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Assessment of the Conclusions of the Joint FAO/WHO Expert Meeting on Tropane Alkaloids

European Food Safety Authority (EFSA) and
Marco Binaglia

Abstract

The European Food Safety Authority (EFSA) was requested to assess the differences in the outcome of the risk assessment of tropane alkaloids (TAs) in food between the CONTAM Panel and the Joint FAO/WHO meeting (FAO/WHO) and to conclude if an update of the EFSA opinion on tropane alkaloids in food and feed would be appropriate. TAs are secondary metabolites occurring in several plants. The main TAs considered in the assessments of EFSA and FAO/WHO were (-)-hyoscyamine and (-)-scopolamine, which exert their pharmacological and toxicological effects by acting as competitive antagonists of the muscarinic acetylcholine receptors. Both EFSA and FAO/WHO considered a study in human volunteers as the key study to assess the effects of TAs. The CONTAM Panel established a group acute reference dose (ARfD) of 0.016 µg/kg body weight (bw) for the sum of (-)-hyoscyamine and (-)-scopolamine, based on decreased heart rate. FAO/WHO concluded that it was not possible to establish an ARfD and instead selected a point of departure of 1.54 µg/kg bw for the sum of the two substances, based on decreased salivary secretion, and applied it in a margin of exposure approach. A detailed assessment of the differences in the two approaches is provided in the report. Overall, it is not straightforward to compare quantitatively the differences emerging from the assessments of the CONTAM Panel and the FAO/WHO, in view of the different approaches applied and the different scopes of the assessments. Given the existing uncertainties, the ARfD established by the CONTAM Panel should be retained without modifications as protective towards the general population including susceptible subgroups. In conclusion, based on the comparison with the FAO/WHO assessment, an update of the CONTAM Panel assessment on the risks to human health related to the presence of tropane alkaloids in food is not considered necessary.

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Keywords: Tropane alkaloids, ARfD, MOE, acute toxicity

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Correspondence: contam@efsa.europa.eu

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Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information (if appropriate).....	4
2. Assessment.....	5
2.1. Selection of the key study.....	6
2.2. Establishment of the Reference Point.....	7
2.3. Hazard characterisation approach.....	7
3. Discussion and conclusions.....	8
References.....	9
Abbreviations.....	9
Appendix A – Additional Benchmark Dose analysis of data on salivatory secretion from the study of Perharič et al. (2013).....	11

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM Panel) has adopted a scientific opinion on tropane alkaloids in food and feed.¹

Since the pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration, the CONTAM Panel concluded that it was appropriate to establish an Acute Reference Dose (ARFD) for these substances. Based on the results for decreased heart rate in a human volunteer study, the CONTAM Panel established a group ARFD of 0.016 µg/kg body weight (bw) expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency.

A Joint FAO/WHO Expert Meeting on Tropane Alkaloids has taken place remotely from 30 March to 3 April 2020.²

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 the uncertainties in the specific sensitivity to tropane alkaloids toxicity of the populations subject to the assessment.

A margin of exposure (MOE) approach based on pharmacological effects in humans and acute dietary exposures was used in the risk characterization. MOEs were calculated for the estimated exposure, compared with the clinically significant minimal acute effect dose of 1.54 µg/kg bw. The expert meeting considered an MOE of 30 (factor of 5 for normal individual variability and an additional factor of 6 for increased sensitivity of the target population) or greater to be of low concern for the target population.

It is appropriate to provide an assessment of the differences in the outcome of the risk assessment between the CONTAM Panel and the Joint FAO/WHO meeting and to conclude if, based on this assessment, an update to the scientific opinion on tropane alkaloids in food and feed would be appropriate or not.

TERMS OF REFERENCE

In accordance with Art. 31 (1) of Regulation (EC) No 178/2002 the Commission asks EFSA for a statement on the conclusions of the Joint FAO/WHO Expert Meeting on Tropane Alkaloids including:

- an assessment of the differences in the outcome of the risk assessment between the CONTAM Panel and the Joint FAO/WHO meeting
- a conclusion if, based on this assessment, an update to the scientific opinion on tropane alkaloids in food and feed would be appropriate or not.

1.2. Additional information (if appropriate)

Tropane alkaloids (TAs) are toxins produced as secondary metabolites by plants from various families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae (including coca). Plants biosynthesise the (-)-enantiomeric forms of TAs, among which (-)-hyoscyamine and (-)-scopolamine are the most studied and predominant ones in TA-producing plants (EFSA, 2018). Atropine, the racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine, is generally used as analytical standard for the quantification of (-)-hyoscyamine in food and as pharmaceutically active substance.

