg/mL, even in the presence of HEPES buffer to maintain pH (NTP, 1997). In vivo, exposure to (-)-scopolamine hydrobromide trihydrate up to doses of 1 200 mg/kg bw per day via gavage for 14 weeks did not increase the frequency of micronucleated polychromatic erythrocytes in peripheral blood samples obtained from male and female B6C3F1 mice (NTP, 1997).

(c) **Combined exposure to hyoscyamine and scopolamine**

In the study by Dugan *et al.* (1989), bone marrow samples were collected following the 90-day exposure to diets and analysed for the presence of micronuclei in polychromatic erythrocytes. Exposure to combined atropine/scopolamine doses of up to 19 mg/kg bw day (approximately 15 mg atropine and 4 mg scopolamine) for 90 days had no effect on micronuclei frequency.

(d) **Summary**

Based on the available in vitro (i.e., negative mutagenicity; negative SCE; weakly positive clastogenicity in the presence of metabolic activation only at highest concentrations) and in vivo (negative clastogenicity in mouse erythrocytes) data, the weight of evidence suggests that (-)-scopolamine is unlikely to exhibit genotoxicity in vivo. There is also no evidence that suggests atropine sulfate would exhibit genotoxicity in vivo.

### 3.2.5 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

(a) **Hyoscyamine/atropine**

**Reproductive toxicity**

No reproductive toxicity studies with hyoscyamine or atropine were identified.

**Developmental toxicity**

Pregnant CF-1 albino mice, 25–30 g, were treated on either gestation day 8 or 9 with a single 50 mg/kg bw dose (s.c.) of atropine sulfate and terminated on gestation day 18 for assessment of fetal anomalies. Treatment with atropine had no effect on maternal or fetal weight, fetal sex ratio and did not induce a significant increase in either soft tissue or skeletal anomalies (Arcuri and Gautieri, 1973).
(b) Scopolamine

Reproductive toxicity

No standard reproductive toxicity studies with (-)-scopolamine were identified. However, no toxicologically significant changes in sperm parameters or in estrous cycle length were reported in mice and rats following exposure to (-)-scopolamine hydrobromide trihydrate for 14 weeks (NTP, 1997).

Developmental toxicity

In a NTP teratology study, pregnant CD-1 mice (n = 23 to 32 per treatment) were exposed to (-)-scopolamine hydrobromide trihydrate at doses of 0, 10, 100, 450 or 900 mg/kg bw per day (equivalent to 6.9, 69.2, 311.4, 622.9 mg/kg bw per day (-)-scopolamine) by oral gavage from gestation day 6 to 15. The dams were euthanized on gestation day 17; and uterine weight and the number and status of uterine implantation sites were recorded. The live fetuses were also weighed and examined for external, visceral, and skeletal malformations. There was no evidence of maternal or fetal toxicity at doses of 6.9 and 69.2 mg/kg bw per day (-)-scopolamine. A marginal reduction in fetal body weight was reported at doses ≥ 311.4 mg/kg bw per day (-)-scopolamine, but only in the presence of maternal toxicity. There were no treatment-related differences in the number of congenital malformations or anatomical variations observed in the fetuses (NTP, 1987a).

In another teratology study conducted by the NTP, scopolamine hydrobromide trihydrate was administered via oral gavage to CD rats (n = 21 to 28 per treatment) at doses of 0, 10, 100, 450 or 900 mg/kg bw per day (equivalent to 6.9, 69.2, 311.4, 622.9 mg/kg bw per day (-)-scopolamine) from gestational day 6 to 15. The dams were euthanized on gestation day 20 and uterine weight and the number and status of uterine implantation sites were recorded. The live fetuses were also weighed and examined for external, visceral, and skeletal malformations. A dose-related reduction in maternal body weight and weight gain were reported at doses ≥ 6.9 mg/kg bw per day (-)-scopolamine. A marginal non-dose-related reduction in fetal body weight and a marginal non-dose-related increase in the incidence of malformations per litter were reported at doses ≥ 69.2 mg/kg bw per day (-)-scopolamine and a significant increase in the incidence of short ribs at doses ≥ 311.4 mg/kg bw per day (-)-scopolamine, but only in the presence of significant dose-related maternal toxicity (NTP, 1987b).

(c) Summary

Based on the results summarized above, there is no clear evidence of developmental toxicity following oral exposure to (-)-scopolamine in mice or rats in the absence of maternal toxicity. In rats, no maternal NOAEL for (-)-scopolamine could be identified, as effects on body weight were observed at all doses. The maternal LOAEL was 6.9 mg/kg bw per day (-)-scopolamine, the lowest dose tested. The fetal NOAEL was 6.9 mg/kg bw per day (-)-scopolamine based on decreased fetal bodyweight at doses ≥ 69.2 mg/kg bw per day (-)-scopolamine in rats, in the
presence of maternal toxicity. There was also no indication that atropine produced
developmental or maternal toxicity following a single s.c. injection during pregnancy.
The maternal and fetal NOAEL was 50 mg/kg bw for s.c. exposure.

3.2.6 SPECIAL STUDIES

(a) Experimental models for neurological effect

(-)-Scopolamine is commonly used as a standard/reference drug for inducing
cognitive impairments in humans and experimental animals (Ebert and Kirch, 1998;
Barak and Weiner, 2009; Klinkenberg and Blokland, 2010). The antidepressant-
like effects of (-)-scopolamine have also been explored extensively in humans and
experimental animals (Hasselmann, 2014). The use of (-)-scopolamine as a model
of cognitive decline and its antidepressant-like effects are not the focus of this
document and will not be discussed further.

3.2.7 OBSERVATIONS IN DOMESTIC ANIMALS/VETERINARY TOXICOLOGY

The ingestion of fresh plants containing TAs by livestock is uncommon due to the
general unpalatability of these plants (Piva and Piva, 1995). Toxicity in livestock
more commonly occurs due to the ingestion of animal feed contaminated with
plant material containing TAs, such as the seeds from Datura spp. The European
Union has regulated the presence of weed seeds, and unground and uncruushed fruits
containing TAs in products intended for animal feed, establishing a maximum limit
of 3 000 mg/kg at 88 percent dry matter (Directive 2002/32/EC). An individual
maximum limit of 1 000 mg/kg at 88 percent dry matter was established for
D. stramonium in products intended for animal feed.

Some consider pigs to be the most sensitive livestock species to TA intoxication,
followed by cattle, horses and chickens (Piva and Piva, 1995). Others have argued
that horses are the most sensitive, followed by pigs, cattle and poultry, based
on susceptibility and chance of exposure (Naidoo, 2012). In pigs, a LOAEL
of approximately 60 μg/kg bw per day has been identified based on a statistically
significant decrease in bodyweight gain following addition of a synthetic mixture
of (-)-scopolamine and (-)-hyoscyamine (98:2; corresponding to the ratio found in
D. ferox) to commercial feed (Piva et al., 1997). At the lowest doses tested
(1 500 μg/kg in feed), Piva et al. (1997) reported an approximate 10 percent decrease
in body weight after 76 days of treatment, which may have been related to a decrease
in mean feed consumption. In general, TAs are unpalatable to most livestock.
Pertinent information related to TA intoxications in livestock is summarized in
Table 13 below. More detailed reviews of the effects of TAs on livestock, can be
found in literature (EFSA, 2008; Naidoo, 2012; EFSA, 2013).
### TABLE 13  TROPANE ALKALOID (TA) INTOXICATION AMONG LIVESTOCK

<table>
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<th>THRESHOLDS OF TOXIC EFFECTS</th>
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<td><strong>Horses</strong></td>
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| > Several reports of toxicosis in horses following the ingestion of animal feed (grains) contaminated with *Datura* plants.  
> The clinical signs and course of intoxication vary widely.  
> 0.01 percent *D. innoxia* plant material in diet (equivalent to 0.037 mg (−)-hyoscyamine and 0.055 mg (−)-scopolamine/kg of feed) was associated with no clinical signs of intoxication.  
> A threshold for toxic effects was observed in horses exposed to 0.5 g of air-dried *D. innoxia* (equivalent to 0.275 mg scopolamine and 0.185 mg atropine)/kg of feed which was associated with gastrointestinal atonia, tachycardia, sweating, and colic approximately 2 hours after dosing. Symptoms had not resolved after 72 hours.  
> Signs of toxicity were observed at oral doses of 100 µg/kg bw of (−)-hyoscyamine. | EFSA 2008;  
EFSA 2013;  
Galey *et al.*, 1996;  
Naude *et al.*, 2005;  
Naidoo, 2012 |
| **Pigs**       |                             | EFSA 2008;  
EFSA 2013 |
| > 1.5 mg TAs/kg of feed was initially considered the threshold limit for *Datura* alkaloid intoxication in pigs (20 to 60 kg), equivalent to 60 µg/kg bw for a 60 kg pig.  
> A follow-up study determined that exposure to 1.5 mg/kg feed of a synthetic mixture of TAs (ratio of 0.98 (−)-scopolamine to 0.02 (−)-hyoscyamine, corresponding to the ratio of these alkaloids in *D. ferox*) led to growth inhibition in the pigs, suggesting a lower limit for *D. ferox* should be considered, particularly for long-term exposure.  
> It is unclear whether *Datura* seed consumption can lead to teratogenic effects. Sows that consumed *D. stramonium* during early gestation (no estimate of intake) showed signs of *Datura* intoxication during the second and third months of pregnancy and gave birth to piglets with bony deformities. Later experimental studies could not reproduce the teratogenic effects. | EFSA 2008;  
EFSA 2013 |
| **Cattle**     |                             | EFSA 2008;  
EFSA 2013 |
| > Signs of toxicity are likely to occur at levels exceeding 500 µg of (−)-hyoscyamine plus 100 µg (−)-scopolamine/kg bw.  
> A level of up 300 µg/kg bw total alkaloids may be safe and has been proposed by EFSA (2013) as a NOAEL. | EFSA 2008;  
EFSA 2013 |
| **Sheep and Goats** |                             | EFSA 2008;  
EFSA 2013 |
| > Considered resistant to the adverse effects of large intakes of *Datura* spp. due to the expression of atropine esterase in plasma, which can hydrolyse TAs.  
> Sensitivity to TAs may vary across breeds due to differences in the level of atropine esterase expression. | EFSA 2008;  
EFSA 2013 |
| **Poultry**    |                             | EFSA 2008;  
EFSA 2013 |
| > May be more resistant to TA exposure due to the expression of an atropine hydroxylase-like enzyme that can inactivate TAs.  
> Levels up to 3 percent *D. stramonium* seeds (containing mainly (−)-hyoscyamine) in feed are not expected to have short-term effects in broiler chicks and hens.  
> Levels up to 150 mg/kg feed of *D. ferox* alkaloids (containing mainly (−)-scopolamine) showed no adverse effects in poultry. | EFSA 2008;  
EFSA 2013 |
| **Rabbits**    |                             | EFSA 2008;  
EFSA 2013 |
| > Considered resistant to the adverse effects of TAs due to the expression of atropine esterase in plasma, which can hydrolyse these alkaloids.  
> Not all breeds appear to produce atropine esterase and in breeds that do express this enzyme, production may be seasonal or vary among individuals. | EFSA 2008;  
EFSA 2013 |

* As summarized in Naude *et al.*, 2005.
3.2.8 MECHANISM OF ACTION

(a) Muscarinic acetylcholine receptors

The majority of therapeutic/toxic effects associated with TAs are considered to be due to their competitive antagonistic binding to muscarinic acetylcholine receptors. Muscarinic acetylcholine receptors (mAChRs), or simply muscarinic receptors, are a subclass of G-protein coupled receptors comprised of at least five subtypes: M₁, M₂, M₃, M₄ and M₅. Muscarinic receptors bind acetylcholine, a neurotransmitter in both the peripheral (PNS) and central nervous systems (CNS) (Brown and Taylor, 2006; Langmead et al., 2008; Haga, 2013). The mAChR subtypes are variably expressed throughout the CNS and the PNS (Brown and Taylor, 2006; Langmead et al., 2008; Haga, 2013; Kohnen-Johannsen and Kayser, 2019).

Within the PNS, muscarinic receptors are located on autonomic ganglia and nerve terminals as well as on target organs of parasympathetic neurons (Brown and Taylor, 2006; Haga, 2013). The M₁ subtype is predominately expressed in the autonomic ganglia, enteric nerves, and gastric and salivary glands, but has also been detected in the heart and smooth muscle (Preiksaitis et al., 2000; Wang et al., 2001; Abrams et al., 2006; Brown and Taylor, 2006). The M₂ subtype is abundant in the heart, smooth muscle and on autonomic nerve terminals (Brown and Taylor, 2006; Preiksaitis et al., 2000; Wang et al., 2001). The M₃ subtype is also found in the heart and smooth muscle as well as the salivary glands (Preiksaitis et al., 2000; Wang et al., 2001; Abrams et al., 2006; Brown and Taylor, 2006). The M₄ and M₅ subtypes are preferentially expressed in the CNS, but the M₅ subtype can also be found at low levels in the periphery in the heart and smooth muscle (Preiksaitis et al., 2000; Wang et al., 2001; Brown and Taylor, 2006).

Within the CNS, M₁ is the most predominant subtype and is located postsynaptically in the cortex, hippocampus, striatum and thalamus (Abrams et al., 2006; Brown and Taylor, 2006; Langmead et al., 2008) and presynaptically as a heteroreceptor on dopaminergic axon terminals of the striatum (Raiteri et al., 1990). Heteroreceptors are presynaptic receptors that can regulate the release of neurotransmitters from that neuron. Heteroreceptors differ from autoreceptors in that they cannot be activated by the neurotransmitter that they release. The M₂ subtype is expressed in multiple brain regions, including the brainstem, thalamus, cortex, hippocampus and striatum (Rouse et al., 1997; Abrams et al., 2006; Langmead et al., 2008). Pharmacological studies have suggested that the M₂ subtype may act as a muscarinic autoreceptor in the basal forebrain, hippocampus and striatum regulating the release of acetylcholine (Raiteri et al., 1990; Rouse et al., 1997) and as a presynaptic muscarinic heteroreceptor in the hippocampus regulating the release of GABA, glutamate and aspartate (Rouse et al., 1997). There is also evidence that M₂ subtype is expressed postsynaptically at cholinergic and non-cholinergic synapses in the striatum, basal forebrain, medial septal nucleus and hippocampus (Rouse et al., 1997). The M₃ subtype is much less abundant in the brain than the M₁ and M₂ subtypes and is predominately located in the cortex, hippocampus and thalamus (Abrams et al., 2006; Langmead et al., 2008). The M₄ subtype has been detected
in multiple brain regions including the hippocampus and cortex but is most abundant in the striatum where it is believed to regulate the release of dopamine (Langmead et al., 2008). The M5 subtype has been detected at very low levels in the CNS including the ventral tegmental area, substantia nigra and hippocampus, and may play a role in the regulation of the nigrostriatal pathway (Vilario et al., 1990; Abrams et al., 2006; Langmead et al., 2008).

Responses under the control of muscarinic receptors include cardiac slowing, contraction of various smooth muscles (gastro-intestinal tract, detrusor muscle of the bladder, bronchioles, urethra, gall bladder and ducts, iris circular muscle, seminal vesicles and vas deferens), vasodilatation and increased secretion from exocrine glands (salivary glands, mucosal glands of the airways, gastric acid secretion and lachrymal secretion) (Broadley and Kelly, 2001). The secretion of sweat is anatomically and physiologically a sympathetic response but is mediated by binding of acetylcholine onto muscarinic receptors.

**Hyoscyamine/atropine**

Competition binding assays carried out with membranes prepared from porcine brains using the non-selective muscarinic receptor antagonist [H] quinuclidinyl benzilate (QNB) indicate that atropine has a high affinity for most muscarinic receptors, IC50 = 0.0047 µM (Schmeller et al., 1995). The IC50 values for tropic acid and tropine are > 500 µM, which would indicate these compounds are almost inactive at muscarinic receptor binding (Schmeller et al., 1995). QNB is a muscarinic receptor nonselective antagonist, which binds with similar affinity to both membrane and intracellular muscarinic receptors. The Ki values for atropine inhibiting [H] QNB binding to cloned human muscarinic receptors are 0.50, 0.90, 1.1, 0.6 and 1.7 nM for the M1 through M5 subtypes, respectively (Bolden et al., 1992). The majority of non-selective muscarinic receptor antagonism for atropine has been associated with binding of the optical active isomer, (-)-hyoscyamine, to muscarinic receptors. For example, in experiments on postganglionic acetylcholine receptors in guinea-pig ileum, Barlow et al. (1973) reported there is a 300-fold difference in binding affinities for (-)-hyoscyamine compared to (+)-hyoscyamine.

Uchida et al. (1978) reported that the affinity of atropine for binding to synaptic plasma membranes from rat brains was 20 times greater than acetylcholine. Similarly, the competition for [H] QNB binding in guinea pig jejunum membrane revealed that atropine was more potent than acetylcholine, pKd = 6.8 verses 5.25, respectively (Yuan et al., 2005).

Low doses of atropine have been demonstrated to slow the heart rate, an effect initially thought to be due to central vagal stimulation by atropine doses too low to block the peripheral muscarinic receptor. However, cardiac slowing is also observed with muscarinic receptor antagonists that do not readily cross the blood brain barrier (Brown and Taylor, 2006) and it has been suggested that the mechanism of decreased heart rate may be due to low plasma concentrations inhibiting prejunctional muscarinic receptors. Inhibition of prejunctional muscarinic receptors, which regulate acetylcholine release from autonomic ganglia and parasympathetic nerve-endings,
may result in a transient release of acetylcholine (Alihanka et al., 1994) thereby resulting in a decreased heart rate. Similarly, Golding and Stott (1997) suggest that the bradycardia induced by antimuscarinics may be due to blockade of M1 receptors on the postganglionic parasympathetic neurons, whereas larger doses cause tachycardia by blocking vagal effects on M2 receptors on the sinoatrial nodal pacemaker. Support for this biphasic heart rate response to atropine was seen in experiments where relatively low doses of the dextro enantiomer of atropine, (+)-hyoscyamine, in vitro and in vivo (10^{-14} to 10^{-12} M and 5 µg/kg bw i.p., respectively) were able to induce significant increases in the release of acetylcholine (Ghelardini et al., 1997). The heart rate is under parasympathetic control of the M2 receptors on the sinoatrial node, which when blocked to a significant degree by atropine causes the typical tachycardia effect. This paradoxical bradycardia is not observed in all human studies and is thought to be related to route and time of dose administration. Earlier studies had demonstrated bradycardia could be induced by doses of atropine < 0.3 mg i.v. whereas higher doses produce the typical tachycardia.

