Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO EXPERT MEETING ON TROPANE ALKALOIDS

Executive Summary

30 March – 3 April 2020

Background

Assisting 86.7 million people in around 83 countries each year, the United Nations World Food Programme (WFP) is the leading humanitarian organisation that saves and changes lives by delivering food assistance in emergencies and working with communities to improve nutrition and build resilience. WFP 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 Specialised 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 CDC/FDA, WHO, 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 soya 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].

Within this context, WFP had requested assistance from FAO/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 HQ in Rome, was held remotely by an electronic platform due to the various global restrictions in place during the COVID-19 pandemic.

Scope

The scope of the Joint FAO/WHO Expert Meeting on Tropane Alkaloids was to: 1) provide a risk assessment for (-)-scopolamine, (-)-hyoscyamine and (+)-hyoscyamine: 2) 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.

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

Conclusions

<u>Chemical and Analytical Characterisation</u>

- Tropane alkaloids are present in many *Solanacae* genera 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 0.01–3%.

- 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* L. cultivated in Morocco. In seeds, hyoscyamine and scopolamine accounted for 66% and 20% (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% of the reported tropane alkaloid content (Mulder *et al.,* 2016).

[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.

- 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% 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 *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 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 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 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 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% of single component flours, 20% of cereal-based food for young children age 6-36 months, 15.8% of bread, 26.2% of legumes and stir-fry mixes, and 14.6% 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, 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.

- 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 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 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 (Perharič *et al.* 2013; Marín-Sáez *et al.* 2019). Decreases reported were 63-70% of hyoscyamine and 42-80% 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.

- One study (List and Spencer 1976) suggests that when processing soybeans, hyoscyamine (90% of the content in unprocessed soybeans) and scopolamine (84%) will be present in the defatted meal. Approximately 0.1% will be present in the unrefined oil, and over 90% 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 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. 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% 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% of (-)-hyoscyamine was converted to (+)-hyoscyamine after one day. Therefore, in most conditions used for food processing minimal enantiomerisation is expected to occur.

- 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.

Toxicological Evaluation of Hyoscyamine and Scopolamine

- As the toxicological and exposure information on tropane alkaloids is generally limited to hyoscyamine and scopolamine, the hazard characterisation described herein necessarily focusses 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%) with a lower bioavailability observed for (-)-scopolamine (27% or lower; range 12 to 48%). Both tropane alkaloids are extensively distributed into tissues and excreted predominantly via the renal system. Only 2.6% of parent (-)-scopolamine is excreted in urine whereas up to 50% 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 breastmilk.

- 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 1-4 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 2 years (6.9 ± 3.3 hours) and in geriatric patients 65-75 years (10.0 ± 7.3 hours), compared to in children over 2 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 (M_1-M_5). 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 1000 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 secretions (digestive, bronchial, cutaneous, lacrimal), as an anti-spasmodic for various GI 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 1.5-3.0 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 Perharič *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 secretions, sweat secretions, 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 secretions, sweat secretions and pupil dilation were observed. Decreased heart rate and salivary secretions 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 secretions was observed, becoming statistically significant at 9.7 µg/kg bw. A benchmark dose lower confidence limit or $BMDL_{05}$ of 0.38 µg/kg bw was derived for the decrease in salivary secretions. 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 secretions observed at the low doses in the Perharič *et al.*, (2013) are of questionable adversity to healthy individuals, the NOEL and $BMDL_{05}$ do represent sensitive indicators of the anticholinergic activity of (-)-hyoscyamine and (-)-scopolamine.

- Since Perharič *et al.*, (2013) reported loss of atropine (37%) and scopolamine (58%) during cooking, the above noted doses were adjusted accordingly. The NOEL and $BMDL_{05}$ from the Perharič *et al.*, (2013) study translate to 0.15 and 0.20 µg/kg bw, respectively, considering the adjustment factors suggested by Perharič *et al.*, (2013).

Hazard characterisation

- 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 characterisation.

- The following points were considered for the hazard characterisation for hyoscyamine and scopolamine:
    1. They do not bioaccumulate;
    2. They have short half-lives in humans (hours);
    3. Peak plasma concentrations are achieved within two hours; the effects generally occur within a short time after administration and are transient;
    4. They are not genotoxic *in vivo*;
    5. They do not cause carcinogenicity or progressive toxicity following repeated oral exposure;
    6. They do not cause developmental toxicity; and
    7. 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 Perharič *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 Perharič *et al.*, (2013) data yielded a model averaged $BMDL_{05}$ 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%) 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% 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 Perharič *et al.*, (2013) study at the LOEL is not likely to cause adverse effects in healthy individuals. At higher doses, statistically significant decreased sweat secretions (≥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 WGP 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 Perharič *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 an 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% 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. Consequently, 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 Europe (99%), with a small number from Singapore (1%). 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% 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 (MCRA). 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% 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 (95^{th} 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% 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%) 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% 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 *t*TA 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 *t*TA 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% of *t*TA exposure was due to hyoscyamine, depending on the time period (before or after the incident), the sub-population group and the treatment of left-censored data.

- Before the incident, mean acute dietary *t*TA 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 and implementation of additional risk management measures (monitoring, raw material source selection and improved grain cleaning), the mean acute dietary *t*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.

- LNS was found to be rarely contaminated with tropane alkaloids and, when contaminated, only at low *t*TA concentrations (<2 µg/kg). Based on the main target population for this product (young children), LNS may result in a maximum acute dietary *t*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 characterisation and Recommendations

- In order to provide guidance for the development of operational limits for hyoscyamine and scopolamine in WFP products, a Margin of Exposure (MOE) approach based on pharmacological effects in humans and acute dietary exposures was used in the risk characterisation.