Accidental contamination of cereals and other vegetables with parts of TA-producing plants during harvesting is the main source for the occurrence of TAs in food. Occasionally high levels of contamination caused poisoning cases and circumscribed outbreaks of intoxication, the most recent one in the EU occurred in Slovakia, where about 100 people reported mild intoxication symptoms related to the consumption spinach puree contaminated by atropine and (-)-scopolamine in the ranges of 850–3,446 and 1,033–3,860 µg/kg, respectively.³

TAs exert their pharmacological and toxicological effects mainly by acting as competitive antagonists of the muscarinic acetylcholine receptors, both at the level of the central and peripheral nervous systems. Several studies showed that (-)-hyoscyamine is mainly responsible of the

¹ <https://www.efsa.europa.eu/en/efsajournal/pub/3386>

² FAO and WHO, 2020. *Joint FAO/WHO Expert meeting on tropane alkaloids - 30 March – 3 April 2020*. Food Safety and Quality Series No. 11. Rome. <https://doi.org/10.4060/cb1857en>

³ RASFF notification 2021.1390.

antimuscarinic activity of atropine, with the (+)-enantiomer having a 30- to 300-time lower affinity for the muscarinic receptors. The main antimuscarinic effects of TAs in the peripheral system are decreased production of secretions from the salivary, bronchial and sweat glands, dilation of the pupils and paralysis of accommodation, increased heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion. At toxic doses, (-)-hyoscyamine and (-)-scopolamine cause stimulation of the central nervous systems with restlessness, disorientation, hallucinations and delirium. As the dose increases, stimulation is followed by central depression leading to death from respiratory paralysis. Below the antimuscarinic dose ranges, atropine and (-)-scopolamine show cholinomimetic activity, resulting in paradoxical effects such as increased gastric contraction frequency and amplitude, and decreased heart rate. The exact mechanism by which the cholinomimetic activity occurs is not known.

In 2013, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) published a scientific opinion on the risks to human and animal health related to the presence of tropane alkaloids (TA) in food and feed (EFSA CONTAM Panel, 2013). The CONTAM Panel established a group acute reference dose (ARfD) of 0.016 µg/kg body weight (bw) for the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. Due to limited data availability, the exposure assessment in the 2013 opinion was only possible for toddlers through the consumption of cereal-based food for infants and young children. The acute exposure levels estimated by means of a probabilistic approach were found to exceed the ARfD with a probability ranging from 11.6% to 17.9% of the consumption days for average and high consumers. The exposure assessment was updated (EFSA, 2018) using a more robust occurrence data set allowing to estimate the exposure for all the age groups. Large differences were observed between the lower and upper bound (LB and UB) estimated acute exposure levels across all age classes. Under the UB assumption, mean acute exposure levels exceeded the ARfD in 'infants', 'toddlers' and 'other children', and at the P95 in all age classes. Under the LB assumption, the group ARfD was exceeded at the P95 in 'toddlers' and 'other children'.

In 2020, a Joint FAO/WHO Expert Meeting (from now onwards FAO/WHO) assessed the risks related to the presence of TAs in food in response to two cases of contamination outbreaks that occurred in the supply chain of food aids in 2019 (FAO/WHO, 2020). In April 2019, the consumption of a batch of fortified cereals contaminated by high levels of (-)-scopolamine and (±)-hyoscyamine led to the hospitalisation of about 300 people and five deaths in the Republic of Uganda. A second incident involved unprocessed sorghum distributed as food aid to the Republic of South Sudan. FAO/WHO 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. The expert committee did not consider this adequate to establish a Health-Based Guidance Value and instead applied a margin of exposure (MOE) approach. The exposure assessment was performed considering two scenarios, one for the general population groups in countries where the World Food Programme (WFP) is active, and another focussing on the specific food products formulated for WFP. In the first scenario, developed using occurrence data mainly from European countries, the mean acute exposure levels (reported for the LB assumption) were lower than 1 ng/kg bw for most of the population, with the exception of children and women from Zambia for whom levels of 18 and 4.6 ng/kg bw were estimated. The highest P95 levels (under the LB assumption) were also estimated for children and women in Zambia (38 and 10 ng/kg bw, respectively), whereas for the other populations ranged from 2.5 to 3.5 ng/kg bw. The scenario on specific foods formulated for WFP considered occurrence data from the monitoring programme in these products, performed both before and after the 2019 incidents (the concentrations of the specific batches associated with cases of intoxication were excluded from the data set). Exposure levels were estimated for three populations considering the specific consumption patterns of the fortified cereals: children from 6 months to < 5 years, children from 5 to 15 years and women from 15 to 44 years. The levels before the 2019 incidents ranged from 26 (mean LB for adult women) to 557 (P95 UB in children < 5 years) ng/kg bw. After the incident, considerably lower exposure levels were estimated, ranging from 6.0 to 53.8 ng/kg bw.

2. Assessment

The differences in the outcome of the risk assessment of the CONTAM Panel and the FAO/WHO are discussed in this section. Regarding the exposure assessment, the occurrence data sets and approach of FAO/WHO for the general population were comparable to those applied by EFSA (EFSA, 2018); however, FAO/WHO based its exposure assessment on consumption data from countries from Africa, Asia and South America. In view of this, a direct comparison of the estimated exposure levels is

considered not pertinent and not discussed in detail. The present assessment therefore focuses on the approaches undertaken for the hazard characterisation of TAs with particular reference to the assessment of the selected key study, the selection of the toxicological reference points (RP) and the hazard characterisation approaches applied by the two scientific bodies.