In the earlier study by Murrin (1973), at low oral doses of atropine (9 µg/kg bw), an initial slowing of the heart rate was observed whereas only tachycardia was evident at a dose of 28 µg/kg bw. Similarly, in the study by Lönnroth and Widerlöv (1975), a low dose of atropine (approximately 3.5 µg/kg bw; i.m.) produced bradycardia whereas higher doses (>10 µg/kg bw) produced tachycardia.

**Scopolamine**

Competition binding assays carried out with membranes prepared from porcine brains using the non-selective mAChR antagonist [³H] QNB indicate that (-)-scopolamine has a high affinity for muscarinic receptors with an IC₅₀ of 0.0020 µM (Schmeller et al., 1995). Affinity estimates (pKᵢ) for (-)-scopolamine derived from [³H] N-methylscopolamine displacement experiments with cloned human M₁, M₂, M₃, and M₄ receptors were 8.95, 8.68, 9.41 and 9.47, respectively (Huang et al., 2001). In a review, Lakstygal et al., (2019) reported that (-)-scopolamine has a high affinity for M₁, M₂, M₃ and M₄ receptor subtypes and a low affinity for the M₅ receptor subtype.

Due to the widespread localization of the muscarinic receptors throughout the PNS and the CNS, (-)-scopolamine can lead to a wide variety of physiological and behavioural effects. The peripheral antimuscarinic effects of (-)-scopolamine are related to the inhibition of the parasympathetic nervous system and include reduced salivary, bronchial and sweat gland secretion, mydriasis, cycloplegia, changes in heart rate, inhibition of micturition, inhibition of gastric acid secretion, and reduced gastrointestinal tone (EFSA, 2013; Kohnen-Johannsen and Kayser, 2019). Similar to hyoscyamine, effects on heart rate appear paradoxical with bradycardia at low doses and tachycardia at high doses. The central antimuscarinic effects of (-)-scopolamine include CNS depression, drowsiness, amnesia, restlessness, disorientation, hallucinations, delirium and death (EFSA, 2013; Kwakye et al., 2018; Lakstygal et al., 2019; Volgin et al., 2019). Although (-)-scopolamine elicits its therapeutic/critical toxic effects via the antagonism of muscarinic receptors, antagonism of the
muscarinic receptors of the heart muscle should theoretically cause an increase in heart rate. The mechanism of action of the paradoxical decrease in heart rate at low doses of scopolamine has not been clearly elucidated and has been discussed above.

Note, the inhibition of muscarinic autoreceptors by (-)-scopolamine can lead to the enhanced release of acetylcholine at cholinergic synapses (Wessler et al., 1987; Toide, 1989; Pfister et al., 1994; Jackson et al., 1995). (-)-Scopolamine can also enhance the release of other neurotransmitters, including serotonin, norepinephrine and dopamine, at CNS synapses (Bhattacharya and Sen, 1992; Di Giovanni and Shi, 2009; Qiu et al., 2016). This enhanced release likely occurs indirectly via muscarinic autoreceptor blockade or directly via muscarinic heteroreceptor blockade and could activate various signalling cascades and/or modulate other neurotransmitter/receptor systems.

The results of the receptor binding assays for (-)-scopolamine described above were carried out using in vitro assays. Therefore, the binding affinities observed in these assays may not correlate with potencies observed in vivo.

(b) Other receptors

Hyoscymine and scopolamine can also act as competitive antagonists at 5-hydroxytryptamine type-3 (5-HT₃) receptors, which are excitatory, ligand-gated ion channels located throughout CNS and PNS (Lochner and Thompson, 2016). The application of atropine or scopolamine with 5-hydroxytryptamine (i.e., serotonin: a 5-HT₃ receptor agonist) resulted in concentration-dependent inhibition of the serotonin-evoked response, Kᵦ₃ = 1.89 µM and 3.23 µM, respectively (Lochner and Thompson, 2016). Additionally, at high concentrations, atropine and scopolamine can also inhibit nicotinic acetylcholine receptors, IC₅₀ = 284 µM and 928 µM, respectively (Schmeller et al., 1995). Binding affinities for atropine and scopolamine were much lower for the nicotinic receptors compared to the muscarinic receptors, which are in the nanomolar range (Schmeller et al., 1995).

3.3 OBSERVATIONS IN HUMANS

3.3.1 HYOSCYNAME/ATROPINE

(a) Oral

Human infants/children of ages ranging from less than 1 year to 12 years (n = 9–15 per dose group) were provided oral doses of atropine sulfate (0.1–0.7 mg) and then assessed 1 hour after dosing for salivary secreting ability. The minimal effective dose (MED), defined as the atropine dose required to cause reduced salivary secretion by 25–33 percent, was somewhat lower in infants of 1 to 12 months of age and toddlers from 12 to 36 months (16 µg/kg bw and 14 µg/kg bw, respectively) compared with older children 3 to 6 years (22 µg/kg bw) and 6 to 12 years (20 µg/kg bw) (Unna et al., 1950). Reduction of salivary secreting ability subsided by 4 hours after dosing.
Comparison of MEDs between oral and i.v. dosing indicated the oral MEDs for atropine sulphate were on average 3.4-times greater than the i.v. MEDs.

In male volunteers (18–30 years old, n = 6) who had been dosed with 3.0 mg atropine sulphate orally, there was an increase in pulse rate (approximately 35 percent), with maximum effect reached 1 hour after dosing, with the pulse rate returning to pre-dose values by 2 hours (Cullumbine et al., 1955). At a higher dose (5 mg), a more pronounced effect on heart rate was observed (50 percent increase) which was reached 45 minutes after dosing and only returned to pre-dosing values after 7 hours. No significant effect on heart rate was observed in subjects provided either a 2 mg dose of atropine or two 2 mg doses, with 4.5 hours between doses. The authors suggested that 1 mg of atropine sulphate i.v. would cause a similar effect on heart rate as 2 mg i.m. or s.c. or 5 mg oral (Shutt and Bowes, 1979).

Nine healthy adult volunteers (three females, six males; age range 27-40 years) were given oral doses of atropine sulphate at 7 µg/kg bw (n = 3), 14 µg/kg bw (n = 9) and 28 µg/kg bw (n = 6) with a minimum of three days between two treatments in the same subject (Murrin, 1973). Beginning at 30 minutes after dosing and up until 5 hours, subjects were monitored for heart rate, blood pressure, salivary secretion and pupillary dilation. A dose dependent reduction in salivary secretion was observed at all the three doses (range 32.5 to 68.0 percent), with maximum effect reached by approximately 1–2 hours post treatment. ED$_{50}$ for inhibition of salivary secretion was estimated at 18 µg/kg bw. Heart rate was statistically significantly higher (27.3 percent) at the highest dose by 30 minutes after dosing and this persisted for up to 2 hours while heart rate was significantly lower in subjects treated with 7 µg/kg bw (19 percent) (starting from 1.5 hours post treatment and persisting for one hour). No significant changes were observed for heart rate in the subjects treated with 14 µg/kg bw atropine or with any dose for blood pressure or pupillary dilation.

Human volunteers (5 males and 1 female; 31.8 ± 4.1 years old) were administered single oral doses of 0, 0.5, 1.0 or 2.0 mg atropine (approximately 8, 16 and 32 µg/kg bw, respectively) and assessed at 30 minutes, 60 minutes and then hourly after dosing for 6 hours for effects on salivary secretion, heart rate, arterial pressure, body temperature, pupillary size, near-point of vision and sweat-gland activity (Mirakhur, 1978). Maximum reduction in salivation was observed 2 hours after dosing (38 percent - 1.0 mg; 57 percent - 2.0 mg), with the decreases significant at 2 and 3 hours following the 1.0 mg dose and between 2 and 6 hours with the 2.0 mg dose. No significant effect on heart rate was observed with the 0.5 mg dose while in subjects treated with 1.0 mg atropine, there was a slight, non-statistically significant decrease (approximately 16 percent) and a slight, non-statistically significant increase in heart rate (approximately 13 percent) at 2.0 mg atropine. Sweat gland activity (number of measured active glands) was reduced at all doses tested with the effect being more pronounced 2 hours after dosing at the 2 highest doses. Only the 2.0 mg atropine dose induced appreciable pupillary dilation, reaching a maximum response by 4–6 hours after dosing. Based on the observed effects, the author reported that the oral to i.m. potency ratio for atropine appeared to be 2:1.
Male volunteers (n=8, 19–23 years old) were provided oral doses (tablet form) of 0.85 mg or 1.7 mg atropine sulphate and then assessed 1, 2 and 4 hours after dosing for a variety of physiologic and psychologic effects (Seppälä and Visakorpi, 1983). Both doses of atropine significantly increased pupil diameter which persisted over the 4 hour observation period while heart rate was only increased in the test subjects provided the 1.7 mg dose (maximum increase of 20 bpm by 2 hours) which also persisted to the end of the observation period. Atropine was also reported to increase errors on tests of coordination and caused a dose-related depression in subjective measures of performance. It was hypothesized that the psychological effects were in part related to the unspecific sedative properties of atropine.

In the study by Saarnivaara et al. (1985), no significant effects on heart rate, blood pressure or temperature were noted in the children provided an oral dose of 0.03 mg/kg bw atropine. The children dosed with 0.02 mg/kg bw atropine i.m. exhibited an increase in heart rate by the first observation period (30 minutes) which was significantly different from controls up until 2 hours after dosing. No significant changes in blood pressure were noted in the i.m. dose group while mean significant temperature increases of 0.5 (oral) and 0.7 °C (i.m.) were noted but described as clinically “hardly significant”.

Eight human volunteers (seven male and one female, mean age 25.5 years) were provided a single oral dose of atropine (1 mg) by capsule and heart rate and salivary secretion measured 1, 3 and 7 hours after dosing (Brion et al., 1988). Atropine significantly reduced salivary output by approximately 54 percent and heart rate was reduced by approximately 13 percent. Maximal effects were seen after 1 hour for heart rate and 3 hours for salivary secretion. Pulse rate had returned to pre-dosing values by 3 hours whereas in 7 of 8 test subjects, salivary secretion was still depressed at the final observation time point (7 hours). Children (n=60, <12 years old) were given an oral dose of atropine sulphate, 0.02 mg/kg bw, and then monitored for changes in heart rate, skin temperature, flushing and degree of dryness of the mouth beginning 20 minutes after dosing and continuing for up to 90 minutes (Chaudhari et al., 1989). For comparison, a similar group received a single i.m. injection of 0.01 mg/kg bw of atropine sulfate. Increase in heart rate was similar with both dosing routes (15–19 bpm), with the only difference noted being a significant initial decrease in heart rate (6 bpm) observed in the oral dose group at 20 minutes after dosing. Inhibition of salivary secretion, as determine by subjective mouth dryness, was also similar in both dose groups with onset occurring more rapidly in the i.m. dose group (35 minutes versus 70 minutes, respectively). Average skin temperature increases of 0.2 °C to 0.5 °C after 40 minutes were noted in both groups.

Male volunteers (21–56 years old, average body weight 87.9 kg) were given a single oral dose of 0.03 mg/kg bw atropine with heart rate, blood pressure and salivary secretion monitored up to 3 hours after dosing (Volz-Zang et al., 1995). Heart rate was slightly increased, but not significantly different from controls. In a comparable dose group given a single i.m. dose of 0.02 mg/kg bw atropine, heart rate did significantly increase (maximum 22 bpm, 45 minutes after dosing). Similar decreases in salivary secretion were observed with both dose groups compared to controls (84.3 percent oral, 87.5 percent i.m.).
In a similar study design, children (n = 15; average age 5.6 years) were provided a single oral dose of atropine sulphate of 0.03 mg/kg bw (average dose 0.61 mg) prior to anaesthetic and heart rate continually monitored by electrocardiography (ECG) (Gervais et al., 1997). There were no significant changes in heart rate and no episodes of bradycardia observed.

Human infants (n = 59; approximately 16 weeks of age) received a single oral dose of 0.04 mg/kg bw atropine sulphate 1 hour prior to elective surgery and then monitored for changes in heart rate (Shaw et al., 2000). There was a significant increase in peroperative heart rate (22 percent or 15 bpm) observed in the test group receiving atropine compared to the control group.

In a review of nine cases of accidental poisoning with oral drops of Eumydrin (0.6 percent atropine methonitrate) in infants between 1–27 weeks of age, relatively severe signs (tachycardia, irritability, dilated and unresponsive pupils) were noted with atropine methonitrate doses ranging from 0.8–16 mg (0.31–2.80 mg atropine/kg bw) (Meerstadt, 1982). Atropine methonitrate was used to treat whooping cough in infants/young children and is considered to be less lipid soluble because of the presence of a quaternary nitrogen atom that limits penetration of the blood-brain barrier and therefore produces fewer CNS effects.

Groups of human volunteers (n = 9-10, age 19–21 years) were administered a single oral dose of atropine sulphate (1.2 mg or approximately 17 µg/kg bw) and monitored for changes in heart rate, pupil diameter and salivary secretion up to 155 minutes after dosing (Fry and Burr, 2011). Pulse rate initially declined by approximately 7 bpm (maximum effect reached 30 minutes after dosing) and then increased to near control values by 130 minutes. Salivary flow decreased, beginning approximately 25 minutes after dosing, reaching a maximum effect by 155 minutes after dosing. No significant change in pupil diameter was noted. The authors suggested that salivary flow was the most sensitive variable measured and the oral dose used produced similar changes as a s.c. dose of 0.84 mg (12.0 µg/kg bw).

An oral dose of 1 mg atropine sulphate was administered to 12 healthy subjects (7 males, 5 females; age 22–29 years, body weight 58–85 kg) and then heart rate, salivary secretion and pupil function monitored for up to 12 hours after dosing (Müller et al., 2012). Heart rate was not affected but salivary secretion was significantly inhibited by approximately 40 percent. Pupil diameter was also significantly increased by an average of 0.22 mm.

There is limited information from the therapeutic use of atropine and scopolamine during human pregnancy. The U.S. Collaborative Perinatal Project, a prospective study of 50 282 mother-child pairs, recruited between 1959 and 1965, studied the effect of medications on pregnancy outcomes. It reported on 401 mothers exposed to atropine, 322 exposed to hyoscyamine and 309 exposed to scopolamine, during the first four months of pregnancy. None of these three drugs were associated with congenital malformations; survival- and race-standardised relative risks ranged from 0.88 to 1.02 (Heinonen et al., 1977).
Earlier studies had demonstrated that fetal bradycardia could be treated by administering atropine to the mother, suggesting effective placental transfer (Hon, 1962). In 51 pregnant women (between 38 weeks gestation and term) who had been administered a single dose of 8.5 µg/kg bw atropine sulphate i.v., no change in fetal heart rate was detected in 10 patients, 26 patients showed a fetal tachycardia response (10–27 percent increase in heart rate), 8 cases showed bradycardia with no recovery within the 30 minute observation period, while the remaining 7 cases demonstrated an initial bradycardia followed by tachycardia (John, 1965). Heart rate changes were initially delayed with 23 percent (n = 6) of tachycardia cases occurring within 5 minutes after maternal dosing and 57 percent (n = 15) between 6–10 minutes after maternal dosing.

Reductions in fetal breathing movements, lasting 5–10 minutes, were described within 2 minutes of administration of 0.5 mg atropine i.v. to mothers between 37 and 42 weeks of gestation undergoing induction of labour. There were no effects on fetal hypoxia, fetal heart rate or beat-to-beat variability in 13 out of 15 fetuses (Roodenburg et al., 1979).

Summarizing all the available data on malformations and fetal effects in utero, Schaefer et al., (2007) concluded that neither atropine nor scopolamine were associated with adverse developmental effects or significant fetotoxicity.

A French database, containing over 43 000 mother-child pairs recruited between 2004 and 2010, has explored the possibility of more subtle postnatal effects of drugs with “atropinic” properties as their main or side effects. None of the listed drugs were atropine, hyoscyamine or scopolamine themselves. The drugs were assigned an atropinic score (0 = null, 1 = low, 3 = strong) and the prenatal atropinic burden (AB) calculated as the sum of atropinic scores of drugs prescribed. Twenty-seven drugs were listed as strong potency, 42 as low potency for atropinic effects. Thirty-four percent of the mothers received at least one atropinic drug during pregnancy. At 24 months of age, more infants of mothers with AB ≥1 had difficulties to ‘name a picture’ (OR = 1.18 [95 percent CI 1.03; 1.36]) and to ‘understand instructions’ (OR = 1.61 [95 percent CI 1.13; 2.30]) compared with infants of unexposed women (Beau et al., 2016).

The same group has investigated the association between prenatal exposure to drugs with atropinic properties and the use of digestive disorder medications in childhood (0–3 years) in 8 372 children. More than 30 percent of the children were exposed prenatally to atropinic drugs. Exposed children used significantly more digestive disorder medications than unexposed children (RR = 1.11 [95 percent C.I.1.06; 1.16]). The strength of the association increased with the prenatal atropinic burden, which was calculated in the same way as in their earlier study (Benevent et al., 2019).

(b) Non-oral exposure routes

Male human volunteers (average age 23 years) were given a single dose of atropine sulphate i.m. (0, 32, 50, 75 or 125 µg/kg bw; n = 4–8 per dose group) and then assessed for various physiological measures, including heart rate, blood pressure, axillary temperature and respiratory rate, over the proceeding 24 hours (Ketchum et al., 1973).
Heart rate increase was observed with all doses, with the ED\textsubscript{50} for producing an increase of greater than 30 bpm calculated as 17.8 µg/kg bw. Blood pressure was reported to be increased (data not shown) while there were minimal changes to both respiration rate and axillary temperature.

Human volunteers (25–35 years of age; n = 9–17 per dose group) were provided doses of 0.1–0.8 mg atropine sulphate by i.v. and then monitored for sinoatrial (S-A) nodal rate (as determined from ECG mean P-P interval) for up to 5 minutes after dosing (Das \textit{et al.}, 1975). At doses less than 0.3 mg (4.3 µg/kg bw), there was a significant decrease in S-A nodal rate by approximately 9 percent whereas at doses greater than 0.4 mg (5.7 µg/kg bw), there was a significant increase (30 percent).