- 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 3080 to 3850 (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 secretions 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 characterise 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 C_{max} 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 ug/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 secretions).

- 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 *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

Uncertainties in the Risk Assessment

Table 1. Summary of the qualitative evaluation of the impact of uncertainties on the risk assessment of hyoscyamine and scopolamine in food

Sources of uncertainty	**Direction** [a,b]
Total measurement uncertainty in chemical analytical results	+/-
Exposure estimates for the general diet used occurrence data almost entirely from European countries	-
Exposure estimates for the general diet were based on lower-bound concentrations[6]	-
Exposure estimates for WFP products and the general diet assumed no degradation of TAs on processing/cooking	+
Lack of accurate information on actual daily amount of WFP products consumed	+/-
Assumption of equivalent potency of (-)-hyoscyamine and (-)-scopolamine	+/-
Assumption that all hyoscyamine in food is present as the active (-) enantiomer	+
Lack of occurrence data on TAs other than hyoscyamine and scopolamine	-
Lack of toxicological data on TAs other than hyoscyamine and scopolamine	-
Limited data to identify PODs at which clearly adverse effects start to occur in humans	+
The actual dose of (-)-hyoscyamine and (-)-scopolamine to which volunteers were exposed in the critical study	+
Lack of information on the adverse effects in humans of incurred (-)-hyoscyamine and (-)-scopolamine in food	+/-
PODs used based on pharmacological endpoints in healthy volunteers that are unlikely to be adverse in the general population	+
Differences in sensitivity in population experiencing adverse effects after consumption of WFP products assumed be due only to differences in sensitivity to tropane alkaloids	+
Appropriateness of the uncertainty factor of 5 to allow for inter-individual variability in toxicokinetics and toxicodynamics, including age dependent differences	+/-
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)	+/-

(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.

[6] 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.

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Annex I – Margin of Exposures (MOEs)

		Acute Dietary Exposure Estimates (µg/kg bw; hyo + scop), mean (95th percentile)															
		Young children (6-59 months)						Children (5-15 years)				Women (15-44 years)					
		General diet				WFP products		General diet		WFP products		General diet				WFP products	
		Mean LB range		p95 LB range		Mean UB	p95 UB	Mean	p95	Mean UB	p95 UB	Mean LB range		p95 LB range		Mean UB	p95 UB
		0.0005	0.018	0.0034	0.038	0.017	0.054	0.0004	0.0035	0.011	0.032	0.0004	0.0046	0.0025	0.01	0.0064	0.018
		Margins of Exposure															
Effect	**POD**	**Perharič *et al*., 2013**															
Decreased HR (NOEL)	0.15	300	8	44	4	9	3	375	43	14	5	375	33	60	15	23	8
Decreased saliva $BMDL_{05}$	0.2	400	11	59	5	12	4	500	57	18	6	500	43	80	20	31	11
Decreased saliva LOEL	1.54	3,080	86	453	41	91	29	3,850	440	140	48	3,850	335	616	154	241	86
Increasing HR with sub symptoms and decreased sweat LOEL	4.62	9,240	257	1,359	122	272	86	11,550	1,320	420	144	11,550	1,004	1,848	462	722	257
Pupil dilation and increased HR LOEL	15.4	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
Effect	**POD**	**Clinical use and Poisonings**															
Min. thera. dose atropine	3.5	7,000	194	1,029	92	206	65	8,750	1,000	318	109	8,750	761	1,400	350	547	194
Min. thera. dose scop.	4	8,000	222	1,176	105	235	74	10,000	1,143	364	125	10,000	870	1,600	400	625	222
Toxic dose for adult	833	1,666,667	46,296	245,098	21930	49,020	15,432	2,083,333	238,095	75,758	26,042	2,083,333	181,159	333,333	83,333	130,208	46,296
Toxic dose for atr. in child	250	500,000	13,889	73,529	6579	14,706	4,630	625,000	71,429	22,727	7,813	625,000	54,348	100,000	25,000	39,063	13,889
WFP poisoning (adversity)	29	58,000	1,611	8,529	763	1,706	537	72,500	8,286	2,636	906	72,500	6,304	11,600	2,900	4,531	1,611
Effect	**POD**	**Experimental Animals**															
Rodent LOEAL (14 wks)	10,400	20,800,000	577,778	3,058,824	273,684	611,765	192,593	26,000,000	2,971,429	945,455	325,000	26,000,000	2,260,870	4,160,000	1,040,000	1,625,000	577,778
Rodent LOEAL (chronic)	692	1,384,000	38,444	203,529	18,211	40,706	12,815	1,730,000	197,714	62,909	21,625	1,730,000	150,435	276,800	69,200	108,125	38,444

Note regarding exposure estimates: For the general diet, the concentration data set contained a high level of left-censored data (>90%) and values below the LOD were assumed to be true zero values (lower bound). For the WFP product concentration data only 19% 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

Annex II – List of Participants

Experts

- Abdul AFGHAN, Canada.
- Gabriel ADEGOKE, Swaziland
- Susan BARLOW, United Kingdom
- Diane BENFORD, United Kingdom
- Alan BOOBIS, United Kingdom *(Chair of the meeting)*
- Polly BOON, The Netherlands
- Peter CRESSEY, New Zealand
- Mark FEELEY, Canada *(Rapporteur of the meeting)*
- Susanne RATH, Brazil
- Josef SCHLATTER, Switzerland
- Sheryl TITTLEMIER, Canada
- Stephan WALCH, Germany

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- Vittorio FATTORI, FAO, Italy
- Markus LIPP, FAO, Italy
- Keya MUKHERJEE, FAO, Italy
- Kim PETERSEN, WHO, Switzerland

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- Suvrat BAFNA
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