2.1. Selection of the key study

The CONTAM Panel and the FAO/WHO selected the human clinical study from Perharič et al. (2013) for the derivation of the acute reference point (RP). In this study, 20 young healthy adult volunteers were exposed to various doses of a mixture of atropine and (-)-scopolamine through the consumption of a boiled buckwheat meal, following a double-blind, placebo-controlled study design. All subjects were randomly exposed to the placebo control and four doses in a cross-over fashion, allowing for at least 2 weeks of wash-over between an exposure and the next one. The meals were spiked with increasing concentrations of a 2:1 atropine/(-)-scopolamine mixture. The final concentrations were measured in the cooked meal to account for the loss of the two substances during the processing. The study design is reported in Table 1.

Table 1: Study design as reported by Perharič et al. (2013)

Number of subjects	Concentrations in buckwheat meals		Average doses (after correction for losses during processing)	
	Atropine ($\mu\text{g}/100\text{ g meal}$)	(-)-Scopolamine ($\mu\text{g}/100\text{ g meal}$)	Atropine ($\mu\text{g}/\text{kg bw}$)	(-)-Scopolamine ($\mu\text{g}/\text{kg bw}$)
20	–	–	–	–
9	25.0	12.5	0.12	0.10
20	75.0	37.5	0.37	0.29
20	250.0	125.0	1.22	0.95
11	750.0	375.0	3.58	2.81
20	2,500.0	1,250.0	12.10	9.50

Following the treatment, the subjects were monitored for 4 h and body temperature, heart rate, salivary secretion, sweat secretion and pupillary dilation were recorded at regular intervals. Subjective symptoms were recorded during the 4-ho period and subjects were requested to report eventual symptoms for at least 24 h after treatment or until the symptoms ceased.

Both the CONTAM Panel and the FAO/WHO derived doses for the sum of (-)-hyoscyamine and (-)-scopolamine, assuming a 50:50 racemic composition for atropine and a negligible activity of the (+)-hyoscyamine enantiomer. This resulted in doses derived by the CONTAM Panel of 0, 0.16, 0.48, 1.56, 4.60 and 15.55 $\mu\text{g}/\text{kg bw}$ for the sum of (-)-hyoscyamine and (-)-scopolamine. The doses derived by FAO/WHO slightly differed (0, 0.15, 0.46, 1.54, 4.62 and 15.41 $\mu\text{g}/\text{kg bw}$ for the sum of (-)-hyoscyamine and (-)-scopolamine), likely due to the different approach used for the calculation. The results of the study are reported in this section considering the doses derived by the CONTAM Panel.

A trend for a slight increase in body temperature was recorded at all dose levels from 1 to 4 h after the treatment, with no clear dose–response relationship.

Heart rate showed a non-monotonic response in relation to dose. A statistically significant increase was observed at the top dose 2–4 h after the treatment (reaching a maximum after 2.75 h), whereas statistically significantly decreased heart rate was observed at 0.48 $\mu\text{g}/\text{kg bw}$ (only 2.75 h after the treatment) and at 1.56 $\mu\text{g}/\text{kg bw}$ at all time points. No statistically significant differences from the control were observed for the other doses (0.16 and 4.60 $\mu\text{g}/\text{kg bw}$).

Decreased salivary and sweat secretion were recorded during the 4-h post-treatment period, both effects achieving statistical significance at > 1.56 $\mu\text{g}/\text{kg bw}$.

Statistically significant pupil dilation was observed after 4 h at the top dose only.

The number and frequency of reported subjective symptoms increased in a dose-related fashion. Drowsiness was reported within the 4-hour post-treatment period with an increasing trend (8/20, 3/9, 8/20, 11/20, 8/11 and 18/20 subjects treated at 0, 0.16, 0.48, 1.56, 4.60 and 15.55 $\mu\text{g}/\text{kg bw}$, respectively). No other symptoms were reported at the lowest treatment dose, whereas one out of 20 subjects treated at 0.48 $\mu\text{g}/\text{kg bw}$ experienced dry mouth and dizziness within 4 h after treatment, and dizziness, headache and nausea within 24 h. Dry mouth was also reported by 2/20 subjects

treated with the placebo, and was the symptom most frequently reported at the three highest doses within 4 h from the treatment (6/20, 9/11 and 20/20 subjects treated at 1.56, 4.60 and 15.55 µg/kg bw, respectively), accompanied by other CNS effects (dizziness, ataxia, nausea headache and speech disturbance), in particular at > 1.56 µg/kg bw. In most of the cases, the effects reported at the two top doses within 4 h post-treatment persisted for at least 24 h.

2.2. Establishment of the Reference Point

Both risk assessment bodies considered the effect on the heart rate as a possible endpoint and concluded that no BMD analysis was possible due to the biphasic nature of the dose–response curve.

