TOX/2019/22

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of potential risks from contaminants in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Background

1. As part of the review by the Scientific Advisory Committee on Nutrition (SACN) of Government recommendations on complementary and young child feeding, the Committee on Toxicity (COT) was asked to review the toxicity of chemicals in the diets of infants and young children aged 1-5 years.

2. As part of this review a risk assessment on tropane alkaloids (TAs) was presented to the Committee in July 2018 (TOX/2018/28) and the members decided a full review on TAs was unnecessary.

3. All estimated acute exposures of infant and young children to (-)-hyoscyamine and (-)-scopolamine or the sum of (-)-hyoscyamine and (-)-scopolamine were close to or below the ARfD of 16 ng/kg bw per day. The exposures are unlikely to be of toxicological concern. In addition, the limited information available indicates no toxicological concern regarding TAs in breast milk.

4. The members however requested additional information on other TAs reported in the FSA's survey, which was presented to the Committee in October 2018 (TOX/2018/36 Matter Arising). At the meeting the members discussed the pharmacological effects of (-)-hyoscyamine and (-) scopolamine and inquired if a) information regarding pharmacological effects of other TAs and b) information regarding structural parts of (-)-hyoscyamine and (-) scopolamine, which are responsible for their pharmacological effects was available and could be provided.

5. A brief literature search was undertaken for all TAs, excluding (-)-hyoscyamine, (-)-scopolamine and atropine, for any data on pharmacological effects of these TAs or any other additional information that might allow the Committee to reduce uncertainties on the total dietary exposure of infants and young children to a combination of all TAs.

6. A closer look was taken at the EFSA opinion (2013) for the mode of action of (-)-hyoscyamine and (-)-scopolamine and a brief literature search was performed for any data on structural effects and their related pharmacological effects.

7. Annex A provides the Committee with a brief context and the additional information requested. For information, the paper providing previous additional information on other TAs reported in the FSA's survey is attached in Annex B, the initial discussion paper on TAs presented to the members at the July meeting is attached in Annex C.

At this meeting the Committee is invited to comment on the additional information.

Questions to be asked to the Committee

i) Do the Committee, agree with their initial assessment that a full review will not be necessary, and TAs can be included in the addendum to the overarching statement?

ii) Do the Committee have any final conclusions about (-)-hyoscyamine, (-)-scopolamine and the potential risk from all TAs reported in the FS's survey?

iii) Are there any points regarding the total risk from TAs exposure the Committee would like to emphasise?

iv) Do the members have any other comments?

Secretariat

May 2019

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of potential risks from contaminants in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Additional information on the pharmacological effects of other TAs reported in the FSA's survey

8. The group of TAs composes of about 200 compounds, the best-known representatives are (-)-hyoscyamine, (-)-scopolamine and atropine, a racemic mix of (-)-hyoscyamine and (+)-hyoscyamine. EFSA (2013) performed a risk assessment on (-)-hyoscyamine and (-)-scopolamine, the TAs for which both, occurrence and toxicity data were available and established an acute reference dose (ARfD) of 0.016 µg/kg bw per day. EFSA considered the ARfD to be protective against long term exposure due to the lack of bioaccumulation, genotoxicity and chronic toxicity of TAs.

9. Both, (-)-hyoscyamine and (-)-scopolamine, are strong antimuscarinic agents and act on receptors in the CNS and ABS; their toxicological effect is closely related to their pharmacological effect. Toxic effects of other TAs are largely unknown and only very limited data on occurrence in food and feed is available.

10. Plant extracts containing TAs have been and are continued to be used in veterinary and human medicine, as are (-)-hyoscyamine, (-)-scopolamine and atropine. Uses include the treatment of wounds, gout, sleeplessness and pre-anaesthesia.

11. No (relevant/pharmacological) information was available for 6-Hydroxytropinone, O-Acetylscopolamine, 2a-hydroxymethylatropine aposcopolamine, fillalbine, norscopolamine, nortropinone, phenylacetoxytropane, scopoline and tropinone; very limited (pharmacological) information was available for the rest of the TAs reported in the FSA's survey.

12. A study by Mattioli et al. (2012) reported a significant decrease in withdrawal syndromes in mice after co-administration of morphine with atropine, apoatropine, and 3α-tigloyl-oxitropane. However, pure alkaloids failed to reduce morphine tolerance. Convolamine has been reported to exhibit antihypoxic, immunomodulating and anti-inflammatory activity (Gapparov et al., 2011), while convolvine has been reported to block the M-receptor of the heart and intestine but raises the sensitivity of the M-receptor of the salivary glands and the central nervous system (CNS) (Mirzaev et al., 1998). Both, convolamine and convolvine and convolidine have been applied as treatment of gastrointestinal diseases, asthma, lung tuberculosis and have analgesic and anticonvulsant effects (Chandel and Kharoliwal 2014). Littorine is structurally closely related to atropine, scopolamine and hyoscyamine, while noratropine is a metabolite of atropine, all sharing a common biosynthetic pathway and act as muscarinic antagonists. Hazard (1939) reported pseudotripin to be a nicotine-like stimulant and block autonomic ganglia, while (pseudo)-scopine showed

weak vasodilatory effects, but otherwise did not show any effects on behaviour of rats at concentrations up to 450 mg/kg (i.p.) (Vidal-Beretevide 1968).