Atropine sulfate (0, 0.25, 0.40, 0.75 and 1.50 mg) was administered i.v. to adult human volunteers (n = 5–7 per dose) and heart rate, systolic and diastolic blood pressure and salivary secretion studied for one hour after dosing (Lönnerholm and Widerlöv, 1975). At the lowest dose, there was a decrease in heart rate (approximately 10 bpm) which occurred 2–4 minutes after dosing and persisted throughout the observation period. No effect on heart rate was seen with the 0.40 mg dose while the two top doses caused a dose-dependent increase in heart rate (30 and 55 bpm, respectively) which peaked after 2–4 minutes and remained elevated through the 60-minute observation time. All doses produced an inhibition of salivary secretion with the maximum affect occurring after 15 minutes with a clear dose response relationship. Estimated ED\textsubscript{50} (from graphed data) for inhibition of salivary secretion was approximately 0.90 mg (13 µg/kg bw based on a 70 kg bw).

Human volunteers (n = 9; 19–32 years old) were provided atropine sulfate doses of 0.4–40 µg/kg bw i.v. and then monitored for changes in heart rate and salivary secretion for up to 6 hours following dosing (Wellstein and Pitschner, 1988). A slowing of the heart rate was observed with doses less than 3.0 µg/kg bw (maximum decrease of 7.6 bpm or approximately 13 percent) while at the highest dose of 40 µg/kg bw, heart rate increased by 41.6 bpm (approximately 67 percent). Inhibition of salivary secretion was observed at all doses. ED\textsubscript{50} values for achieving maximum heart rate increase and inhibition of salivary secretion were reported as 7.42 µg/kg bw and 2.98 µg/kg bw, respectively.

Atropine sulphate was administered to male human volunteers (n = 7; mean ages and body weights 26.3 years and 73.9 kg, respectively) as a single i.m. dose of 0, 1.5, 3.0 or 6.0 mg (0, 21, 42 and 84 µg/kg bw) with a minimum time of 72 hours between doses. Heart rate, blood pressure and pupil diameter were measured, starting 30 minutes after dosing and continually at regular intervals for up to 24 hours (Higgins \textit{et al.}, 1989). Maximum effect on heart rate (increase) was reached by 30 minutes post dosing and was dose-dependent (low dose-26.7 percent; mid dose-37.5 percent; high dose-38.9 percent). Heart rate declined and returned to values below placebo levels within 7 to 9 hours after dosing. Pupil diameter also increased, with the effects beginning by 30 minutes after dosing and reaching a maximum by 1.5 hours. There was no significant difference in pupil effects between dose groups (average change of 0.5 mm compared to placebo) whereas slight decreases in systolic pressure significantly below placebo levels were noted with the 1.5 and 3.0 mg doses, but not the 6 mg dose.
In a similar study, anesthetized human infants (n = 22–40 per dose group; average age 2.3–3.0 years), were provided atropine sulphate doses by i.v. of 0, 5, 10, 20, 30 or 40 µg/kg bw. Based on body weight, total administered doses ranged from 75–480 µg. Peak heart rate increased with increasing atropine doses up to 30 µg/kg bw, with ED₅₀ and ED₉₀ values for maximal response estimated from the dose response curve as 9 µg/kg bw and 26 µg/kg bw, respectively (Palmisano et al., 1991). In 15 percent (n = 6) of children in the low dose group (5 µg/kg bw), there was an initial slowing of the heart rate by an average of 7 bpm which was described as clinically insignificant.

Two groups of human male volunteers, average ages of 26 and 60 years, were provided bolus doses (i.v.) of 0.03, 0.06, 0.12, 0.24, 0.48 and 0.96 mg atropine sulphate (5 minute infusion per dose) with increasing doses every 20 minutes. Blood pressure was continually monitored beginning at 5 minutes after the first dose while heart rate was monitored throughout the experiment by simultaneous recordings of an ECG lead (Poller et al., 1997). Atropine caused an initial decrease in heart rate for volunteers in the younger age group at doses less than 0.12 mg (1.7 µg/kg bw) (maximum decrease 14 bpm or 23 percent) while, in the older age group, atropine only marginally decreased HR at these same doses. At doses greater than 0.12 mg, atropine caused a similar increase in heart rate which was not significantly different between the 2 groups (maximum increase of approximately 30 bpm). Atropine caused a slight increase in blood pressure at similar doses as the increased heart rates were observed but there were no differences between the two age groups. It was postulated by the authors that the decrease in heart rate by low doses of atropine was due to the inhibition of presynaptic inhibitory M₁-cholinoceptors in the heart, thereby enhancing the release of acetylcholine (Poller et al., 1997).

Anesthetized human infants (n = 60), median age 6.5 months, were provided a dose of atropine sulphate, 5 µg/kg bw (average dose 40.9 µg) i.v. and monitored for heart rate effects by simultaneous ECG. An increase in heart rate was detected as early as 30 seconds after dosing (7 percent), with further increases after 1 minutes (14 percent) and after 5 minutes (25 percent) (Eisa et al., 2015). Twenty-nine infants (48 percent) experienced tachycardia (heart rate increase greater than 20 percent above baseline rate) after atropine, which persisted up until the end of the 5-minute observation period. No indication of bradycardia, defined as a minimum 20 percent decrease in heart rate from baseline, was observed in this study.

In a study investigating the use of atropine autoinjectors in 240 children (74 percent male) under the age of 16 years during the Gulf War, atropinization signs were recorded after atropine doses (majority s.c in the fingers or palm) ranging from 0.01–0.17 mg/kg bw (Amitai et al., 1992). The most common symptoms observed in the children were dilated pupils (43 percent), tachycardia (39 percent), dry membranes (35 percent) and flushed skin (20 percent). Seizures and life-threatening arrhythmias were not reported, and there were no fatalities. It was estimated by the authors that doses up to 0.045 mg/kg bw produced no signs of atropine intoxication while doses less than 0.175 mg/kg bw produced only mild signs.
### TABLE 14 HUMAN OBSERVATION SUMMARIES FOR ATROPINE

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>DOSE(^a) (mg)</th>
<th>EFFECTS(^b)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants/children</td>
<td>0.1–0.7</td>
<td>S.S. ↓ (MED&lt; 3 years = 14-16 µg/kg bw) ↓ (MED&gt; 3 years = 20-22 µg/kg bw)</td>
<td>Unna et al., 1950</td>
</tr>
<tr>
<td>Adults</td>
<td>7–28 (µg/kg bw)</td>
<td>S.S. ↓ (ED(_{50}) = 18 µg/kg bw) H.R. ↓ (7 µg/kg bw) (19%) n.e. (14 µg/kg bw) ↑ (28 µg/kg bw) (27.3%)</td>
<td>Murrin, 1973</td>
</tr>
<tr>
<td>Adults</td>
<td>0.5–2.0</td>
<td>S.S. ↓ 1.0 mg-38% ↓ 2.0 mg-57% H.R. n.e.</td>
<td>Mirakhur et al., 1978</td>
</tr>
<tr>
<td>Adults</td>
<td>1.0</td>
<td>S.S. ↓ 54% H.R. ↓ 13%</td>
<td>Brion et al., 1988</td>
</tr>
<tr>
<td>Adults</td>
<td>1.0</td>
<td>S.S. ↓ 405 H.R. n.e.</td>
<td>Müller et al., 2012</td>
</tr>
<tr>
<td>Adults</td>
<td>2.0–5.0</td>
<td>H.R. ↑ 3.0 mg (35%) ↑ 5.0 mg (50%)</td>
<td>Cullumbine et al., 1955</td>
</tr>
<tr>
<td>Adults</td>
<td>3.0</td>
<td>H.R. ↑ 35%</td>
<td>Shutt and Bowes, 1979</td>
</tr>
<tr>
<td><strong>Non-Oral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>32–125 µg/kg bw (i.m.)</td>
<td>H.R. ↑ (ED(_{50}) = 17.8 µg/kg bw)</td>
<td>Ketchum, 1973</td>
</tr>
<tr>
<td>Adults</td>
<td>0.25–1.5 (i.v.)</td>
<td>S.S. ↓ all doses (ED(_{50}) = 0.9 mg) H.R. ↓ 0.25 mg n.e. 0.4 mg ↑ 0.75, 1.5 mg</td>
<td>Lönnerholm and Widerlöv, 1975</td>
</tr>
<tr>
<td>Adults</td>
<td>0.03–0.96 (i.v.)</td>
<td>H.R. ↓ (&lt;0.12 mg; x 26 years) H.R. ↑ (&gt;0.12 mg; x 26, 60 years)</td>
<td>Poller et al., 1997</td>
</tr>
<tr>
<td>Adults</td>
<td>0.1–0.8 (i.v.)</td>
<td>H.R. ↓ 9% (&lt;0.3 mg) H.R. ↑ 30% (&gt;0.3 mg)</td>
<td>Das et al., 1975</td>
</tr>
<tr>
<td>Adults</td>
<td>1.5–6.0 (i.m.)</td>
<td>H.R. ↑ (all doses)</td>
<td>Higgins et al., 1989</td>
</tr>
<tr>
<td>Infants</td>
<td>0.075–0.4 (i.v.)</td>
<td>H.R. ↑ (ED(_{50}) = 0.13 mg)</td>
<td>Palmisano et al., 1991</td>
</tr>
<tr>
<td>Infants</td>
<td>0.043 (i.v.)</td>
<td>H.R. ↑ (25%)</td>
<td>Eisa et al., 2015</td>
</tr>
</tbody>
</table>

\(^a\) unless indicated as i.m. (intramuscular) or i.v. (intravenous), all doses were oral.

\(^b\) S.S. = salivary secretion; H.R. = heart rate; n.e. = no effect; MED = minimal effective dose.

### 3.3.2 SCOPOLAMINE

According to Corallo et al. (2009) the range of toxic effects of scopolamine is variable and unpredictable, e.g., there are reports of some adults surviving an oral dose of more than 100 mg, whereas lethal oral doses as low as 10 mg in children and 50 mg in adults have also been reported. In humans, the typical triphasic time course of action of high dose acute exposure to (-)-scopolamine consists of (Ketchum, 1973):

- peripheral parasympatholytic effects (e.g. change in heart rate; decreased saliva production);
- disturbances of basic neuroregulatory functions (e.g. somnolence, restlessness, ataxia, incoordination, hyperreflexia, hyperthermia and hypertension); and
- disruption of higher integrative functions (e.g. disruption of awareness and loss of ability to pay attention, to carry out instructions, to speak coherently, or to interpret stimuli realistically).

In cases of overdose or extreme poisoning, central depression and death from respiratory paralysis may be observed (EFSA, 2013; McLendon and Preuss, 2020). Mirakhur (1978) compared the effects of oral and i.m exposure to (-)-scopolamine in healthy volunteers (5 males and 1 female; ages 29 to 40 years).
Doses of (-)-scopolamine (material specifications not provided) were administered orally (0.25, 0.5 or 1 mg) or i.m. (0.25, 0.5 or 1 mg). Effects on heart rate (measured from the radial pulse), arterial pressure (measured indirectly as diastolic arterial pressure + one-third of pulse pressure), oral temperature (measured with a standard clinical thermometer), pupil (measured using a transparent pupil gauge), near-point vision (measured using an R.A.F. near-point scale), sweat-gland activity (measured according to methods described in Wada, 1950), and salivary secretion (measured according to a modification of the method described by Mushin et al. 1953) were recorded. Assuming that the test material was pure (-)-scopolamine, the administered doses correspond to approximately 6, 12 and 23 µg/kg bw (-)-scopolamine for the female subject (based on the reported bodyweight) and to 4, 8 and 15 µg/kg bw (-)-scopolamine for the male subjects (based on the mean bodyweight). Following oral exposure to (-)-scopolamine, all three oral doses caused bradycardia (~20 bpm) within 2 to 3 hours of dosing. Additionally, a dose related peak reduction in salivary secretion of 83, 76 and 55 percent was observed between 1- and 2-hours following dosing. In contrast to salivation, which was more significantly reduced via i.m. exposure, the effects on the heart were similar in magnitude following oral exposure and i.m. exposure. Oral administration induced drowsiness but it occurred later and persisted longer than that following i.m. administration. Oral exposure to (-)-scopolamine had minimal and inconsistent effects on sweat gland activity (decreased but not in a dose related pattern), ocular effects (no change in pupil dilation or near point vision), mean arterial pressure and oral temperature. According to Mirakhur (1978), if the antisialogogue action (reduced salivary secretion) is the main objective, oral administration of (-)-scopolamine is effective since the degree of tachycardia is less and the effects on the eye are minimal.

Golding and Stott, (1997) investigated the effectiveness of oral (-)-scopolamine to treat motion sickness in a group of eighteen healthy male volunteers (ages 19 to 46 years; no bw reported). During (-)-scopolamine treatment, volunteers received 0.6 mg (-)-scopolamine hydrobromide (equivalent to approximately 7 µg/kg bw (-)-scopolamine assuming 70 kg bw). Volunteers were then subjected to ‘cross-coupled motion’ 90 minutes following treatment to elicit motion sickness. Volunteers were monitored for possible side effects, and for effects on heart rate, short term memory, skin conductance and visual performance. (-)-Scopolamine significantly decreased symptoms of sweating and bodily warmth approximately 5 minutes following motion challenge. At 2 hours post administration, the only other symptom reported by volunteers was dry mouth. A significant decrease in skin conductance was observed and heart rate decreased significantly 1- and 2-hours post dosing. At the 2-hour reading the heart rate had decreased by a mean of approximately 9 bpm compared to placebo. (-)-Scopolamine also significantly impaired visual performance in one of the measures- i.e. visual pursuit gain. No severe side effects were recorded and no effects on short-term memory were observed.

Golding et al. (1991) investigated the effectiveness of exposure to (-)-scopolamine via oral and buccal administration in ten healthy men (mean age 33.9 years; mean weight 79.4 kg). Volunteers were exposed either i) mouth only (buccal)
(allowed to dissolve in mouth but not swallowed) to 0.6 mg standard tablets of \((-\text{-scopolamine hydrobromide; or ii) orally to 0.6 mg standard tablets of \((-\text{-scopolamine hydrobromide (equivalent to approximately 6 µg/kg bw \((-\text{-scopolamine). According to the Golding et al. (1991) data, mouth only exposure produced similar peak plasma concentrations and similar T_{max} values as did the ingestion of standard tablets. Additionally, Golding et al. (1991) reported that oral and buccal administration induced a similar magnitude decrease in heart rate 1 to 4 hours following exposure (i.e. ~15 bpm).

Brown et al. (2016) examined the role of the central noradrenergic and cholinergic systems in temporal attention using a randomized, double-blind, counterbalanced double-dummy crossover design experiment with single oral doses of clonidine (alpha2 adrenoceptor agonist) or \((-\text{-scopolamine (muscarinic antagonist; material specifications not provided). \((-\text{-Scopolamine was administered to eighteen healthy volunteers (15 women, 3 men; ages 18 to 26 years; no bw reported) via a single oral dose of 1.2 mg (equivalent to approximately 17 µg/kg bw \((-\text{-scopolamine; assuming 70 kg bw). One-hundred- and twenty-minutes following dosing, a dual-target attentional blink task was administered. Only participants with a blood pressure above 100/70 mmHg and a heart rate of over 65 bpm were included in the study. In addition to the attentional blink task, volunteers were monitored for sedation (simple reaction time task), blood pressure and heart rate. \((-\text{-Scopolamine impaired temporal attention through a decrease in tonic alertness, and induced signs of sedation (slowed down simple reaction time), and decreased heart rate (-10 bpm) compared to placebo. No significant change in blood pressure was observed.

Parrot (1986) compared the effects of transdermal \((-\text{-scopolamine to oral \((-\text{-scopolamine (material specifications not provided) in twelve male volunteers (ages 19 to 38 years; no body weights were reported) using a double-blind design. Single oral \((-\text{-scopolamine doses of 0.15, 0.3, 0.6 and 1.2 mg \((-\text{-scopolamine (equivalent to approximately 2, 4, 8 and 17 µg/kg bw; assuming 70 kg/bw) were administered, followed by the assessment of memory, visual information processing, reaction time, cognitive information processing, target tracking and resting heart rate. One to two hours following oral administration of \((-\text{-scopolamine, significant linear dose-related decrements on tasks involving continuous attention, continuous performance, memory storage for new information, and on self-rated feelings of alertness and sociability were observed. Transdermal exposure (dose information not provided) to \((-\text{-scopolamine produced significant impairments on these same measures and both methods of administration decreased resting heart rate. Specifically, transdermal exposure decreased heart rate by up to 14 bpm, while oral exposure induced a dose-dependent decrease in heart rate of 7, 8, 10 and 12 bpm from low to high dose, respectively. Dry mouth and blurred vision were reported at low doses while higher doses significantly increased the incidence of self-reported dizziness, 1 to 2 hours post dosing. Transdermal exposure to \((-\text{-scopolamine induced similar side effects compared to intermediate and high oral doses.
Weerts *et al.* (2012) investigated the therapeutic effects of (-)-scopolamine (material specifications not provided) on motion sickness in sixteen healthy volunteers (7 males and 9 females; no bw reported) using a randomized, placebo controlled, single blind study design. The study specifically focused on the effect of (-)-scopolamine administration on the canal and otolith functions of the vestibular system. Subjects were administered 0.4 mg of (-)-scopolamine (equivalent to approximately 6 µg/kg bw (-)-scopolamine; assuming 70 kg bw) orally and evaluated by means of electronystagmography and unilateral centrifugation (rotating chair). (-)-Scopolamine significantly reduced utricular sensitivity without any effect on the semicircular canal parameters.

Franco *et al.* (2018) describe a series of cases where the administration of high doses of (-)-scopolamine (material specifications not provided) via gastrostomy significantly decreased hypersalivation in 4 children (ages 7 to 15 years old) with neurological sequelae on mechanical ventilation via tracheostomy with a history of recurrent pneumonia. Children ages 7 and 8 years of age received 20 drops (assumed 0.05 ml) of (-)-scopolamine (10 mg/ml) every 12 hours (equivalent to 667 µg/kg bw per day; assuming 30 kg bw) and a 15 year old child received 35 drops of (-)-scopolamine (10mg/ml) every 12 hours (equivalent to 583 µg/kg bw per day; assuming 60 kg bw). All of the children experienced a significant decrease in sialorrhea 24 hours after the start of treatment with no complications.