The CONTAM Panel selected the decrease in heart rate as the critical effect of the Perharič et al. (2013) study occurring at lower doses than the other peripheral antimuscarinic effects. For this effect, the Panel identified the lowest dose of 0.16 µg/kg bw as a no observed adverse effect level (NOAEL) and selected it as the acute reference point (RP) for the sum of (-)-hyoscyamine and (-)-scopolamine, noting uncertainties associated with the small population of the study. The Panel acknowledged that the decreased heart rate observed in the study is not adverse in healthy individuals, but could be adverse in more susceptible individuals, such as those with bradycardia. The Panel considered this dose also as the study NOAEL for subjective CNS symptoms, since one subject reported dizziness, headache and nausea when exposed at the next dose (0.48 µg/kg bw).

The FAO/WHO performed benchmark dose (BMD) analyses for several study endpoints, including salivary secretion at 1.5 and 3.5 h, sweat secretion at 1.5 and 3.5 h and pupil size at 4 h, in all cases using a benchmark response (BMR) of 5%. For the decrease in salivary secretion BMDL₀₅ of 0.3 and 0.2 µg/kg bw per day were calculated at 1.5 and 3.5 h, respectively. For the other endpoints, the FAO/WHO considered the BMDL₀₅ estimates of low confidence in view of the wide BMDL/BMDU intervals. In parallel, the no observed effect levels (NOEL) and lowest observed effect levels (LOEL) were identified for all the endpoints, and points of departure⁴ (PODs) were derived as summarised in Table 2.

Table 2: Summary of critical effect levels (µg/kg bw) identified by FAO/WHO for the sum of (-)-hyoscyamine and (-)-scopolamine. Points of departure considered for the risk characterisation are indicated in bold

Effect	NOEL	LOEL	BMDL ₀₅
Decreased heart rate	0.15	0.46	_(a)
Decreased salivary secretion	0.46	1.54	0.2–0.3
Decreased sweat secretion	1.54	4.62	_(b)
Increased pupil size	4.62	15.41	_(b)

(a): BMD analysis not possible due to the biphasic dose–response.

(b): Low confidence in the BMDL estimate (wide BMDL–BMDU interval).

The decreased heart rate and salivary secretion were considered the most sensitive biological effects.

In relation to the decreased heart rate, the FAO/WHO concluded that although it represented a sensitive indicator of biological effects, the magnitude observed was not likely to cause adverse effects in healthy individuals.

The FAO/WHO acknowledged that the applied BMR of 5% did not represent a level of adversity in relation to the effect on salivary secretion, but it was rather used as a sensitive biomarker of antimuscarinic effects. The lowest dose at which a non-statistically significant decrease in salivary secretion was observed in the Perharič et al. (2013) study (i.e. the LOEL of 1.54 µg/kg bw, cfr. Table 2) was eventually considered by FAO/WHO as ‘a clinically significant minimal acute effect dose’ for the antimuscarinic effects of (-)-hyoscyamine and (-)-scopolamine in healthy male adults and carried forward as POD for the risk characterisation.

2.3. Hazard characterisation approach

Following the identification of the critical effect(s) and derivation of the relevant reference point, the CONTAM Panel and the FAO/WHO differed in the approach for hazard characterisation.

⁴ The definition of point of departure coincides with that of reference point, used by EFSA.

The CONTAM Panel concluded that the RP of 0.16 µg/kg bw provided the preferred basis for establishing a group acute reference dose (ARfD) for the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equal potency for the two substances. For the ARfD derivation, the CONTAM Panel decided to apply an uncertainty factor (UF) of 10 for interindividual differences, considering that the key study providing the RP was a small study in young healthy male volunteers. Therefore, a group ARfD of 0.016 µg/kg bw expressed as the sum of (-)-hyoscyamine and (-)-scopolamine was derived. The CONTAM Panel noted that group ARfD is approximately two orders of magnitude lower than the lowest single therapeutic doses for (-)-hyoscyamine and (-)-scopolamine.

The FAO/WHO considered that the determination of a health-based guidance value (HBGV) based on results from the general population studied by Perharič et al. (2013) was not appropriate. Instead, the expert meeting concluded that the use of a margin of exposure (MOE) approach would be most appropriate in consideration of the several PODs identified (cfr. Table 2 of this document). Considering the LOEL of 1.54 µg/kg bw as the relevant POD, an acceptable MOE of ≥ 30 was established based on the following rationale:

- A factor of 5 to account for intraindividual variability. Since the critical effects for TAs are dependent effects on the systemic maximal peak concentrations (C_{max}), according to JECFA guidance (FAO/WHO, 2017), a 50 percent reduction in the default safety factor for interindividual differences in toxicokinetics is deemed appropriate (e.g. $3.16 \div 2 = 1.58$), resulting in a composite uncertainty factor of 5 ($3.16 \times 1.58 = 5$).
- Noting that during the incident with fortified cereals described in Section 1 of this document the subpopulations consuming WFP products showed severe effects at lower doses than would otherwise have been expected in the general population, FAO/WHO deemed appropriate to include an additional factor of 6 to account for the perceived additional sensitivity of the affected subpopulation under the scope of the assessment.

3. Discussion and conclusions

The comparison of the hazard characterisations of the toxicological and pharmacological effects of (-)-hyoscyamine and (-)-scopolamine performed by the CONTAM Panel and the FAO/WHO evidenced main differences in the interpretation of the results of the human clinical study performed by Perharič et al. (2013).

The CONTAM Panel ultimately considered the decreased heart rate observed at 0.48 µg/kg bw and 1.56 µg/kg bw as a relevant effect for hazard characterisation, acknowledging that the effect is not adverse in healthy adults but could be adverse in more susceptible individuals. Due to the biphasic nature of the effect, quantitative dose response analysis was not performed and the lowest dose of 0.16 µg/kg bw was selected as an NOAEL.

The FAO/WHO also concluded that the decreased heart rate is not adverse in healthy adults, but did not identify a possible group of the population with higher susceptibility to this effect and ultimately considered it as a non-adverse effect and identified 0.15 and 0.46 µg/kg bw as NOEL and LOEL, respectively. FAO/WHO established PODs for most of the clinical effects observed by Perharič et al. (2013), concluding that the already mentioned decreased heart rate and the decreased salivary secretion (NOEL and LOEL identified at 0.46 and 1.54 µg/kg bw, respectively) are the most sensitive biological effects of (-)-hyoscyamine and (-)-scopolamine. For the latter, FAO/WHO derived BMDL₀₅ of 0.2–0.3 µg/kg bw, noting however that the applied BMR of 5% did not represent a level of adversity. Instead of using a larger BMR, FAO/WHO selected the LOEL of 1.54 µg/kg bw as POD, concluding that this would be a 'clinically significant minimal acute effect dose'. Although both effects were considered as non-adverse by FAO/WHO, a rationale on the final selection of a 'clinically significant minimal acute effect dose' based on the effect on salivary secretion instead of the effect on heart rate is not detailed in the report. In addition, the rationale why the LOEL and not the NOEL for decreased salivatory secretion was selected as a POD is also not completely clear.

It is noted that the LOEL of 1.54 µg/kg bw for the sum of (-)-hyoscyamine and (-)-scopolamine (or 1.56 µg/kg bw, considering the dose conversion applied by the CONTAM Panel), selected as POD by FAO/WHO, falls in the range of the minimum therapeutic doses of (-)-hyoscyamine (0.1 mg/person, corresponding to 1.4 µg/kg bw for 70 kg bw), atropine (0.42 mg, corresponding to 6 µg/kg bw) and (-)-scopolamine (0.17 mg corresponding to 2.5 µg/kg bw). In its Scientific Opinion, the CONTAM Panel noted that these doses can be associated with adverse side effects, such as cardiac slowing or dryness of the mouth, and do not apply to individuals with contraindications who are likely to be more sensitive to some effects (EFSA CONTAM Panel, 2013).

In addition, it is unclear why instead of identifying a relevant BMR higher than 5%, possibly indicative of a 'clinically significant minimal acute effect dose' in relation to the salivary secretion, FAO/WHO decided to select the LOEL of 1.54 µg/kg bw. It is noted from the BMD analysis of the data set that this LOEL would fall in the BMDL range associated with BMRs of 40–50% for decreased salivary secretion (see Appendix A) and corresponded to an increased reporting of subjects with 'dry mouth' during the 4-h post-treatment period of the Perharič et al. (2013) study.

The CONTAM Panel and the FAO/WHO had also different approaches towards the possible setting of a health-based guidance value. The CONTAM Panel considered the information from the Perharič et al. (2013) study adequate to establish an ARfD, although acknowledging uncertainties related to the small size of the study and on the adversity of the heart rate decrease. The application of an UF of 10 for interindividual variability took into account the small size of the study on the one hand, and on the other hand, the relevance of the key effect identified only for the most susceptible part of the population.

Conversely, the FAO/WHO selected the MOE approach in the absence of a clear POD based on an adverse effect. The established 'acceptable MOE' of ≥ 30 considered both interindividual variability (accounting for a factor of 5) and an additional factor of 6 for the sensitivity due to 'malnutrition and underlying comorbidities' of the specific population targeted in the assessment. It is unclear from the FAO/WHO document whether a factor of 5 only would have been considered sufficient to assess the general population or whether an additional factor to account for the presence of a more susceptible population within the general population would have been also considered.

Overall, it is not straightforward to compare quantitatively the differences emerging from the assessments of the CONTAM Panel and the FAO/WHO, in view of the different approaches applied (ARfD vs. MOE approach) and the different scopes of the assessments (general population vs. a target population with higher susceptibility to develop severe effects from exposure to TAs).