### TABLE 15 HUMAN OBSERVATION SUMMARIES FOR SCOPOLAMINE

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>DOSE (µg/kg bw -)-scopolamine)</th>
<th>EFFECTS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>~4–15 (single dose)</td>
<td>saliva ↓ H.R. ↓</td>
<td>Mirakhur <em>et al.</em>, 1978</td>
</tr>
<tr>
<td>Adults</td>
<td>~7 (single dose)</td>
<td>H.R. ↓ Sweat ↓ Visual performance ↓ ‘dry mouth’</td>
<td>Golding and Stott, 1997</td>
</tr>
<tr>
<td>Adults</td>
<td>~6 (single dose)</td>
<td>H.R. ↓</td>
<td>Golding <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>Adults</td>
<td>~17 (single dose)</td>
<td>H.R. ↓ Temporal attention ↓ Sedation</td>
<td>Brown <em>et al.</em>, 2016</td>
</tr>
<tr>
<td>Adults</td>
<td>~2–17 (single dose)</td>
<td>H.R. ↓ Attention ↓ Memory ↓ Alertness ↓ ‘dry mouth and blurred vision’</td>
<td>Parrot, 1986</td>
</tr>
<tr>
<td>Adults</td>
<td>~6 (single dose)</td>
<td>Utricular sensitivity ↓</td>
<td>Weerts <em>et al.</em>, 2012</td>
</tr>
<tr>
<td>Children</td>
<td>~583 to 667 per day</td>
<td>saliva ↓</td>
<td>Franco <em>et al.</em>, 2018</td>
</tr>
</tbody>
</table>

---

16 0.05 ml/drop × 20 drops × 2/day × 10 mg/ml × 30 kg bw × 1000 µg/mg = 667 µg/kg bw/day.
3.3.3 Combined Exposure to Hyoscyamine and Scopolamine

A 2:1 mixture of atropine/scopolamine (25.0/12.5; 75.0/37.5; 250.0/125.0; 750.0/375.0; 2500.0/1250.0 µg) was administered as a single oral dose in cooked buckwheat flour to 9–20 fasted male volunteers (mean age of 22.7 years, mean bw 77.05 kg). Each volunteer consumed a control meal and an atropine/scopolamine dose in random order separately with a 2 week wash out period. Doses of atropine and scopolamine ingested per kg bw were reported as 0.12–12.10 µg and 0.10–9.50 µg (adjusted for breakdown of tropane alkaloids during processing). Measurements of body temperature, heart rate, pupil size, near-point vision, salivary secretion and sweat secretion were taken for up to 4 hours after dosing and the volunteers were also asked to record various subjective symptoms for at least 24 hours after dosing or until the symptoms ceased (Perharic et al., 2013). Statistically significant incidences of decreased salivary secretion were observed in the two highest dose groups (i.e., by approximately 63 percent and 97 percent, respectively), by 1.5–3.5 hours after dosing, with similar effects occurring on sweat secretion. A biologically relevant, but not statistically significant, decrease in salivary secretion was also observed at the intermediate dose (i.e., approximately 38 percent decrease in mean salivary secretion at 3.5 hours). A transient but significant decrease in heart rate (bpm) was noted in the second lowest dose group but only 2.75 hours after dosing whereas the 3rd highest dose group demonstrated an approximate 12.8 percent decrease in heart rate which persisted throughout the entire 4-hour observation period. While no effects on heart rate were seen in the second highest dose group, a consistent increase of approximately 26 percent in heart rate was observed in the highest dose group between 2–4 hours after dosing. Inconsistent changes in body temperature were noted in all dose groups, including controls. A significant increase in pupil size was noted only in the highest dose group, 4 hours after dosing. One individual out of 20 reported subjective symptoms (dry mouth, dizziness, headache, and nausea) in the 2nd lowest dose group whereas no symptoms were reported in the lowest dose group. According to the authors, subjective symptoms observed at the highest dose were characteristic of an anti-muscarinic agent; however, the symptoms observed at lower doses were less likely to be due to exposure based on low incidence and similar reports following exposure to placebo. In the two highest dose groups, subjective symptoms that were reported within the 0–4 hour observation period by at least 40 percent of participants included drowsiness (90 percent), dry mouth (100 percent), ataxia (40 percent) and dizziness (40 percent).

3.4 Clinical Uses

3.4.1 Hyoscyamine/Atropine

Clinical use of hyoscyamine is typically in the form of atropine. Indications for uses of atropine as a muscarinic receptor antagonist in clinical settings include, or have included, as a mydriatic agent, to reduce secretion (digestive, bronchial, cutaneous, lacrimal), as an anti-spasmodic for various GI tract conditions.
Atropine has different effects on the various organs of the body, associated with extent of dosing; salivary and bronchial secretion are usually suppressed by initial low doses, followed by pupil dilation, heart rate increase and decreases in bladder and gastrointestinal tract motility. Finally, large doses are required to inhibit gastric acid secretion and motility (Broadley and Kelly, 2001).

While i.m and i.v. are the preferred route of administration for clinical uses of atropine, atropine sulphate continues to be used to treat various GI tract disorders, with oral dosing in adults recommended as 0.4 mg every 4–6 hours and in children, 0.01 mg/kg bw every 4–6 hours, with a maximum dose of 0.4 mg. Atropine can also be used prior to anesthesia in neonates and children to inhibit salivation and excessive secretion of the respiratory tract with recommended oral doses of 20–40 µg/kg bw for neonates and children (Martindale, 2014). Purified hyoscyamine, the active (-) isomer, is still used to treat conditions associated with visceral spasm, rhinitis and was formerly used in the treatment of parkinsonism. Recommended oral doses, usually in the form of hyoscyamine sulfate or hydrobromide, are 125 to 300 µg up to four times daily, up to a maximum of 1.5 mg in 24 hours.

### Table 16: Atropine Symptoms Following Oral Dosing

<table>
<thead>
<tr>
<th>DOSE</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg</td>
<td>Slight cardiac slowing; some dryness of mouth; inhibition of sweating</td>
</tr>
<tr>
<td>1 mg</td>
<td>Definite dryness of mouth; thirst; acceleration of heart rate, sometimes preceded by slowing; mild dilation of pupils</td>
</tr>
<tr>
<td>2 mg</td>
<td>Rapid heart rate; palpitation; marked dryness of mouth; dilated pupils; some blurring of near vision</td>
</tr>
<tr>
<td>5 mg</td>
<td>All the above signs and symptoms marked; difficulty in speaking and swallowing; restlessness and fatigue; headache; dry, hot skin; difficulty in micturition; reduced intestinal peristalsis</td>
</tr>
<tr>
<td>≥10 mg</td>
<td>Above signs and symptoms more marked; pulse rapid and weak; iris practically obliterated; vision very blurred; skin flushed, hot, dry, and scarlet; ataxia, restlessness, and excitement; hallucinations and delirium; coma</td>
</tr>
</tbody>
</table>


Overtly toxic reactions to atropine, including death, have been reported following doses of approximately 100 mg or less in adults and 10 mg in children. Reported oral lethal doses of atropine sulphate have also been suggested as 10 to 20 mg/kg body weight for adults and from 1 to 10 mg/kg bw for children (Adamse et al., 2014).

The main effect of atropine on the heart is to alter the rate. Although the typical response is tachycardia, the heart rate often decreases transiently with average clinical doses (0.4 to 0.6 mg, oral). The slowing is typically not clinically significant and is usually absent after rapid i.v. injection. There are no accompanying changes in blood pressure or cardiac output. The slight bradycardia effect was thought to be due to central vagal stimulation; however, cardiac slowing due to low doses of atropine has been seen with other muscarinic receptor antagonists that do not cross the blood brain barrier (Wellstein and Pitschner, 1988). More likely, low doses of atropine
cause a selective blockade of prejunctional muscarinic receptors which stimulates acetylcholine release from postganglionic parasympathetic fibers innervating the heart (Dowd, 2017). The heart rate usually increases significantly in humans given more than 0.4 mg atropine i.v. or 1 mg orally. Larger doses of atropine cause progressively increasing tachycardia by blocking vagal effects on M2 muscarinic receptors on the sinoatrial nodal pacemaker. The resting heart rate is typically increased by up to a maximum of 35 to 40 bpm in young males given 2 mg of atropine i.m. The maximal heart rate (e.g., in response to exercise) is not altered by atropine.

### 3.4.2 SCOPOLAMINE

Scopolamine has a long history of use therapeutically. Typically, (-)-scopolamine is administered as (-)-scopolamine hydrobromide trihydrate either orally or by transdermal patch. Orally administered (-)-scopolamine is effective at quickly preventing motion sickness (e.g. symptom relief within 30 minutes of exposure and lasting 6 hours; maximum of 3 doses in 24 hours). However, due to limited oral bioavailability, short half-life and dose-dependent adverse effects (i.e. hallucinations and less serious reactions, e.g., vertigo, dry mouth and drowsiness), the clinical use of oral (-)-scopolamine is relatively limited. In adults, the usual daily oral dose range of (-)-scopolamine hydrobromide soluble tablets is 17 to 46 µg/kg bw per day.\(^{17}\) For treating motion sickness specifically, (-)-scopolamine at recommended doses of between approximately 4 to 11 µg/kg bw\(^{18}\) can be administered one hour prior to travel. Recommended subsequent oral doses of 4 to 11 µg/kg bw may be given 3 times per day as needed and as tolerated (US DHHS, 2013). Dosing in children is typically not recommended by the drug manufacturer since children appear to be particularly susceptible to the side effects of belladonna alkaloids, which include (-)-scopolamine (US DHHS, 2013); however, according to EFSA (2013) daily oral and sublingual doses of (-)-scopolamine hydrobromide trihydrate of up to 40 µg/kg bw is possible during palliative care.

Adverse reactions that have been reported at therapeutic doses include dry mouth, dilated pupils, blurred vision, angle-closure glaucoma, ocular dryness or pruritis or conjunctival injection (hyperemia), and CNS effects (disorientation, memory disturbances, dizziness, restlessness, giddiness, hallucinations, delirium, and confusion). In general, transdermal (-)-scopolamine has been associated with fewer adverse side effects than oral (-)-scopolamine. Scopolamine derivatives such as scopolamine butylbromide (Buscopan) have been designed to limit the central effects associated with oral (-)-scopolamine.

The most frequent adverse effect associated with transdermal (-)-scopolamine is dry mouth, seen in about 67 or 29 percent of patients being treated for prevention of motion sickness or postoperative nausea and vomiting, respectively; less frequently, adverse CNS effects have been reported (e.g. disorientation, memory disturbances,

\(^{17}\) 0.4-0.8 mg 3 to 4 times per day (70 kg bw).
\(^{18}\) 0.25-0.8 mg (70 kg bw).
dizziness, restlessness, giddiness, hallucinations, delirium, and confusion). Irritation, delayed contact dermatitis and withdrawal symptoms (e.g. nausea, vomiting, headache, dizziness, and disturbances of equilibrium) have also been reported with transdermal application (US DHHS, 2013).

### 3.5 TROPANE ALKALOID POISONINGS

Signs and symptoms of TA toxicosis include both peripheral and CNS involvement and include increased respiratory and cardiac rates, mydriasis, mouth dryness, excessive thirst, diarrhoea, confusion, hallucinations, ataxia, convulsions, loss of consciousness and, in severe cases, death from respiratory failure (Gaire and Subedi, 2013; Diaz, 2015). Typical signs and symptoms that have been observed related to the anticholinergic effects (Anticholinergic Syndrome) of both atropine and scopolamine include tachycardia, high blood pressure, fever, agitation, anxiety, memory impairment, sweating, palpitations and blurred vision (Lakstygal et al., 2019). TA poisoning with *Datura* species has been described as the “10 Ds”; 1) dryness of mouth, thirst, and slurred speech; 2) dysphagia; 3) dilated pupils; 4) diplopia; 5) dry hot skin, with flushing and hyperpyrexia; 6) drunken gait (ataxia), hyperreflexia, and convulsions; 7) delirium with hallucinations, agitation, amnesia, and incoherence; 8) delusions; 9) dysuria, urinary retention, and bladder distention; and 10) death, preceded by tachycardia, arrhythmias, coma, and respiratory depression (Kanchan and Atreya, 2016). Typically, TA poisoning is diagnosed based on symptomology and patient history presented at medical centres, with limited confirmation using analysis of biological samples. The first effects exhibited by patients usually present within 30–60 minutes after ingestion and include hallucinations, dryness of mucous membranes, thirst, dilated pupils, and visual and speech disorders. In the further stages, effects including tachycardia, urinary retention, and ileus occur. At higher doses, hyperthermia, respiratory arrest, and convulsions may be observed. Mortality due to CNS depression, circulatory collapse and hypotension are rare but may also occur (Vanderhoff and Moser, 1992). Effects may not resolve for up to 48 hours as the TAs delay gastric motility. Exposure to TAs can be through unintended ingestion of contaminated foods or natural health products, improper or accidental use of Solanaceae plants for medicinal purposes and deliberate use to induce an anticholinergic delirium (Chan, 2017). Some examples of previous incidences of TA poisonings involving contaminated foods are provided in Table 17. Additional reports from poisoning cases involving food, herbal medicines, teas and deliberate/accidental ingestion can be found in literature (Fuchs et al., 2011; EFSA, 2013; Adamse et al., 2014; Chan, 2017; Fatur and Kreft, 2020; Kerchner and Farkas, 2020).
TABLE 17 TROPANE ALKALOIDS IN POISONINGS INVOLVING CONTAMINATED FOOD

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>COMMODITY</th>
<th>CASES</th>
<th>YEAR</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>Flour</td>
<td>(?)</td>
<td>1949</td>
<td>Pulewka and Saygin, 1949</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Corn</td>
<td>688</td>
<td>1984</td>
<td>Aga and Geyid, 1992</td>
</tr>
<tr>
<td>Botswana</td>
<td>Sorghum</td>
<td>92</td>
<td>1998</td>
<td>Onen et al., 2002</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Buckwheat</td>
<td>73</td>
<td>2003</td>
<td>Perharicí, 2005</td>
</tr>
<tr>
<td>Austria</td>
<td>Millet grain</td>
<td>8</td>
<td>2006</td>
<td>Fretz et al., 2007</td>
</tr>
<tr>
<td>France</td>
<td>Buckwheat</td>
<td>19</td>
<td>2012</td>
<td>Glaizal et al., 2013</td>
</tr>
</tbody>
</table>

It was reported that inhabitants of a Turkish village presented with severe intoxications consistent with typical anticholinergic effects which was due to the consumption of bread made from flour estimated to contain almost one percent of *D. stramonium* seeds (Pulewka and Saygin, 1949).

Retrospective review of medical records in Venezuela between 1984–98 identified 15 cases of suspected atropine poisoning due to consumption of honeycomb or wasp honey (Ramirez et al., 1999). Main symptoms reported in the cases included hallucination/confusion (93 percent), dermal/face flushing (66 percent), hyperpyrexia (66 percent), tachycardia (60 percent) and hyperactivity (60 percent). Inspection of the areas where the honey was collected from identified *D. innoxia* as the predominant plant species.

Following reports of acute foodborne toxicity in an agricultural labour force in Eastern Ethiopia, a review of medical records in 5 health units identified 688 patients who met the case definition (occurrence of one or more peripheral or CNS symptoms), 33 of which required hospitalization with 9 reported deaths (Aga and Geyid, 1992). Main symptoms reported included dryness of throat and mouth (94 percent), blurred vision and difficulty in swallowing (88 percent), dry and flushed skin (61 percent), palpitations (71 percent), restlessness (50 percent) and delirium (53 percent). A subsequent inspection of corn used to manufacture a corn flour distributed to the workers showed the presence of *D. stramonium* seeds. Estimated intake of TAs per person, based on amount of seeds per kg of flour, ranged from 13.5–90 mg/meal.

In 1998, 92 patients presented at Botswana health care facilities with a variety of symptoms previously associated with *D. stramonium* poisoning. The main reported symptoms included mental confusion (50 percent), dry mouth (42 percent), restlessness (26 percent), dizziness (23 percent), abdominal pain/discomfort (22 percent), uncontrolled talking (18 percent), headache (15 percent) and nausea/vomiting (11–13 percent) (Onen et al., 2002). Visual inspection of suspected sorghum grain indicated the presence of 1–3 seeds of *D. stramonium* per 20 seeds of...
Super cereal plus – food aid product.
sorghum grain. Atropine and scopolamine concentrations in *D. stramonium* seeds have been reported as 1.28 mg/g and 0.68 mg/g, respectively (Caligiani *et al.*, 2011).

In Slovenia 2003, 73 individuals presented (self-reported) with symptoms of TA toxicity associated with the consumption of buckwheat flour. Signs and symptoms reported included dry mouth, hot red skin, blurred vision, tachycardia, urinary retention, ataxia, speech disturbance, disorientation, and visual hallucinations (Perharič, 2005). Victims reported ingestion of a dish made of buckwheat flour a few hours prior to the onset of effects, which resolved within 48 hours after ingestion. Subsequent examination of the whole buckwheat grain used in the flour production showed the presence of up to 190 *D. stramonium* seeds/kg of grain, with one sample of flour having 26 mg of atropine/kg and 12 mg scopolamine/kg. Intake of TAs by a family of eight who consumed this flour was estimated at between 53–137.6 µg atropine/kg bw and between 24.5–63.5 µg scopolamine/kg bw.

In 2006, 8 employees, on average 45 minutes after consuming a millet grain carrot dish, presented with a variety of symptoms of food poisoning, including nausea, dry mouth and projectile emesis. One person was hospitalized due to unconsciousness and auditory hallucinations (Fretz *et al.*, 2007). Analysis of left-over millet seed demonstrated the presence of *D. stramonium* seeds at 50 seeds/kg of millet with an estimated dose of three seeds per person.

In the Fall of 2012, the Poison Control Centre of Marseille, France was contacted by local health authorities about several cases of food poisoning that had been presented at emergency departments. A common element to all the cases was consumption of products made with a local organic buckwheat flour (Glaizal *et al.*, 2013). The main effects reported in 19 patients who met the case definition were xerostomia and dry mucous membranes, visual disorders, ataxia and dizziness, urine retention and digestive disorders. The onset of effects was rapid (one to four hours after ingestion) and of variable duration (six hours to ten days during repeated consumption). Reported concentrations of atropine and scopolamine detected in a sample of suspect flour were 16 467 µg and 7 042 µg/kg flour, respectively.
Woman preparing a meal with Super Cereal for her family in Nigeria.
CHAPTER 4

FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

The major toxicological concerns from exposure to tropane alkaloids (TAs) are their acute effects and hence exposure over an acute timeframe (single meal or single day). Therefore, the expert consultation considered acute dietary exposure only. Acute dietary exposure techniques seek to quantify the probability of high single exposure events.

The assessment considered dietary exposure through two scenarios; the general diet of population groups in countries where WFP is active and from consumption of the specific food products formulated for WFP. These specific food products are Super Cereal, Super Cereal Plus (for young children, 6–59 months) and Lipid-based Nutrient Supplements. WFP also distributes cereal grains and processed cereal and legume products (flours, meal). However, no monitoring data for TAs in these grains and products were available from WFP. Therefore, these grains and products were considered only in the context of the general diet.

For both exposure scenarios, TA concentration data were available for the two most-studied alkaloids; hyoscyamine (reported as atropine) and scopolamine. While a large number of other TAs may be present in food samples, hyoscyamine and scopolamine are typically the dominant compounds, accounting for approximately 85 percent of TAs from *D. stramonium* (section 2.7.2). The toxicity of the other TAs has not been well characterized. The analytical methods commonly used were unable to separate the enantiomers, (-)-hyoscyamine and (+)-hyoscyamine. The available evidence suggests that (-)-hyoscyamine is the dominant enantiomer in most plant material. The expert consultation concluded that there would be little opportunity for enantiomerisation during most food processing (section 2.2). Consequently, it was assumed that results reported as atropine referred to (-)-hyoscyamine.
(-)-Hyoscyamine and scopolamine appear to be approximately equipotent and dose additivity was assumed for mixtures of the two compounds. The acute dietary exposure assessment was carried out for the sum of the concentrations reported for hyoscyamine and scopolamine (referred to here as \( t \)TA). It should be noted that this nomenclature was adopted solely for the current assessment.

There is conflicting evidence on the thermal stability of hyoscyamine and scopolamine and for the current assessment the concentration of \( t \)TA in foods, as consumed, was assumed to be the same as in the foods as analysed prior to further processing, including cooking (section 2.8).

While WFP supplies food aid to the general population, three particular nutritional risk groups were identified; pregnant and lactating women, children (5–15 years), and young children (6–59 months). Acute dietary exposures were calculated for these sub-populations. With respect to dietary habit, pregnant and lactating women were represented by women of childbearing age (15–44 years).

### 4.1 Dietary Exposure from the General Diet

#### 4.1.1 Concentrations in Food Used in the Dietary Exposure Assessment

Concentration data submitted to the GEMS/Food contaminants database in response to a call for data on TAs were considered in the exposure assessment for the general diet. These data were submitted by Singapore and the European Food Safety Authority (EFSA). Exposure assessments were performed for the sum of hyoscyamine and scopolamine (\( t \)TA). The data of EFSA included single sample identifiers, making it possible to sum the concentrations per sample. In case of the data from Singapore, samples could be identified based on the product description. Only the samples in which both hyoscyamine and scopolamine were analysed were included in the exposure assessment.

The concentration data were checked and recoded for mapping with the foods coded in the food consumption databases. The concentration data were either reported as a numerical value above the LOQ or between the LOD and LOQ, or as undetectable.\(^{19}\)

A lower-bound (LB) scenario was considered regarding the hyoscyamine and scopolamine concentrations used in the exposure assessment: samples with an undetectable result were assumed to contain no hyoscyamine or scopolamine, and concentrations reported as a numerical value between LOD and LOQ were considered to contain the substances at the level of the LOD. Concentrations reported as numerical values at or above the LOQ were used as such. No upper bound (UB) scenario was considered, because the high proportion of undetectable data suggested that TAs were likely to be truly absent from many food samples and the UB scenario resulted in unrealistically high exposure levels. Unless otherwise stated all summary statistics given in this section are LB estimates. All concentrations were reported as µg/kg. Analysed products that were not sufficiently described were excluded.

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\(^{19}\) In the exposure assessment, undetectable values were defined as values below the LOD.
The data set contained some undetectable results with very high LOQs. Currently available LC-MS/MS methods can achieve LOQs of 1 µg/kg or less. Analytical results with up to 10-fold higher LOQs were included in the analysis but results with LOQs above 10 µg/kg were considered to be insufficiently sensitive to yield useful data.

The GEMS/Food contaminants database contained hyoscyamine and scopolamine concentrations that were labelled as obtained via random sampling or unknown sampling protocol. Both sources of data were included in the exposure assessment. In 2018, EFSA reported on the acute exposure to TAs in Europe and identified that bread and other grain milling products were the main contributors to acute dietary exposure in all age classes (Arcella et al., 2018). To simplify the dietary exposure assessment, only cereals and cereal-based products, as well as legumes were considered in this assessment. Foods with numerical values above the LOQ that were excluded were tea (including herbal tea), different seeds (hempseed, fennel seed, pumpkin seed, coriander seed, linseed and sunflower seed), peppermint and pumpkin. As these foods are not expected to be consumed in high amounts by the relevant populations, the dietary exposure assessment was not expected to be affected significantly.

(a) Hyoscyamine and scopolamine

In total, 2,933 of the 3,708 samples analysed for hyoscyamine and scopolamine were considered in the exposure assessment for the general diet (99 percent from EFSA, 1 percent from Singapore). Table 17 lists the LB mean concentrations of hyoscyamine and scopolamine in the foods considered in the dietary exposure assessment. The summed concentrations were calculated by adding the concentrations of hyoscyamine and scopolamine per sample and are expressed as $t_{TA}$.

Of all samples, 24 percent referred to cereal-based foods for infants and young children; 7.6 percent to buckwheat bread/flour/milling products; 7.0 percent to millet grain/groats; 6.0 percent to corn bread/flour/milling products; and 5.8 percent to biscuits (cookies), wheat-based. Hyoscyamine was detected at concentrations at or above the LOD in 272 samples (9 percent) and scopolamine in 170 samples (7 percent). The overall mean concentrations of hyoscyamine and scopolamine across all analysed samples were 3.7 and 1.1 µg/kg, respectively. The three foods with the highest concentrations of $t_{TA}$ were porridge, rye bread/flour/milling products, and sorghum flour/milling products (Table 18). Porridge had the highest concentrations of $t_{TA}$, ranging from 113 µg/kg to 2,000 µg/kg for the samples with a concentration at or above the LOD in 272 samples (9 percent) and scopolamine in 170 samples (7 percent). Corresponding concentrations for rye bread/flour/milling products were 0.6 to 1,960 µg/kg and 1.4 to 377 µg/kg for sorghum flour/milling products.

The concentrations of $t_{TA}$ in Table 18 were used in the combined acute dietary $t_{TA}$ exposure assessment for the general diet.
TABLE 18  LOWER BOUND\(^{a}\) CONCENTRATIONS OF HYOSCYAMINE, SCOPOLAMINE AND SUM OF HYOSCYAMINE AND SCOPOLAMINE OF SAMPLES INCLUDED IN THE EXPOSURE ASSESSMENT FOR THE GENERAL DIET (continue)

<table>
<thead>
<tr>
<th>FOOD CATEGORY</th>
<th>FOOD NAME</th>
<th>TOTAL SAMPLES (n)</th>
<th>&lt;LOD(^{b}) (%)</th>
<th>MEAN CONCENTRATION (µg/kg)</th>
<th>tTA</th>
<th>HYS</th>
<th>SCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley (products)</td>
<td>Barley flakes</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Barley grain</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Buckwheat (products)</td>
<td>Buckwheat bread/flour/milling products</td>
<td>222</td>
<td>90</td>
<td>5.0</td>
<td>2.4</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buckwheat grain/groats</td>
<td>153</td>
<td>97</td>
<td>1.3</td>
<td>0.6</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Cereal-based foods for infants and young children</td>
<td>Biscuits, rusks and cookies for children</td>
<td>127</td>
<td>91</td>
<td>0.03</td>
<td>0.03</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cereal-based food for infants and young children</td>
<td>692</td>
<td>90</td>
<td>0.51</td>
<td>0.36</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Corn (products)</td>
<td>Corn bread/flour/milling products</td>
<td>176</td>
<td>90</td>
<td>0.66</td>
<td>0.57</td>
<td>0.09</td>
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</tr>
<tr>
<td></td>
<td>Corn chips/curls</td>
<td>11</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corn flakes</td>
<td>68</td>
<td>97</td>
<td>0.13</td>
<td>0.10</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Corn grain</td>
<td>54</td>
<td>54</td>
<td>3.4</td>
<td>2.9</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corn starch</td>
<td>5</td>
<td>80</td>
<td>0.01</td>
<td>0.01</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maize, popped</td>
<td>25</td>
<td>56</td>
<td>1.3</td>
<td>1.1</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Tortilla/tortilla chips</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grains</td>
<td>Grain milling products</td>
<td>12</td>
<td>00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grains as crops</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grains for human consumption</td>
<td>56</td>
<td>88</td>
<td>9.2</td>
<td>7.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Legumes and beans</td>
<td>Beans (Phaseolus vulgaris)</td>
<td>12</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chickpea flour</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Legumes, beans, dried</td>
<td>15</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lentils/lentils, green</td>
<td>26</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soybean flour</td>
<td>8</td>
<td>63</td>
<td>2.2</td>
<td>1.5</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Millet (products)</td>
<td>Millet flakes</td>
<td>35</td>
<td>80</td>
<td>0.48</td>
<td>0.27</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Millet flour/milling products</td>
<td>62</td>
<td>69</td>
<td>4.5</td>
<td>2.8</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Millet grain/groats</td>
<td>204</td>
<td>88</td>
<td>2.4</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Mixed cereal products</td>
<td>Breakfast cereals</td>
<td>20</td>
<td>95</td>
<td>0.003</td>
<td>0.003</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cereal bars</td>
<td>14</td>
<td>86</td>
<td>7.8</td>
<td>6.5</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Cereal flakes</td>
<td>33</td>
<td>91</td>
<td>0.44</td>
<td>0.31</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Cereal-based dishes</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Fine bakery wares</td>
<td>21</td>
<td>95</td>
<td>0.01</td>
<td>0.01</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flour mix. wheat/rye/barley/oats</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass noodle</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muesli/muesli bars</td>
<td>110</td>
<td>96</td>
<td>0.01</td>
<td>0.005</td>
<td>0.0005</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Pasta</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Popped cereals</td>
<td>17</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Porridge</td>
<td>12</td>
<td>42</td>
<td>524</td>
<td>443</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Rusk, bread and rolls</td>
<td>64</td>
<td>95</td>
<td>0.42</td>
<td>0.26</td>
<td>0.16</td>
<td>0.16</td>
</tr>
</tbody>
</table>
TABLE 18  LOWER BOUND Concentrations of Hyoscyamine, Scopolamine and Sum of Hyoscyamine and Scopolamine of Samples Included in the Exposure Assessment for the General Diet (continued)

<table>
<thead>
<tr>
<th>FOOD CATEGORY</th>
<th>FOOD NAME</th>
<th>TOTAL SAMPLES (n)</th>
<th>(&lt;LOD^a) (%)</th>
<th>MEAN CONCENTRATION (µg/kg)</th>
<th>tTA</th>
<th>HYS</th>
<th>SCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat (products)</td>
<td>Biscuits, oat meal</td>
<td>12</td>
<td>75</td>
<td>0.01</td>
<td>0.008</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat (bran) flakes</td>
<td>26</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat flour/milling products</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat grain</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat porridge</td>
<td>4</td>
<td>100</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rice (products)</td>
<td>Rice</td>
<td>21</td>
<td>95</td>
<td>0.04</td>
<td>0.04</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice bread</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice flakes</td>
<td>7</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice porridge</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rye (products)</td>
<td>Rye bread/flour/milling products</td>
<td>30</td>
<td>77</td>
<td>118</td>
<td>90</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rye flakes</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rye grain</td>
<td>11</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sorghum (products)</td>
<td>Sorghum flour/milling products</td>
<td>8</td>
<td>63</td>
<td>48</td>
<td>38</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sorghum grain</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Spelt (products)</td>
<td>Biscuits, spelt meal</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasta, spelt based</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spelt flakes</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
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<td>Spelt flour/milling products</td>
<td>20</td>
<td>100</td>
<td>0</td>
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<tr>
<td></td>
<td>Spelt grain</td>
<td>18</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wheat (products)</td>
<td>Biscuits (cookies), wheat based</td>
<td>169</td>
<td>89</td>
<td>0.03</td>
<td>0.03</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fine bakery wares, wheat based</td>
<td>10</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Noodles, wheat flour</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancakes</td>
<td>3</td>
<td>67</td>
<td>0.63</td>
<td>0.41</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasta, wheat based</td>
<td>91</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat bran</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat bread/flour/milling products</td>
<td>132</td>
<td>91</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat flakes</td>
<td>18</td>
<td>94</td>
<td>0.04</td>
<td>0.04</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>97</td>
<td>0.03</td>
<td>0.03</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

HYS: hyoscyamine; LOD: limit of detection; LOQ: limit of quantification; SCP: scopolamine; tTA: sum of the concentrations of hyoscyamine and scopolamine, assuming equi potency.

* Analytical results below the LOD were substituted by a value of zero and analytical results with a level between LOD and LOQ were substituted by a value equal to the LOD.

+ Percentage of samples with an analytical level below the LOD for both hyoscyamine and scopolamine.
4.1.2 FOOD CONSUMPTION DATA USED IN THE DIETARY EXPOSURE ESTIMATES

An acute dietary exposure assessment was required for tTA. Due to this, food consumption data from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss) database and the GEMS/Food cluster diets were not suitable, as the data in these databases is suitable only for chronic dietary exposure assessments. For an acute dietary exposure assessment, preferably consumption levels of foods at an individual level from a single consumption day should be used. Therefore, the expert consultation used individual food consumption data from seven countries as included in the FAO/WHO Global Individual Food consumption data Tool (GIFT). At the time of the assessment, this tool contained such data from nine countries, which were the Republic of Italy, Burkina Faso, the Republic of Zambia, the People’s Republic of Bangladesh, the Republic of Uganda, the Plurinational State of Bolivia, the Lao People’s Democratic Republic, the Republic of the Philippines, and the Argentine Republic. These data also included characteristics of the individuals, including age, sex and body weight. Data from six of these countries were identified as relevant for the current assessment. The following subpopulations were identified as relevant for the exposure assessment based on the target populations of the WFP:

- Women of childbearing age (15–44 years)
- Young children (6–59 months)
- Children (5–15 years)

Some characteristics of these sub-populations per country are listed in Table 19. Burkina Faso was also identified as a country relevant for the current assessment. However, inconsistencies with the data set for Burkina Faso meant that this data set was considered to be unreliable and no acute dietary exposure estimates were calculated for this country.

TABLE 19  CHARACTERISTICS OF THE SELECTED SUB-POPULATIONS FOR FOOD CONSUMPTION DATA OBTAINED FROM THE GLOBAL INDIVIDUAL FOOD CONSUMPTION TOOL (GIFT) AND USED IN THE EXPOSURE ASSESSMENT FOR THE GENERAL DIET

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>SAMPLE SIZE</th>
<th></th>
<th></th>
<th>YEAR</th>
<th>TITLE SURVEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WOMEN</td>
<td>YOUNG CHILDREN</td>
<td>CHILDREN</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(15-44 years)</td>
<td>(6-59 months)</td>
<td>(5-15 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolivia (Plurinational State of)</td>
<td>57</td>
<td>5</td>
<td>45</td>
<td>2009–2012</td>
<td>Bolivia - 2009/2012 - Lund University</td>
</tr>
<tr>
<td>Uganda</td>
<td>503</td>
<td>-</td>
<td>1</td>
<td>2007</td>
<td>HarvestPlus Reaching End Users (REU) Orange-Fleshed Sweet Potato (OFSP) Project</td>
</tr>
<tr>
<td>Zambia</td>
<td>271</td>
<td>484</td>
<td>32</td>
<td>2009</td>
<td>The 2009 Food consumption and Vitamin A status survey in Zambia</td>
</tr>
</tbody>
</table>

a  The food consumption data were recorded during two days in all surveys.
b  In the exposure assessments consumption data of children aged 0 years were included.
4.1.3 ASSESSMENT OF DIETARY EXPOSURE

The expert consultation estimated national acute dietary \( r \)TA exposures using data from GIFT (see section 4.1.2) combined with the summed occurrence data of hyoscyamine and scopolamine analysed in the same sample from the GEMS/Food contaminants database (see section 4.1.1). Acute dietary exposures were calculated per country and population group. Only population groups with at least 100 person-days (= sample size \( \times \) two days) per country were included in the exposure assessment. Children aged 5 to 15 years from the Plurinational State of Bolivia, the Republic of Uganda, and the Republic of Zambia, and children aged 6 to 59 months from the Plurinational State of Bolivia were therefore excluded (Table 19). No information on body weight was included in the data of the Republic of the Philippines. An average body weight of 60 kg was therefore assumed. Information on body weights was partly missing in the data from the Republic of Uganda and the Republic of Zambia. These body weights were imputed using the mean body weight for the same age and sex per survey.

The acute dietary \( r \)TA exposure was calculated using Monte Carlo sampling as implemented in the Monte Carlo Risk Assessment tool (version 8.3; de Boer et al., 2019). For this, randomly drawn daily consumed amounts of foods from the food consumption databases were multiplied with randomly drawn sample-based \( r \)TA concentrations for the relevant foods. This was done 100,000 times, resulting in a distribution of 100,000 person-day acute dietary \( r \)TA exposures. The acute dietary \( r \)TA exposure estimates were divided by the individual body weights. The acute dietary \( r \)TA exposure was summarised as the mean of the combined acute exposure distribution, and as the 95th percentile (P95). Also, the contributions of the food and substance combinations and that of hyoscyamine to the combined exposure expressed as percentages were calculated per sub-population and country.

The mean and P95 acute dietary \( r \)TA exposure estimates are listed in Table 20. The mean acute dietary \( r \)TA exposure was lower than 1 ng/kg bw per day in all countries and sub-populations, except in the Republic of Zambia where, the mean acute dietary exposure was 18 ng/kg bw per day in children of 6–59 months and 4.6 ng/kg bw per day in women of child-bearing age (15–44 years).
### Table 20: Combined Acute Dietary Exposure to the Sum of Hyoscyamine and Scopolamine (tTA) for the Selected Sub-Populations from the General Diet According to the Lower Bound Scenario<sup>a</sup>

<table>
<thead>
<tr>
<th>Country</th>
<th>Acute Dietary tTA Exposure (ng/kg bw per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Children 6–59 months</td>
<td></td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>0.5</td>
</tr>
<tr>
<td>Zambia</td>
<td>18</td>
</tr>
<tr>
<td>Children 5–15 years</td>
<td></td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>0.4</td>
</tr>
<tr>
<td>Women 15–44 years</td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>0.6</td>
</tr>
<tr>
<td>Bolivia (Plurinational State of)</td>
<td>0.6</td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>0.4</td>
</tr>
<tr>
<td>Philippines</td>
<td>0.7</td>
</tr>
<tr>
<td>Uganda</td>
<td>0.5</td>
</tr>
<tr>
<td>Zambia</td>
<td>4.6</td>
</tr>
</tbody>
</table>

tTA: sum of hyoscyamine and scopolamine; bw: body weight; P95: 95th percentile; P99: 99th percentile.