Given the existing uncertainties, in particular related to the small size of the study in human volunteers used in the derivation of the acute RP, the ARfD established by the CONTAM Panel is considered protective towards the general population including susceptible subgroups and as such should be retained without modifications. A more robust data set informing on the dose-response relationship of TAs in humans would be needed to reduce the level of uncertainty and for a possible revision of the ARfD. In conclusion, based on the comparison with the FAO/WHO assessment, an update of the CONTAM Panel assessment on the risks to human health related to the presence of tropane alkaloids in food is not considered necessary.

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Abbreviations

ARfD	Acute Reference Dose
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower confidence Level
BMR	Benchmark Response
bw	Bodyweight
FAO	Food and Agriculture Organisation
HBGV	Health-based Guidance Value
LB	Lower Bound
LOAEL	Low Observed Adverse Effect Level

LOEL	Low Observed Effect Level
MOE	Margin of Exposure
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
P95	95 percentile
POD	Point of Departure
RP	Reference Point
TAs	Tropane alkaloids
UB	Upper Bound
UF	Uncertainty Factor
WHO	World Health Organisation

Appendix A – Additional Benchmark Dose analysis of data on salivatory secretion from the study of Perharič et al. (2013)

A.1. Summary

The data on salivary secretion in human volunteers measured at 3.5 h in the study by Perharič et al. (2013) were considered for the analysis. The study had a cross-over design with no independent control group and volunteers treated with different doses of TAs. The BMD analyses were performed assuming that the 2-week wash-out periods undertaken between sequential treatments were sufficiently long to avoid residual effects from the previous treatment. The results of the analysis show that the LOEL selected by FAO/WHO would fall in the BMDL range related to BMR of 40–50% for decreased salivary secretion.

A.2. Data description

The endpoint analysed was the mean salivary secretion measured at 3.5 h after the treatments.

Data used for analysis:

Dose (mg TA/kg bw)	Mean salivary secretion (mL)	Standard error (mL)	Number of subjects
0.00	6.64	0.80	20
0.16	7.36	1.50	9
0.48	6.36	0.93	20
1.56	4.14	0.58	20
4.60	2.76	0.90	11
15.55	0.12	0.07	20

A.3. Selection of the Benchmark Response

Benchmark responses (BMR) of 40% and 50% changes in mean response compared to the controls were used to indicatively replicate the effect size observed at the study LOEL (1.56 mg/kg). The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

A.4. Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 70.0, for the underlying calculations.