* Analytical results below the limit of detection (LOD) were substituted by a value of zero and analytical results with a level between LOD and limit of quantification (LOQ) were substituted by a value equal to the LOD.

The acute dietary tTA exposure was dominated by hyoscyamine; 84–97 percent of the exposure could be attributed to this compound (Table 21). The foods contributing most to the combined exposure were rice, corn products and sorghum products (Table 21). The high estimated acute dietary exposure for population groups from Zambia could be explained by the consumption of sorghum flour/milling products for which high levels of TAs were present in the concentration database. The contribution of rice and corn or corn-based products were due to high consumption levels.
## Table 21: Contribution of Top 3 Products and Hyoscyamine to the Combined Dietary Exposure Distribution to Hyoscyamine and Scopolamine (\( \text{tTA} \)) from the General Diet for the Selected Sub-populations in the Lower Bound Scenario

<table>
<thead>
<tr>
<th>Country and Sub-population</th>
<th>Contribution to the Total Combined Acute Exposure Distribution</th>
<th>Hyoscyamine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top 3 Substance - Food Combinations (%)</td>
<td>1</td>
</tr>
<tr>
<td>Children 6–59 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>rice – hyoscyamine (67%)</td>
<td>corn grain – hyoscyamine (15%)</td>
</tr>
<tr>
<td>Zambia</td>
<td>corn bread/flour/milling products – hyoscyamine (46%)</td>
<td>sorghum flour/milling products – hyoscyamine (32%)</td>
</tr>
<tr>
<td>Children 5–15 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>rice – hyoscyamine (82%)</td>
<td>corn grain – hyoscyamine (14%)</td>
</tr>
<tr>
<td>Women 15–44 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>rice – hyoscyamine (72%)</td>
<td>corn grain – hyoscyamine (23%)</td>
</tr>
<tr>
<td>Bolivia (Plurinational State of)</td>
<td>corn grain – hyoscyamine (55%)</td>
<td>rice – hyoscyamine (22%)</td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
<td>rice – hyoscyamine (63%)</td>
<td>corn grain – hyoscyamine (30%)</td>
</tr>
<tr>
<td>Philippines</td>
<td>rice – hyoscyamine (35%)</td>
<td>corn grain – hyoscyamine (27%)</td>
</tr>
<tr>
<td>Uganda</td>
<td>corn bread/flour/milling products – hyoscyamine (84%)</td>
<td>corn bread/flour/milling products – scopolamine (13%)</td>
</tr>
<tr>
<td>Zambia</td>
<td>corn bread/flour/milling products – hyoscyamine (52%)</td>
<td>sorghum flour/milling products – hyoscyamine (27%)</td>
</tr>
</tbody>
</table>

* Analytical results below the limit of detection (LOD) were substituted by a value of zero and analytical results with a level between LOD and limit of quantification (LOQ) were substituted by a value equal to the LOD.*
4.2 DIETARY EXPOSURE FROM WFP PRODUCTS

4.2.1 CONCENTRATIONS IN FOOD USED IN THE DIETARY EXPOSURE ASSESSMENT

Information on the concentrations of TAs in foods were available to the expert consultation from monitoring of hyoscyamine and scopolamine in Super Cereal, Super Cereal Plus, Lipid-based Nutrient Supplements and ingredients, carried out by WFP and some of their suppliers.

Table 22 summarizes data from WFP monitoring on TA contamination of Super Cereal and Super Cereal Plus.

<table>
<thead>
<tr>
<th></th>
<th>SUPER CEREAL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SUPER CEREAL PLUS&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HYS</td>
<td>SCP</td>
</tr>
<tr>
<td>Pre-incident</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>530</td>
<td>337</td>
</tr>
<tr>
<td>N (excluding samples causing illness)</td>
<td>526</td>
<td>337</td>
</tr>
<tr>
<td>Mean concentration, all samples (µg/kg)</td>
<td>101–101</td>
<td>12.0–12.1</td>
</tr>
<tr>
<td>Mean concentration, excluding samples causing illness (µg/kg)</td>
<td>10.5–10.5</td>
<td>2.1–2.3</td>
</tr>
<tr>
<td>Mean concentration, samples causing illness only (µg/kg)</td>
<td>12 000</td>
<td>1 300</td>
</tr>
<tr>
<td>Maximum concentration, all samples (µg/kg)</td>
<td>15 530</td>
<td>1 860</td>
</tr>
<tr>
<td>Maximum concentration, excluding samples causing illness (µg/kg)</td>
<td>192</td>
<td>24</td>
</tr>
<tr>
<td>Post-incident</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>64</td>
<td>37</td>
</tr>
<tr>
<td>Mean concentration (µg/kg)</td>
<td>2.4–2.5</td>
<td>0.47–0.61</td>
</tr>
<tr>
<td>Maximum concentration (µg/kg)</td>
<td>7.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

N: number of samples, HYS: hyoscyamine, SCP: scopolamine, tTA: sum of the concentrations of hyoscyamine and scopolamine, assuming equipotency.

<sup>a</sup> Mean values were calculated as lower bound (LB) – upper bound (UB). LB estimates substituted by a value of zero for all analytical results below the limit of detection. UB estimates substituted by a value equal to the limit of detection for all analytical results below the limit of detection.

When samples of cereal products known to have caused illness were excluded, the mean concentrations of TAs in Super Cereal and Super Cereal Plus were very similar. However, a marked difference was apparent between TA content of Super Cereal and Super Cereal Plus after the incident, compared to TA content before the incident.

TA monitoring data for Lipid-based Nutrient Supplements products were not available for material produced before the contamination incident. WFP monitoring did not detect TAs in any Lipid-based Nutrient Supplements samples (n = 36) sampled after the incident. Post-incident monitoring by a supplier of Lipid-based Nutrient Supplements products occasionally detected TAs in the raw materials used
in the manufacture of Lipid-based Nutrient Supplements, particularly soya flour, with concentrations up to 67 µg/kg total TAs detected. However, TAs were detected only at very low concentrations in 5 of 22 batches: two at trace levels (<1 µg/kg) and the remaining three at concentrations of tTA of 1.21–1.66 µg/kg. Four of the five positive detections were in the Lipid-based Nutrient Supplements-MQ product.

4.2.2 FOOD CONSUMPTION DATA USED IN THE DIETARY EXPOSURE ASSESSMENT

The WFP cereal products, Super Cereal and Super Cereal Plus, are intended to be consumed by the general population, not including infants, as part of a food basket, at a rate of 100 g/person/day, with the products intended to be consumed in the form of a porridge, after addition of water. It is recognised that Super Cereal and Super Cereal Plus may occasionally be shared with family members of the primary recipient, resulting in lower than intended consumption. In other instances, Super Cereal or Super Cereal Plus may be an individual’s sole source of nutrition, with an individual’s allocation exhausted in a shorter than intended time period. A maximum consumption of 200 g/person/day has been estimated by WFP. Two statistical distributions are available to represent data in the form minimum, most likely and maximum; the triangular and the beta pert distribution. The beta pert distribution is often preferred, as this distribution gives less statistical weight to the right-hand tail of the distribution. For the current assessment, no minimum consumption value was available. It was assumed that the minimum would be half of the daily recommended consumption (50 g/person/day). The resulting triangular and beta pert distributions are shown in Figure 2. The beta pert distribution was used for the current assessment.

The Lipid-based Nutrient Supplements product is targeted at children 6 months and older. Dietary guidelines differ for the three product formulations: Ready-to-use Supplementary Foods (RUSF) 100 g/day, Lipid-based Nutrient Supplements-MQ 50 g/day and Lipid-based Nutrient Supplements-SQ 20 g/day. Lipid-based Nutrient Supplements is distributed as an alternative to Super Cereal Plus, but not in addition to Super Cereal Plus.
4.2.3 BODY WEIGHT DATA USED IN THE EXPOSURE ASSESSMENT

In order that the acute dietary exposure calculations were based on body weights relevant to countries receiving food aid from WFP, body weight data in the seven relevant GIFT datasets were amalgamated for the same three sub-populations as identified for the general diet. Body weight statistics for these sub-populations are summarized in Table 23.

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>COUNTRIES WITH DATA</th>
<th>BODY WEIGHT (KG)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MEAN (SD)</td>
<td>MEDIAN</td>
<td>P5</td>
<td>P95</td>
</tr>
<tr>
<td>Women, 15–44 years</td>
<td>BGD, BFA, BOL, LAO, UGA, ZMB</td>
<td>53.8 (9.7)</td>
<td>53.2</td>
<td>39.5</td>
<td>71.3</td>
</tr>
<tr>
<td>Children, 6–59 months</td>
<td>BOL, BFA, LAO, ZMB</td>
<td>12.7 (2.9)</td>
<td>13.0</td>
<td>7.6</td>
<td>17.1</td>
</tr>
<tr>
<td>Children, 5–15 years</td>
<td>BOL, BFA, LAO</td>
<td>25.5 (10.9)</td>
<td>22.5</td>
<td>13.9</td>
<td>47.7</td>
</tr>
</tbody>
</table>


Source: Authors’ own elaboration.
The distribution of body weights within each age group conformed well to a normal distribution and the resulting distributions were used in the simulation of acute dietary exposure from consumption of Super Cereal and Super Cereal Plus.

### 4.2.4 Assessments of Dietary Exposure

Concentration data were available for four samples of Super Cereal known to have caused illness, with mean and maximum \( t \)TA concentrations of 13 300 and 17 390 µg/kg, respectively. While these samples were excluded from the main analysis, for a Super Cereal consumption of 100 g and an adult body weight of 60 kg, these concentrations would equate to exposure doses of 22 000 and 29 000 ng/kg bw (22 and 29 µg/kg bw), respectively. For a young child of 15 kg consuming 100 g of this product, these doses would be 89 and 116 µg/kg bw.

Acute dietary exposure was estimated for each of the three sub-populations by simulation modelling using the Microsoft Excel add-in @Risk (Palisades Corporation). Concentrations of \( t \)TA from WFP monitoring were treated as an empirical distribution. Results from products processed prior to the incident were analysed separately to results from products processed after the incident.

The quantity of Super Cereal or Super Cereal Plus consumed on each occasion was drawn from the beta pert distribution shown in Figure 2. WFP nutrient guidelines state that consumption of Super Cereal Plus by children 6–59 months “will depend on the age of the child”. However, in the absence of further information, a conservative approach was taken and it was assumed that the amount of Super Cereal Plus consumed by young children would be drawn from the same distribution as Super Cereal consumption by older children or women of childbearing age. Discussion with WFP representatives suggested that this was a reasonable assumption.

Body weights were drawn from normal distributions fitted to the data summarized in Table 23 and truncated at the minimum and maximum body weights observed in the source data. It was assumed that there was no significant correlation between consumption amount and body weight.

Simulations were run for 100 000 iterations. Summary statistics from the simulations are shown in Table 24.

Due to the low occurrence of TAs in Lipid-based Nutrient Supplements products, the potential acute dietary \( t \)TA exposure was assessed using a ‘worst case’ scenario. The products are primarily intended for children 6–59 months of age. Quantifiable concentrations of TAs were detected only in the Lipid-based Nutrient Supplements-MQ product, with a targeted consumption of 50 g/day. The highest \( t \)TA concentration detected was 1.66 µg/kg, while the mean body weight for this sub-population is 12.7 kg. On this basis, the maximum expected acute dietary exposure from consumption of Lipid-based Nutrient Supplements products would be 6.5 ng/kg bw. Higher acute dietary exposures may occur for below-average weight children and above target level consumption.
### Table 24: Summary Statistics of Acute Dietary Exposure to rTA for the Selected Sub-Populations from Consumption of Super Cereal or Super Cereal Plus

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>WFP FOOD PRODUCT</th>
<th>ACUTE DIETARY TTA EXPOSURE (NG/KG BW)</th>
<th>BEFORE INCIDENT</th>
<th>AFTER INCIDENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEAN (LB-UB)</td>
<td>P95 (LB-UB)</td>
</tr>
<tr>
<td>Children, 6–59 months</td>
<td>Super Cereal Plus</td>
<td>130–132</td>
<td>554–557</td>
<td>15.2–17.3</td>
</tr>
<tr>
<td>Children, 5–15 years</td>
<td>Super Cereal</td>
<td>45.3–45.7</td>
<td>214–218</td>
<td>10.3–11.1</td>
</tr>
</tbody>
</table>

WFP: World Food Programme, P95: 95th percentile, LB: Lower bound estimates substituted by a value of zero for all analytical results below the limit of detection, UB: Upper bound estimates substituted by a value equal to the limit of detection for all analytical results below the limit of detection, rTA: the sum of the concentrations of hyoscyamine and scopolamine.
*D. stramonium* growing as a weed among vegetable crops.
Adverse health effects reported following consumption of cereal products distributed by the WFP in 2019 resembled typical effects of TA toxicity. Subsequently, *D. stramonium* seeds were identified in a soy component of the cereal products. While *D. stramonium* plant parts are known to contain at least 67 separate TAs, hyoscyamine (atropine) and scopolamine tend to predominate in seeds. Analysis of solvent extracts of *D. stramonium* seeds by GC-MS demonstrated that hyoscyamine (atropine) and scopolamine accounted for the majority of total detected alkaloids (El Bazaoui *et al.*, 2011). Human poisonings due to consumption of food contaminated with TAs generally lack quantitative dose-response data and usually provide only confirmation of the presence of the plant parts in the food with self-reported intake estimates.

Of the toxicological data most relevant for the risk assessment of TAs, in the study by Dugan *et al.*, 1989, dried seeds from *Datura stramonium* were added to standard rodent diets at 0.5, 1.58 and 5.0 percent by weight which were estimated to provide doses of 13.6, 42.8 and 135.5 mg hyoscyamine and 3.3, 10.4 and 33.0 mg scopolamine/kg diet, in the low-, medium- and high-dose diets. Sprague-Dawley rats (n = 20 per sex) were fed each diet for 90 days with access to food ab libitum. A consistent effect noted in all dose groups was a dose-dependent decrease in body weight gain, which was not associated with feed intake. Relative testes weight in males and relative brain and liver weights in both male and female rats were significantly increased but no histological changes were noted in any organs. There were also dose-related changes in some clinical chemistry parameters. Significant effects were apparent in the lowest dose group (0.5 percent w/w seeds), which provided total hyoscyamine/scopolamine doses of 0.81–1.45 mg/kg bw/day (males) and 1.08–1.63 mg/kg bw/day (females) (approximately 80 percent of the dose would be hyoscyamine). In comparison, a review of human observations caused by atropine exposure has demonstrated effects on salivary secretion (inhibition) and changes in heart rate in adults at oral doses of atropine ranging from approximately 10–20 µg/kg bw. Similarly, oral doses of scopolamine, ranging from 4–15 µg/kg bw, have resulted in effects on salivary secretion (inhibition) and changes in heart rate in humans.
Human dose-response data for combined exposure to relatively low doses of atropine and scopolamine is available from the study by Perharic et al. (2013). This investigation followed a report of TA poisonings where estimated exposures for hyoscyamine and scopolamine ranged from 0.7–138 µg/kg bw and 0.4–64 µg/kg bw, respectively. The randomized, double-blind, placebo controlled cross-over study exposed volunteers to a cooked buckwheat flour meal to which atropine and scopolamine had been added prior to cooking in order to provide doses ranging from 0.32 to 32.45 µg/kg bw for atropine and 0.16 to 16.22 µg/kg bw scopolamine. Quantitative changes in body temperature, heart rate, salivary secretion, sweat secretion and pupil size were recorded for up to 4 hours after dosing.

The following points were considered for the hazard characterization for (-)-hyoscyamine and (-)-scopolamine:

- They do not bioaccumulate;
- They have short half lives in humans (hours);
- Peak plasma concentrations are achieved within two hours; the effects generally occur within a short time after the administration and are transient;
- They are not genotoxic in vivo;
- They do not cause carcinogenicity or progressive toxicity following repeated oral exposure;
- They do not cause developmental toxicity; and
- The effects of toxicological concern are due to the antagonism of acetylcholine binding to the PNS and CNS muscarinic receptors leading to acute effects.

Considering the aforementioned points, protection from acute effects should also cover any anticipated effects following chronic oral exposure.

5.1 PIVOTAL DATA FROM HUMAN CLINICAL/Epidemiological Studies

The randomized, double blind, placebo controlled, crossover study with human volunteers by Perharic et al. (2013) with combined oral exposures to atropine and (-)-scopolamine was considered the most relevant for determining a point of departure. See Table 25 below for dose calculations of total (-)-scopolamine and (-)-hyoscyamine added to the food by Perharic et al. (2013) without adjustment for processing degradation/losses. Assuming the atropine added to the food by Perharic et al. (2013) was a 1:1 racemic mixture, the atropine dose was adjusted to account for only the active (-)-enantiomer (i.e., atropine dose was divided by 2). The expert meeting determined that inadequate data were available to derive relative potency factors for (-)-hyoscyamine and (-)-scopolamine, but based on the limited data available, (-)-hyoscyamine and (-)-scopolamine were considered equipotent via the oral exposure route and a dose additivity approach was used.
<table>
<thead>
<tr>
<th>ATROPINE ADDED TO MEAL (µg/meal)</th>
<th>SCOPOLAMINE ADDED TO MEAL (µg/meal)</th>
<th>ATROPINE DOSEa (µg/kg bw)</th>
<th>(-)-HYOSCYAMINE b DOSE (µg/kg bw)</th>
<th>(-)-SCOPOLAMINE DOSe (µg/kg bw)</th>
<th>TOTAL DOSe (µg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>25</td>
<td>12.5</td>
<td>0.32</td>
<td>0.16</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>75</td>
<td>37.5</td>
<td>0.97</td>
<td>0.49</td>
<td>0.49</td>
<td>0.97</td>
</tr>
<tr>
<td>250</td>
<td>125</td>
<td>3.24</td>
<td>1.62</td>
<td>1.62</td>
<td>3.24</td>
</tr>
<tr>
<td>750</td>
<td>375</td>
<td>9.73</td>
<td>4.87</td>
<td>4.87</td>
<td>9.73</td>
</tr>
<tr>
<td>2,500</td>
<td>1,250</td>
<td>32.45</td>
<td>16.22</td>
<td>16.22</td>
<td>32.45</td>
</tr>
</tbody>
</table>

a Calculated based on average body weight of 77.05 kg for participants in the Perharicˇ et al. (2013) study.
b Calculated by dividing the atropine dose by 2 (assuming a 1:1 racemic mixture).
c Calculated based on average body weight of 77.05 kg for participants in the Perharicˇ et al. (2013) study and assuming all scopolamine added to the meal was the active (-)-enantiomer.
d The sum of (-)-hyoscyamine and (-)-scopolamine doses added to the food.

The buckwheat meals consumed by the volunteers in Perharicˇ et al. (2013) were prepared as follows: buckwheat flour mixed with the atropine/scopolamine was added to boiling water and cooked covered for 6 minutes and uncovered for an additional 6 minutes. According to Perharicˇ et al. (2013) the concentrations of atropine and (-)-scopolamine in the cooked buckwheat meal were reduced by approximately 58 and 37 percent, respectively, during food processing. Taking into account this loss, Table 26 summarizes the doses of (-)-hyoscyamine and scopolamine estimated in the finished meals.

<table>
<thead>
<tr>
<th>TOTAL DOSE BEFORE ADJUSTING FOR PROCESSING (µg/kg bw)</th>
<th>TOTAL DOSE AFTER ADJUSTING FOR PROCESSING (µg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.32</td>
<td>0.15</td>
</tr>
<tr>
<td>0.97</td>
<td>0.46</td>
</tr>
<tr>
<td>3.24</td>
<td>1.54</td>
</tr>
<tr>
<td>9.73</td>
<td>4.62</td>
</tr>
<tr>
<td>32.45</td>
<td>15.41</td>
</tr>
</tbody>
</table>

In this study, Perharicˇ et al. (2013) recorded quantitative changes in body temperature, heart rate, salivary secretion, sweat secretion and pupil size up to 4 hours after dosing whereas a variety of subjective symptoms (drowsiness, ataxia, headache, nausea, etc.) were monitored for up to 24 hours after dosing. Although no dose-dependent effect on body temperature was observed, dose-dependent changes in heart rate, salivary secretion, sweat secretion and pupil size were observed.
5.2 DOSE RESPONSE ANALYSIS

Experts agreed that as a first step, points of departure (PODs) should be identified, to the extent possible, for each effect. As a separate step, a conclusion would be reached on the adversity of the effect at or around the POD. Hence, in the first instance PODs identified from discrete dose spacings are referred to as no (low) observed effect levels (N(L)OELs). Dose response modelling, using the EFSA online tool (PROAST v 67.0; 200 bootstraps), was attempted for heart rate, salivary secretion, sweat secretion and pupil size (prior to adjusting for processing). Adequate model fit was achieved for salivary secretion, sweat secretion and pupil size using the entire dataset; however, due to a relatively large degree of uncertainty in the data for sweat secretion and pupil size (large BMDU:BMDL ratio) the confidence in the BMDLs estimates for sweat secretion and pupil size was considered low. The results of the dose response analysis are summarized in Table 27 below and additional details regarding the data used in this analysis, individual model results and visual plots can be found in Annexes A and B.

<table>
<thead>
<tr>
<th>EFFECT (TIME OF OBSERVATION)</th>
<th>NOEL</th>
<th>LOEL</th>
<th>MODEL AVERAGE CONFIDENCE INTERVALS</th>
<th>INDIVIDUAL MODEL BMD RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>BMDL05</td>
<td>BMDU05</td>
</tr>
<tr>
<td>Heart rate (2.75 hrs)</td>
<td>0.32</td>
<td>0.97</td>
<td>Not amenable to BMD</td>
<td></td>
</tr>
<tr>
<td>Salivary secretion (1.5 hrs)</td>
<td>0.97</td>
<td>3.24</td>
<td>0.62</td>
<td>3.75</td>
</tr>
<tr>
<td>Salivary secretion (3.5 hrs)</td>
<td>0.97</td>
<td>3.24</td>
<td>0.38</td>
<td>2.89</td>
</tr>
<tr>
<td>Sweat secretion (1.5 hrs)</td>
<td>3.24</td>
<td>9.73</td>
<td>0.23</td>
<td>4.79</td>
</tr>
<tr>
<td>Sweat secretion (3.5 hrs)</td>
<td>3.24</td>
<td>9.73</td>
<td>0.013</td>
<td>1.3</td>
</tr>
<tr>
<td>Pupil Size (4 hrs)</td>
<td>9.73</td>
<td>32.45</td>
<td>0.96</td>
<td>27.1</td>
</tr>
</tbody>
</table>

* Due to the known biphasic dose response for heart rate, adequate model fit was not achieved for any of the models considering the entire dataset.
5.3 CRITICAL EFFECT LEVELS

Table 28 below summarizes the adjusted points of departure (PODs) for each endpoint using the adjustment figures suggested by Perharic et al. (2013).

<table>
<thead>
<tr>
<th>EFFECT (TIME OF OBSERVATION)</th>
<th>NOEL</th>
<th>LOEL</th>
<th>MODEL AVERAGE BMDL05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (2.75 hrs)</td>
<td>0.15</td>
<td>0.46</td>
<td>Not amenable to BMD(^a)</td>
</tr>
<tr>
<td>Salivary Secretion (1.5 hrs)</td>
<td>0.46</td>
<td>1.54</td>
<td>0.3</td>
</tr>
<tr>
<td>Salivary Secretion (3.5 hrs)</td>
<td>0.46</td>
<td>1.54</td>
<td>0.2</td>
</tr>
<tr>
<td>Sweat secretion (1.5hr)</td>
<td>1.54</td>
<td>4.62</td>
<td>Uncertainty in the BMDL(^b)</td>
</tr>
<tr>
<td>Sweat secretion (3.5hr)</td>
<td>1.54</td>
<td>4.62</td>
<td>Uncertainty in the BMDL(^b)</td>
</tr>
<tr>
<td>Pupil Size (4hrs)</td>
<td>4.62</td>
<td>15.41</td>
<td>Uncertainty in the BMDL(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Due to the known biphasic dose response for heart rate, adequate model fit was not achieved for any of the models considering the entire dataset;  
\(^b\) Due to wide confidence intervals, confidence in the BMDL estimate was low.

Based on the analysis summarized above, decreases in heart rate and salivary secretion were considered the most sensitive biological effects induced by low doses of atropine and scopolamine. Additionally, both effects are commonly observed following low dose therapeutic use of atropine and (-)-scopolamine.

The paradoxical effects of atropine and scopolamine on heart rate are well documented, with slowing of the heart rate reported at low doses and tachycardia reported at higher doses. Due to the biphasic dose response for heart rate, these data were not amenable to benchmark dose modelling. Based on the transient, mild decrease in heart rate observed at 0.97 µg/kg bw (0.46 µg/kg bw adjusted for processing), the lowest dose of 0.32 µg/kg bw (0.15 µg/kg bw adjusted for processing) for the combined sum of (-)-hyoscyamine and (-)-scopolamine was considered to be the no observed effect level (NOEL). The mean heart rate of the volunteers exposed to control, 0.32, 0.97, 3.24, 9.73 and 32.45 µg/kg bw doses were 60.58 ± 3.55, 59.83 ± 3.22, 52.05 ± 1.65, 49.85 ± 1.65, 55.00 ± 2.19 and 84.38 ± 4.43 bpm, respectively at the 2.75 hours observation interval. By the 4 hours observation time point, the heart rate of the 0.97 µg/kg bw (0.46 µg/kg bw adjusted) dose group was not statistically different from control. However, the decreased heart rate observed with the 3.24 µg/kg bw (1.54 µg/kg bw adjusted) dose and the increased heart rate at 32.45 µg/kg bw (15.41 µg/kg bw adjusted) persisted to the 4 hour observation period (55.19 ± 3.64 and 81.60 ± 3.08 bpm, respectively). Although decreased heart rate represents a sensitive indicator of biological effect in the Perharic et al. (2013) study, the magnitude observed is not likely to cause adverse effects in healthy individuals. For example, based on an analysis of four population studies, the American College of Cardiology/American Heart Association (ACC/AHA) Task Force on Clinical Practice Guidelines (Kusumoto et al., 2019) considers that a heart rate of less than 50 bpm is consistent with the condition of bradycardia.
The effect of atropine and scopolamine on salivary secretion inhibition is also well documented and follows a typical monotonic dose response pattern. Benchmark dose modelling of the Perharicˇ et al. (2013) data resulted in a model averaged BMDL$_{05}$ value of 0.38 µg/kg bw (0.2 µg/kg bw adjusted for processing) when comparing absolute salivary secretion observed 3.5 hours after meal consumption (see Annexes A and B for details). This represents a dose where a minimal change in salivary secretion may occur. Although a small decrease such as 5 percent in salivary secretion (the BenchMark Response) is not considered adverse in healthy individuals, for the purposes of hazard characterization the BMDL$_{05}$ is considered a sensitive indicator of biological effect. When comparing postprandial secretion (e.g. magnitude of change in salivary secretion before and 3.5 hours after the meal), volunteers exposed to control, 0.32, 0.97, 3.24, 9.73 and 32.45 µg/kg bw doses showed mean changes in salivary secretion of approximately +25 percent, +34 percent, +13 percent, -15 percent, -65 percent, and -98 percent, respectively. While the lowest dose had no effect on postprandial secretion 3.5 hours after meal consumption, postprandial secretion appear to be significantly reduced at doses greater than or equal to 3.24 µg/kg bw (1.54 µg/kg bw adjusted).

At doses greater than or equal to 9.73 µg/kg bw (4.62 µg/kg bw adjusted for processing), there were statistically significant decreased sweat secretion (e.g. >65 percent) and increased reports of subjective symptoms, such as dry mouth, drowsiness and ataxia. At the highest dose (32.45 µg/kg bw; 15.41 µg/kg bw adjusted for processing) pupil dilation and increased heart rate (>80 bpm) were observed.
See Table 29 below for a summary of the points of departure considered for risk characterization.

**TABLE 29** POINTS OF DEPARTURE FROM THE PERHARIĆ ET AL. (2013) STUDY AFTER ADJUSTING THE SUM OF (-)-HYOSCYAMINE AND (-)-SCOPOLAMINE DOSE FOR PROCESSING

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>POINT OF DEPARTURE (µg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased HR (NOEL)</td>
<td>0.15</td>
</tr>
<tr>
<td>Decreased salivary secretion (BMDL₀₅)</td>
<td>0.2</td>
</tr>
<tr>
<td>Decreased salivary secretion (LOEL)</td>
<td>1.54</td>
</tr>
<tr>
<td>Increasing HR with subjective symptoms and decreased sweat secretion (LOEL)</td>
<td>4.62</td>
</tr>
<tr>
<td>Pupil dilation (LOEL)</td>
<td>15.4</td>
</tr>
</tbody>
</table>
Woman receives Super Cereal Plus from WFP for her child in Nigeria
Hyoscyamine and scopolamine induce both pharmacological and toxicological effects, however determining a clear point of demarcation between transient non-adverse effects and toxicological effects, from the information available, was not considered possible. Additionally, as populations typically consuming WFP products could have various underlying health conditions (e.g., malnourishment, malaria and tuberculosis) that may make them overly sensitive to TA toxicity, the expert meeting considered that the determination of a health based guidance value (HBGV) based on results from the general population studied by Perharic et al. (2013) was not appropriate. However, in order to support food security in populations receiving the WFP products, the expert meeting concluded that consideration of several PODs, ranging from a no observed effect level for non-adverse anti-muscarinic effects to a potentially adverse effect level, and the use of a Margin of Exposure (MOE) approach would be most appropriate.

The randomised, double blind, placebo controlled, crossover study with adult male volunteers by Perharic et al. (2013) with combined oral exposures to atropine and (-)-scopolamine was considered the most relevant for determining a POD. Decreases in heart rate and salivary secretion were considered the most sensitive indicators of anticholinergic effects and both effects are commonly observed following low therapeutic doses of atropine and (-)-scopolamine.

The effects of atropine and scopolamine on heart rate are well documented, with slowing of the heart rate at low doses and increases in heart rate at higher doses. Due to the biphasic dose response for heart rate, these data were not amenable to benchmark dose modelling. Based on the transient, mild decrease in heart rate observed at 0.97 µg/kg bw (0.46 µg/kg bw adjusted for processing), the lowest dose of 0.32 µg/kg bw (0.15 µg/kg bw adjusted for processing) for the combined sum of (-)-hyoscyamine and (-)-scopolamine was considered to be NOEL. Heart rate increased to a rate similar to that in controls at 9.7 µg/kg bw (4.62 µg/kg bw adjusted for processing) while a statistically significant increase in heart rate was observed at 32.4 µg/kg bw (15.4 µg/kg bw adjusted for processing) in the same study.

The effect of atropine and scopolamine on inhibition of salivary secretion is also well-documented and follows a typical monotonic dose response pattern. Benchmark dose modelling of the Perharic et al. (2013) data yielded a model averaged BMDL05 value of 0.38 µg/kg bw (0.2 µg/kg bw adjusted for processing) at the 3.5 hours observation interval. However, this represents a dose at which a minimal change in salivary
secretion might occur (5 percent) and dry mouth was not apparent until 9.7 µg/kg bw (4.62 µg/kg bw adjusted for processing). It is further acknowledged that in the case of a decrease in salivary secretion the default benchmark response (BMR) of 5 percent for continuous variables does not represent a level of adversity. However, here it was used as a sensitive biomarker of antimuscarinic effects. At doses of greater than or equal to 1.54 µg/kg bw decreases in salivary secretion were evident, becoming statistically significant in the two highest dose groups. Similarly, the magnitude of decreased heart rate induced in the Perharić et al. (2013) study at the LOEL is not likely to cause adverse effects in healthy individuals. At higher doses, statistically significant decreased sweat secretion (≥9.7 µg/kg bw; 4.62 µg/kg bw adjusted for processing), pupil dilation (32.4 µg/kg bw; 15.4 µg/kg bw adjusted for processing) and increased heart rate (32.4 µg/kg bw; 15.4 µg/kg bw adjusted for processing) were observed.

Based on this analysis, the expert meeting determined that in healthy male adults, a dose of 1.54 µg/kg bw was considered to be a clinically significant minimal acute effect dose, based on the reduction of salivary secretion.

In order to provide guidance for the development of operational limits for hyoscyamine and scopolamine in WFP products, an MOE approach (Table 30) based on pharmacological effects in humans and acute dietary exposures was used in the risk characterization.

For the general diet, compared to a clinically significant minimal acute effect dose of 1.54 µg/kg bw, MOEs for the general population (children and women of reproductive
age) ranged from 3,080 to 3,850 (mean) and 440–616 (95th percentile) for combined exposures to hyoscyamine and scopolamine. These MOEs were not considered to be of concern by the expert meeting. For doses required to produce potentially adverse effects (e.g., increased heart rate, decreased saliva, dry mouth and sweat secretion and pupil dilation at 4.62 µg/kg bw), the MOEs would have to be three times greater.

During the incident, the subpopulations consuming WFP products showed severe anticholinergic effects at lower doses than would otherwise have been expected in the general population. Comparing the exposures from consuming the WFP products containing the highest concentrations of TAs (17.4 mg/kg) would lead to estimated intakes of 7.6–32.4 µg/kg bw (100 g intake, body weights as per Table 20) for children and adults. At these doses, relatively severe effects, including coma and mortality, were reported in the affected populations. In comparison, severe effects, including mortality, have usually been associated only with atropine doses of 10 mg in children (approximately 250 µg/kg bw (−)-hyoscyamine) and 100 mg in adults (approximately 920 µg/kg bw (−)-hyoscyamine). This increased sensitivity of one to two orders of magnitude is likely due to any toxicokinetic differences and a variety of toxicodynamic factors that could influence the health outcomes specific to the subpopulations consuming the WFP products, such as malnutrition and underlying comorbidities. In an attempt to establish acceptable MOEs for the PODs based on pharmacological and toxicological effects, various factors affecting inter-individual sensitivity were considered. For example, human inter-individual differences are typically accounted for using uncertainty factors of 3.16 for toxicokinetics and 3.16 for toxicodynamics.
The basis for these default inter-individual uncertainty factors considers protection from long-term exposure to chemicals, and as such are area-under-the-curve of concentration–time (AUC)-dependent. However, since the critical effects for hyoscyamine and scopolamine are $C_{\text{max}}$ dependent effects, according to JECFA guidance (2008), a 50 percent reduction in the safety factor for inter-individual differences in toxicokinetics is deemed appropriate (e.g. $3.16 \div 2 = 1.58$), resulting in a composite uncertainty factor of 5 ($3.16 \times 1.58 = 5$). Given that hyoscyamine and scopolamine can cause pharmacological and toxicological effects, establishing a POD that clearly demarcated adverse reactions from pharmacological reactions was not considered possible. Comparing the clinically significant minimal acute effect dose of 1.54 μg/kg bw and the perceived additional sensitivity of the affected subpopulation, an additional uncertainty factor of six was deemed appropriate, although the expert meeting recognized that accounting for the impacts of existing nutritional and health conditions on effects related to TA exposure are difficult to define and measure. This composite target MOE of 30 (5x6) encompasses allowance for the inter-individual differences in toxicokinetics, perceived additional sensitivity of the affected subpopulations, and the nature of the pharmacological/toxicological effects upon which the clinically significant minimal acute effect dose was based.