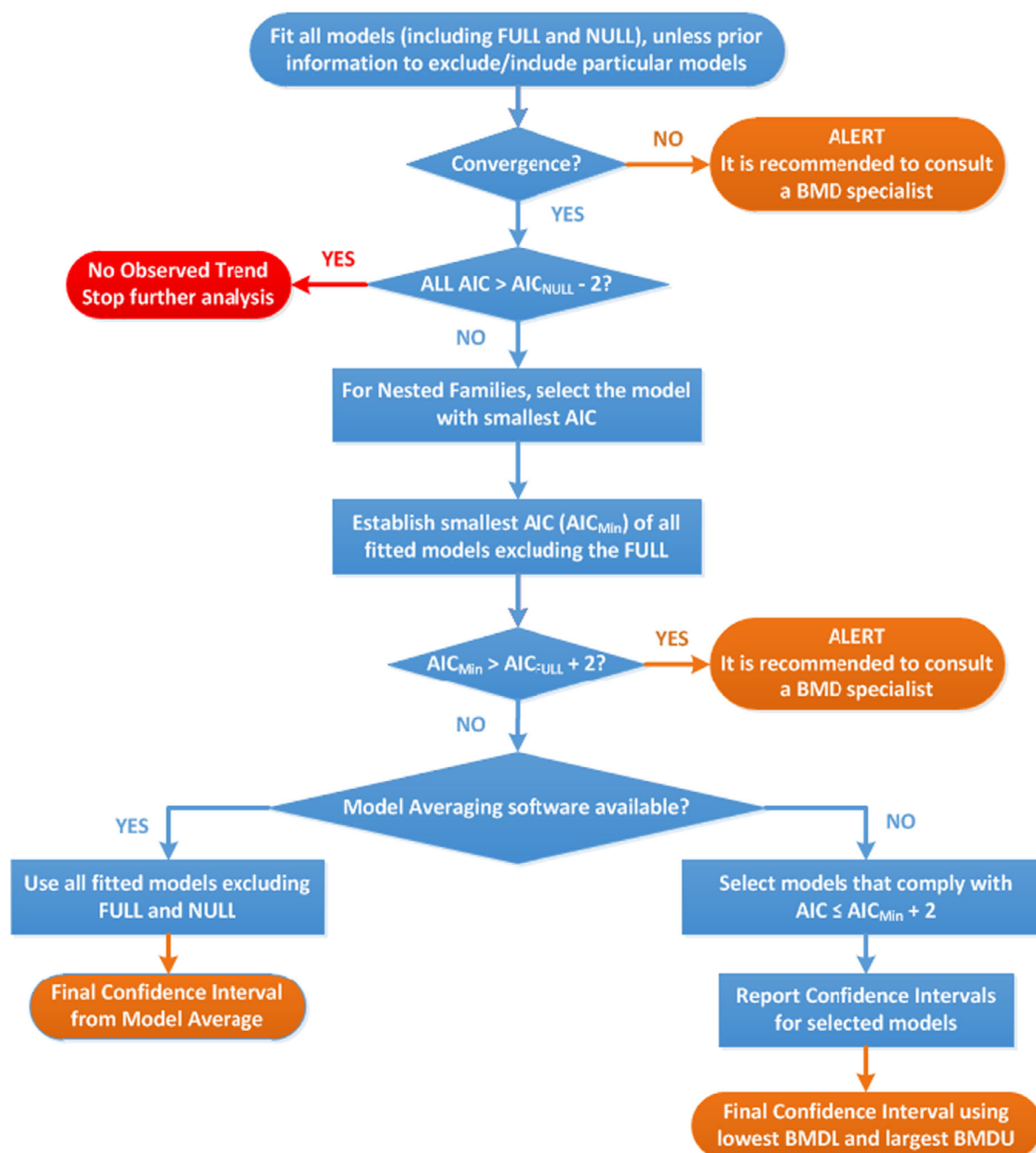
A.5. Specifications

Dose-response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	No. of groups	$y = \text{group mean}$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c - (c - 1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 4	4	$y = a \cdot \left(1 - \frac{(c-1)x^d}{b^d + x^d}\right)$
Inverse Exponential	4	$y = a \cdot (1 + (c - 1)\exp(-bx^{-d}))$
Log-Normal Family	4	$y = a \cdot (1 + (c - 1)\phi(\ln b + d \ln x))$

Procedure for selection of BMDL



Flowchart for selection of BMDL

A.6. Results

Fitted Models

Model	Converged	loglik	npar	AIC
Full model	Yes	-122.04	7	258.08
Null model	Yes	-213.69	2	431.38
Expon. m3-	Yes	-122.53	4	253.06
Expon. m5-	Yes	-122.63	5	255.26
Hill m3-	Yes	-122.57	4	253.14
Hill m5-	Yes	-122.80	5	255.60

Model	Converged	loglik	npar	AIC
Inv. Expon. m3-	Yes	-123.45	4	254.90
Inv. Expon. m5-	Yes	-127.28	5	264.56
LN m3-	Yes	-122.96	4	253.92
LN m5-	Yes	-123.35	5	256.70

A.6.1. BMR of 04

Estimated Model Parameters

EXP1

estimate for var- : 0.6789
 estimate for a- : 5.765
 estimate for CED- : 2.112
 estimate for d- : 1.16

HILL

estimate for var- : 0.6794
 estimate for a- : 5.737
 estimate for CED- : 2.149
 estimate for d- : 1.198

INVEXP

estimate for var- : 0.6914
 estimate for a- : 5.365
 estimate for CED- : 2.619
 estimate for d- : 0.4004

LOGN

estimate for var- : 0.6847
 estimate for a- : 5.529
 estimate for CED- : 2.391
 estimate for d- : 0.5639