For dietary exposures related to WFP products post-incident, compared to a clinically significant minimal acute effect dose of 1.54 μg/kg bw, the MOEs ranged from 91 to 241 (mean) and 29 to 86 (95th percentile). Taking into account the sensitivity and variability between individuals, these MOEs are considered to be of low concern for the target population. The concentrations of hyoscyamine and scopolamine in WFP products detected after the incident and the absence of adverse effects in those consuming them, support the inference of low concern resulting from these MOEs.
TABLE 30 MARGINS OF EXPOSURES FOR ACUTE DIETARY EXPOSURE ESTIMATES (µg/kg bw; HYOSCYAMINE + SCOPOLAMINE) * VS. POINT OF DEPARTURE (POD) FROM PERHARIĆ ET AL. (2013)

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>POD (µg/kg bw)</th>
<th>MARGINS OF EXPOSURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased Heart rate (NOEL)</td>
<td>0.15</td>
<td>300 8 44 4 9 3 375 43 14 5 375 33 60 15 23 8</td>
</tr>
<tr>
<td>Decreased salivary secretion (BMDL05)</td>
<td>0.2</td>
<td>400 11 59 5 12 4 500 57 18 6 500 43 80 20 31 11</td>
</tr>
<tr>
<td>Decreased salivary secretion (LOEL)</td>
<td>1.54</td>
<td>3 080 86 453 41 91 29 3 850 440 140 48 3 850 335 616 154 241 86</td>
</tr>
<tr>
<td>Increasing HR with subjective symptoms and decreased sweat secretion (LOEL)</td>
<td>4.62</td>
<td>9 240 257 1 359 122 272 86 11 550 1 320 420 144 11 550 1 004 1 848 462 722 257</td>
</tr>
<tr>
<td>Pupil dilation (LOEL)</td>
<td>15.4</td>
<td>30 800 856 4 529 405 906 285 38 500 4 400 1 400 481 38 500 3 348 6 160 1 540 2 406 856</td>
</tr>
</tbody>
</table>

* For the general diet, the concentration data set contained a high level of left-censored data (>90 percent) and values below the LOD were assumed to be true zero values (lower bound). For the WFP product concentration data only 19 percent of results were left-censored and lower and upper bound estimates of acute dietary exposure were derived. The resulting estimates were similar, and the upper bound estimates have been included.
Children receiving super cereal plus from WFP in Zimbabwe.
CHAPTER 7
RECOMMENDATIONS

Based on the recommended intake of various WFP products of 100 g/day, a combined hyoscyamine/scopolamine concentration in dry food of less than approximately 30 μg/kg (in Super Cereal)\(^{21}\) or 10 μg/kg (in Super Cereal Plus and Lipid-based Nutrient Supplements)\(^{22}\) should be health protective for adults and children respectively. These concentrations are proposed as operational limits that may be extended to other cereal and grain products when consumed in comparable quantities. If higher quantities are consumed, appropriate adjustment of the values would be necessary.

For emergency situations where food security needs to be taken into consideration it would be expected that guidance levels of 90 μg/kg (Super Cereal)\(^{23}\) and 30 μg/kg (Super Cereal Plus and Lipid-based Nutrient Supplements)\(^{24}\) should still be protective against severe toxicity for adults and children respectively. These emergency guidance levels were derived from a clinically significant minimal acute effect dose (i.e. based on increasing heart rate, decreased salivation, and decreased sweat secretion).

It was further considered by the expert meeting that it would be difficult to define these proposed operational limits/guidance levels based on numbers of *Datura* seeds in grain used in the production of WFP products mainly due to the large variability in TA concentrations in *Datura* species.

A summary of uncertainties considered by the expert meeting are provided in Annex C.

\(^{21}\) Concentration = 1.54 μg/kg bw ÷ 30 × 60 kg bw (adult body weight) ÷ 0.1 kg of dry food.
\(^{22}\) Concentration = 1.54 μg/kg bw ÷ 30 × 20 kg bw (child body weight) ÷ 0.1 kg of dry food.
\(^{23}\) Concentration = 4.62 μg/kg bw ÷ 30 × 60 kg bw (adult body weight) ÷ 0.1 kg of dry food.
\(^{24}\) Concentration = 4.62 μg/kg bw ÷ 30 × 20 kg bw (child body weight) ÷ 0.1 kg of dry food.
Porters offloading WFP commodity bags meant for distribution in South Sudan.


REFERENCES


Parrot, A.C. 1986. The effects of transdermal scopolamine and four dose levels of oral scopolamine (0.15, 0.3, 0.6, and 1.2 mg) upon psychological performance. *Psychopharmacology.* 89: 347-354.


WFP food in a warehouse in Yemen.
The data considered for dose response analysis are listed in Tables A to D below.

**TABLE A1**  **HEART RATE DATA MEASURED 2.75 HOURS AFTER DOSING, FROM PERHARIĆ ET AL. (2013)**

<table>
<thead>
<tr>
<th>DOSE (µg/kg bw)</th>
<th>NUMBER</th>
<th>MEAN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>60.58</td>
<td>3.55</td>
</tr>
<tr>
<td>0.32</td>
<td>9</td>
<td>59.83</td>
<td>3.22</td>
</tr>
<tr>
<td>0.97</td>
<td>20</td>
<td>52.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65</td>
</tr>
<tr>
<td>3.24</td>
<td>20</td>
<td>49.85&lt;sup&gt;5a&lt;/sup&gt;</td>
<td>1.65</td>
</tr>
<tr>
<td>9.73</td>
<td>11</td>
<td>55.00</td>
<td>2.19</td>
</tr>
<tr>
<td>32.45</td>
<td>20</td>
<td>84.38&lt;sup&gt;5a&lt;/sup&gt;</td>
<td>4.43</td>
</tr>
</tbody>
</table>

<sup>a</sup> P<0.05.  
<sup>b</sup> P<0.001.

**TABLE A2**  **SALIVARY SECRETION DATA FROM PERHARIĆ ET AL. (2013)**

<table>
<thead>
<tr>
<th>DOSE (µg/kg bw)</th>
<th>NUMBER</th>
<th>1.5 HOURS</th>
<th>3.5 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MEAN</td>
<td>SEM</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>6.64</td>
<td>0.8</td>
</tr>
<tr>
<td>0.32</td>
<td>9</td>
<td>7.36</td>
<td>1.5</td>
</tr>
<tr>
<td>0.97</td>
<td>20</td>
<td>6.36</td>
<td>0.93</td>
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<td>3.24</td>
<td>20</td>
<td>4.14</td>
<td>0.58</td>
</tr>
<tr>
<td>9.73</td>
<td>11</td>
<td>2.76&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>32.45</td>
<td>20</td>
<td>0.12&lt;sup&gt;5a&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> P<0.001.
Dose response modelling, using the EFSA online tool (PROAST v 67.0; 200 bootstraps), was attempted for heart rate, salivary secretion, sweat secretion and pupil size. Adequate model fit was achieved for salivary secretion, sweat secretion and pupil size using the entire dataset. Additional details regarding the individual model results and visual plots can be found in the Annex B.
ANNEX B

ADDITIONAL DOSE RESPONSE DATA-VISUAL FIT PLOTS AND MODEL PREDICTIONS

(A) HEART RATE

At the lowest combined dose tested, 0.15 µg/kg bw (adjusted for processing and expressed as atropine), no significant difference was observed in heart rate (mean +/- SEM bpm) compared to the controls over the 4-hour observation period. Mean heart rate was decreased by approximately 14 percent (P<0.05) in the second highest dose group, 0.46 µg/kg bw, but only at the 2.75-hour observation period. In the third highest dose group, 1.54 µg/kg bw, heart rate was significantly decreased by an average of approximately 16 percent (P<0.05) over the entire 4-hour observation period. At the highest dose tested, 15.41 µg/kg bw, mean heart rate was increased by an average of approximately 32 percent (P<0.001) during the 2 to 4-hour observation periods (not after 1 hour). No significant difference in heart rate was noted in the 4.62 µg/kg bw dose group. For additional details, see Fig. 2, Perharič et al. (2013). Heart rate data was determined not to be amenable to BMD analysis.
(B) SALIVARY SECRETION 1.5 HRS (EFSA BMD OUTPUT)

**TABLE A5  DATA USED FOR ANALYSIS**

<table>
<thead>
<tr>
<th>DOSE</th>
<th>MEAN</th>
<th>SEM</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>6.21</td>
<td>0.78</td>
<td>20</td>
</tr>
<tr>
<td>0.32</td>
<td>6.71</td>
<td>1.29</td>
<td>9</td>
</tr>
<tr>
<td>0.97</td>
<td>6.03</td>
<td>0.83</td>
<td>20</td>
</tr>
<tr>
<td>3.24</td>
<td>4.51</td>
<td>0.70</td>
<td>20</td>
</tr>
<tr>
<td>9.73</td>
<td>2.95</td>
<td>0.83</td>
<td>11</td>
</tr>
<tr>
<td>32.45</td>
<td>0.13</td>
<td>0.07</td>
<td>20</td>
</tr>
</tbody>
</table>

**TABLE A6  FITTED MODELS**

<table>
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<th>NPAR</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7</td>
<td>252.80</td>
</tr>
<tr>
<td>Null model</td>
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<td>-209.99</td>
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<td>249.74</td>
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<tr>
<td>Hill m5-</td>
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<td>-119.98</td>
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<td>249.96</td>
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<td>5</td>
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<td>LN m3-</td>
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<td>-120.14</td>
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<td>248.28</td>
</tr>
<tr>
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<td>yes</td>
<td>-120.39</td>
<td>5</td>
<td>250.78</td>
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FIGURE A1. PLOTS OF VARIOUS PROAST MODELS - EXPONENTIAL (EXPON), HILL (HILL), INVERSE EXPONENTIAL (INV EXP) AND LOG-NORMAL (LN) MODELS FOR SALIVARY SECRETION 1.5 HOURS
FIGURE A2. AVERAGED DOSE–RESPONSE MODEL FOR SALIVARY SECRETION 1.5 HOURS

TABLE A7  WEIGHTS FOR MODEL AVERAGING

<table>
<thead>
<tr>
<th>EXP</th>
<th>HILL</th>
<th>INVEXP</th>
<th>LOGN</th>
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</thead>
<tbody>
<tr>
<td>0.31</td>
<td>0.3</td>
<td>0.16</td>
<td>0.22</td>
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</table>
### (C) Salivary Secretion 3.5 Hrs (EFSA BMD Output)

**Table A8: Data Used for Analysis**

<table>
<thead>
<tr>
<th>DOSE</th>
<th>MEAN</th>
<th>SEM</th>
<th>NUMBER</th>
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</thead>
<tbody>
<tr>
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<td>6.64</td>
<td>0.80</td>
<td>20</td>
</tr>
<tr>
<td>0.32</td>
<td>7.36</td>
<td>1.50</td>
<td>9</td>
</tr>
<tr>
<td>0.97</td>
<td>6.36</td>
<td>0.93</td>
<td>20</td>
</tr>
<tr>
<td>3.24</td>
<td>4.14</td>
<td>0.58</td>
<td>20</td>
</tr>
<tr>
<td>9.73</td>
<td>2.76</td>
<td>0.90</td>
<td>11</td>
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<td>32.45</td>
<td>0.12</td>
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**Table A9: Fitted Models**

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<th>AIC</th>
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<td>Expon. m3-</td>
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<td>255.36</td>
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<td>LN m3-</td>
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<td>4</td>
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<td>5</td>
<td>256.86</td>
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FIGURE A3. PLOTS OF VARIOUS PROAST MODELS - EXPONENTIAL (EXPON), HILL (HILL), INVERSE EXPONENTIAL (INV EXP) AND LOG-NORMAL (LN) MODELS FOR SALIVARY SECRETION 3.5 HOURS
FIGURE A4. AVERAGED DOSE–RESPONSE MODEL FOR SALIVARY SECRETION 3.5 HOURS

TABLE A10 WEIGHTS FOR MODEL AVERAGING

<table>
<thead>
<tr>
<th></th>
<th>EXP</th>
<th>HILL</th>
<th>INVEXP</th>
<th>LOGN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.34</td>
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<td>0.13</td>
<td>0.21</td>
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### (D) SWEAT SECRETION 1.5 HRS (EFSA BMD OUTPUT)

#### TABLE A11  DATA USED FOR ANALYSIS

<table>
<thead>
<tr>
<th>DOSE</th>
<th>MEAN</th>
<th>SEM</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.10</td>
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<td>20</td>
</tr>
<tr>
<td>0.32</td>
<td>0.10</td>
<td>0.040</td>
<td>9</td>
</tr>
<tr>
<td>0.97</td>
<td>0.08</td>
<td>0.010</td>
<td>20</td>
</tr>
<tr>
<td>3.24</td>
<td>0.09</td>
<td>0.020</td>
<td>20</td>
</tr>
<tr>
<td>9.73</td>
<td>0.03</td>
<td>0.010</td>
<td>11</td>
</tr>
<tr>
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<td>0.001</td>
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</tbody>
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#### TABLE A12  FITTED MODELS

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<th>NPAR</th>
<th>AIC</th>
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</thead>
<tbody>
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<td>224.88</td>
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<td>5</td>
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<td>4</td>
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<td>Hill m5-</td>
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<td>5</td>
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<td>-105.32</td>
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<tr>
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<td>5</td>
<td>220.64</td>
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</tbody>
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FIGURE A5. PLOTS OF VARIOUS PROAST MODELS - EXPONENTIAL (EXPON), HILL (HILL), INVERSE EXPONENTIAL (INV EXP) AND LOG-NORMAL (LN) MODELS FOR SWEAT SECRETION 1.5 HOURS.
FIGURE A6. AVERAGED DOSE–RESPONSE MODEL FOR SWEAT SECRETION 1.5 HOURS

TABLE A13  WEIGHTS FOR MODEL AVERAGING

<table>
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## (E) SWEAT SECRETION 3.5 HRS (EFSA BMD OUTPUT)

### Table A14  Data Used for Analysis

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<th>DOSE</th>
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<th>NUMBER</th>
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<tbody>
<tr>
<td>0.00</td>
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<td>20</td>
</tr>
<tr>
<td>0.32</td>
<td>0.10</td>
<td>0.030</td>
<td>9</td>
</tr>
<tr>
<td>0.97</td>
<td>0.09</td>
<td>0.020</td>
<td>20</td>
</tr>
<tr>
<td>3.24</td>
<td>0.08</td>
<td>0.010</td>
<td>20</td>
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<td>0.04</td>
<td>0.020</td>
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### Table A15  Fitted Models

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<td>238.40</td>
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FIGURE A7. PLOTS OF VARIOUS PROAST MODELS - EXPONENTIAL (EXPN), HILL (HILL), INVERSE EXPONENTIAL (INV EXP) AND LOG-NORMAL (LN) MODELS FOR SWEAT SECRETION 3.5 HOURS
FIGURE A8. AVERAGED DOSE–RESPONSE MODEL FOR SWEAT SECRETION 3.5 HOURS

TABLE A16  WEIGHTS FOR MODEL AVERAGING

<table>
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<th>LOGN</th>
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</thead>
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### (F) PUPIL SIZE 4 HRS (EFSA BMD OUTPUT)

#### TABLE A17  DATA USED FOR ANALYSIS

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<td>5.00</td>
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<td>9</td>
</tr>
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<td>0.97</td>
<td>4.98</td>
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<td>20</td>
</tr>
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<td>20</td>
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<td>9.73</td>
<td>5.28</td>
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<td>11</td>
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#### TABLE A18  FITTED MODELS

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<th>NPAR</th>
<th>AIC</th>
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<td>-99.08</td>
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<td>53.63</td>
<td>5</td>
<td>-97.26</td>
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</table>
FIGURE A9. PLOTS OF VARIOUS PROAST MODELS - EXPONENTIAL (EXPO), HILL (HILL), INVERSE EXPONENTIAL (INV EXP) AND LOG-NORMAL (LN) MODELS FOR PUPIL SIZE 4.0 HOURS.
**FIGURE A10. AVERAGED DOSE–RESPONSE MODEL FOR PUPIL SIZE 4.0 HOURS**

```
version: 0.2
model averaging results
shapes: 10
selected all
dose scaling: 1
conf level: 0.9
number of runs: 200
CIS: 0.01
BMD CI
0.96 27.1
```

**TABLE A19  WEIGHTS FOR MODEL AVERAGING**

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### ANNEX C

## UNCERTAINTIES IN THE RISK ASSESSMENT

Summary of the qualitative evaluation of the impact of uncertainties on the risk assessment of hyoscyamine and scopolamine in food.

### TABLE A20 SUMMARY OF THE QUALITATIVE EVALUATION OF THE IMPACT OF UNCERTAINTIES ON THE RISK ASSESSMENT OF HYOSCYAMINE AND SCOPOLAMINE IN FOOD

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<th>DIRECTION a, b</th>
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<td>Total measurement uncertainty in chemical analytical results</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Exposure estimates for the general diet used occurrence data almost entirely from European countries</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Exposure estimates for the general diet were based on lower-bound concentrations25</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Exposure estimates for WFP products and the general diet assumed no degradation of TAs on processingcooking</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lack of accurate information on actual daily amount of WFP products consumed</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Assumption of equivalent potency of (-)-hyoscyamine and (-)-scopolamine</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Assumption that all of hyoscyamine in food is present as the active (-) enantiomer</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lack of occurrence data on TAs other than hyoscyamine and scopolamine</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lack of toxicological data on TAs other than hyoscyamine and scopolamine</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Limited data to identify PODs at which clearly adverse effects start to occur in humans</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>The actual dose of (-)-hyoscyamine and (-)-scopolamine to which volunteers were exposed in the critical study</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lack of information on the adverse effects in humans of incurred (-)-hyoscyamine and (-)-scopolamine in food</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>PODs used based on pharmacological endpoints in healthy volunteers that are unlikely to be adverse in the general population</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Differences in sensitivity in population experiencing adverse effects after consumption of WFP products assumed be due only to differences in sensitivity to TAs</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Appropriateness of the uncertainty factor of 5 to allow for inter-individual variability in toxicokinetics and toxicodynamics, including age dependent differences</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>Appropriateness of the uncertainty factor of 6 to allow for differences in sensitivity between the general and target sub-populations due to underlying health conditions (malnutrition, malaria, TB, etc)</td>
<td>+/-</td>
<td></td>
</tr>
</tbody>
</table>

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

b Only qualitative estimates of uncertainty are provided and the pluses and minuses should not be added.

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25 Analytical results below the limit of detection (LOD) were substituted by a value of zero; analytical results with a level between LOD and limit of quantification (LOQ) were substituted by a value equal to the LOD.
Between March and April 2019, many cases of suspected food poisoning were reported by health care workers in the Karamoja region of the Republic of Uganda. Consumption of food products that had high levels of tropane alkaloids was identified as the cause. This group of compounds occur in several plant genera that belong to the Solanaceae family and can contaminate staples like cereals and grains.

Given the absence of international guidance and regulations, a Joint FAO/WHO Expert Meeting on Tropane Alkaloids was convened remotely between 30 March – 3 April 2020.

This publication captures the discussions of the expert meeting and provides risks assessments of tropane alkaloids (hyoscyamine and scopolamine) as well as recommendations outlining appropriate risk management options.