Weights for Model Averaging

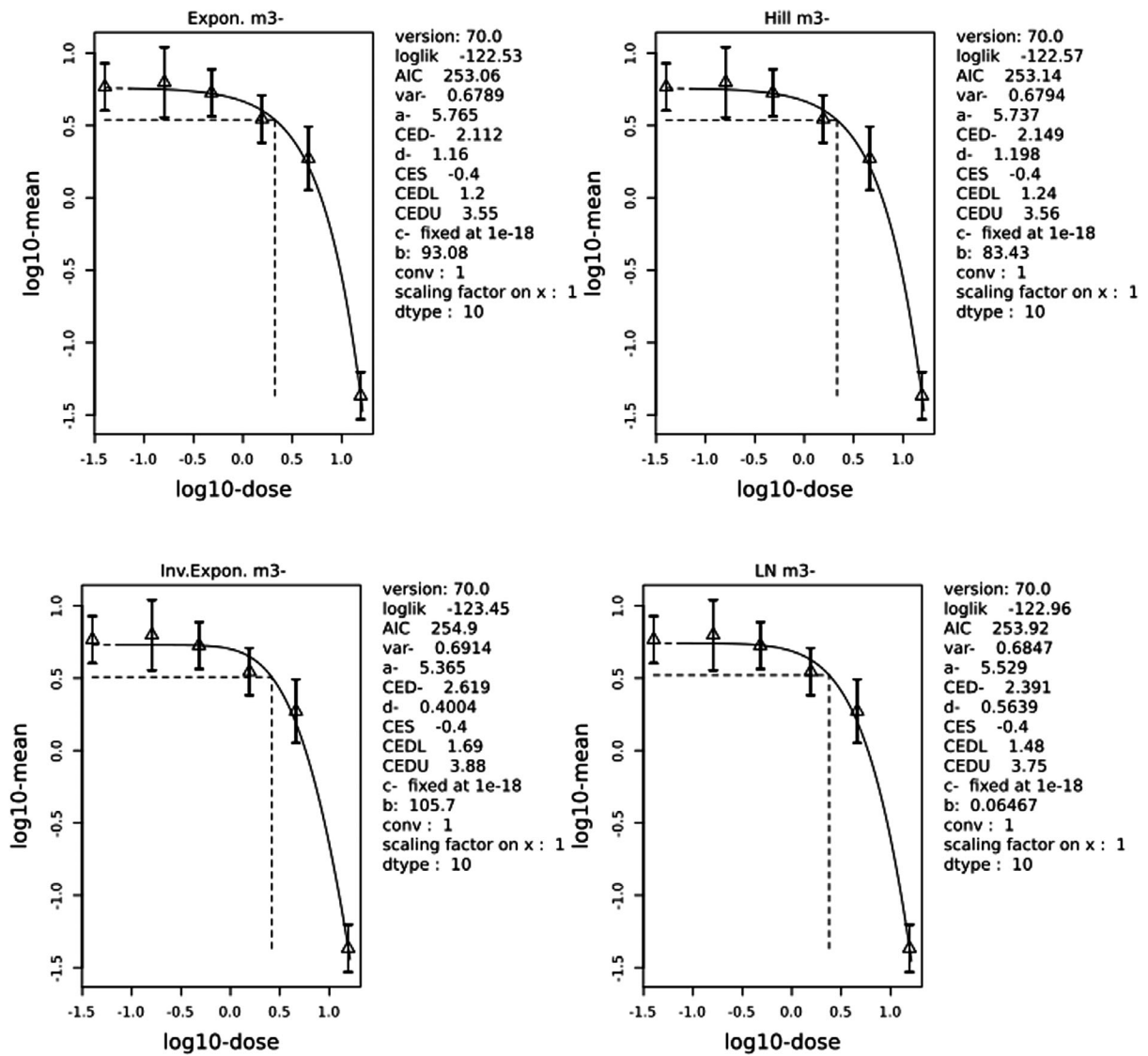
EXP	HILL	INVEXP	LOGN
0.33	0.32	0.13	0.22

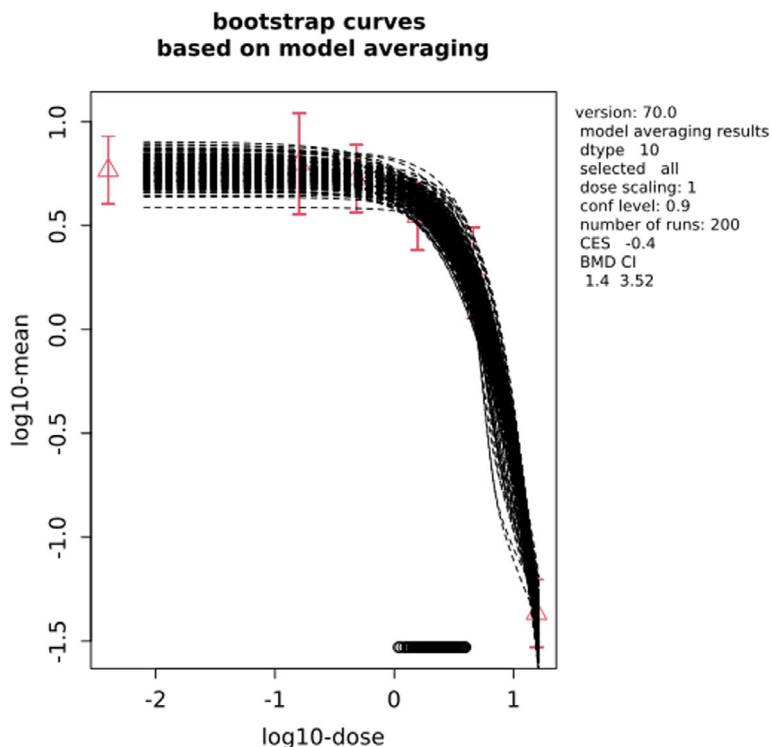
Final BMD Values

endpoint	subgroup	BMDL	BMDU
mean	All	1.44	3.52

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Visualisation





A.6.2. BMR of 0.5

Estimated Model Parameters

EXP

estimate for var- : 0.6789
 estimate for a- : 5.765
 estimate for CED- : 2.753
 estimate for d- : 1.16

HILL

estimate for var- : 0.6794
 estimate for a- : 5.737
 estimate for CED- : 2.783
 estimate for d- : 1.198

INVEXP

estimate for var- : 0.6914
 estimate for a- : 5.365
 estimate for CED- : 3.134
 estimate for d- : 0.4004

LOGN

estimate for var- : 0.6847
 estimate for a- : 5.529
 estimate for CED- : 2.959
 estimate for d- : 0.5639

Weights for Model Averaging

EXP	HILL	INVEXP	LOGN
0.33	0.32	0.13	0.22

Final BMD Values

Endpoint	Subgroup	BMDL	BMDU
Mean	All	1.95	4.18

Confidence intervals for the BMD are based on 200 bootstrap data sets.

Visualisation