13. Tropine esters increased the potency against nicotine-induced tremors and showed some anaesthetic activity (Farquarson and Johnston 1959) as well as potential for new drug development for respiratory alignments (Grynkiewicz and Gadzikiwska 2008). No information was available on tropine itself.

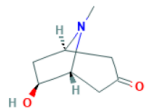
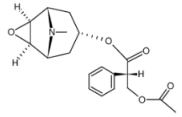
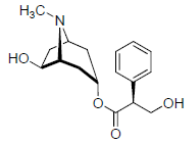
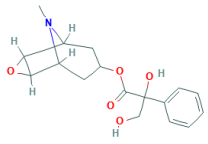
14. Homatropine is an antagonist of the muscarinic acetylcholine receptor and thus the parasympathic nervous system (Leung and Mitchelson 1982). It is used as an atropine substitute to reverse muscarinic and CNS effects, however, it is less potent than atropine and has a shorter duration of action (Cantor et al., 1983). It has also been shown to have cycloplegic and mydriac effects in the eye (Fusco et al., 1990; Mehrotra et al., 1992; Sander et al., 2010, 2014; Whelan et al., 2011).

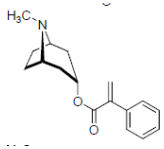
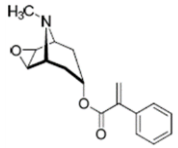
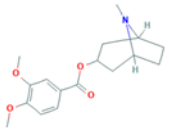
15. Anisodamine is a non-subtype selective muscarinic and nicotinic cholinreceptor antagonist with similar pharmacological effects as atropine/hyoscyamine and scopolamine. It is however less potent than atropine/hyoscyamine and less toxic (toward the CNS) than scopolamine; no toxicity was observed at concentrations of up to 500 mg/day (i.p.) (Li et al., 1999; Poupko et al., 2007). Anisodamine has been used therapeutically, in China, against e.g. septic shock, various circulatory disorders gastrointestinal issues, organophosphate poisoning (Poupko et al., 2007; Verma and Yue, 1986). It has been shown to have antithrombotic and vasodilating effects (Poupko et al., 2007) and to be effective in ameliorating myocardial reperfusion and to protect cardiac function in patients with acute inferior myocadiac infarction (AIMI) (Chen et al., 2015; Eisenkraft and Falk, 2016).

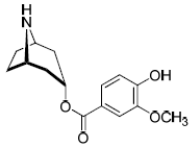
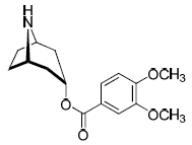
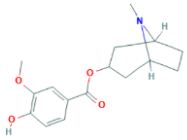
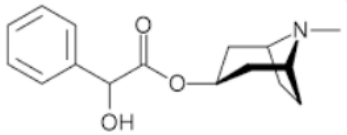
16. Anisodine acts as a muscarinic acetylcholine receptor antagonist and andrenergic receptor agonist and is similar to scopolamine in chemical structure and pharmacological properties (Han and Chen 1983; Penget al., 1982; Xu and Chen, 1994). It has been used as treatment of acute circulation shock in China since decades (Verma and Yue, 1986). Han and Chen (1983,1984) reported effects on acquisition, consolidation and retrieving of memory at concentrations of 0.25 to 1 mg/kg in rabbits and 1 to 5 mg/kg in mice, by inhibiting physiological functions of the central cholinergic system. Remote memory did not seem to be affected. Xu and Chen (1994) reported stimulating effects of anisodine on the respiratory system through the cholinergic M pathway in rabbits while Xu and Deng (1996) reported protective effects from ischemia insults through decreased intracellular calcium accumulation at concentrations of 0.5 mg/kg. Verma and Yue (1986) investigated a number of TAs, including anisodamine and anisodine and found the order of potency for the effects of phenylalanine on aortic strips and left atria to be atropine/hyoscyamine > anisodamine > scopolamine > anisodine.

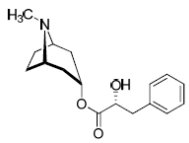
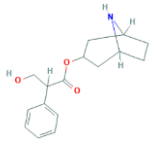
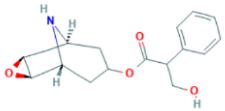
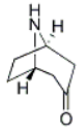
17. Given the limited information available on the pharmacological effects of the TAs reported in the FSA's survey, Table 1 provides the Committee with the molecular formula and chemical structure of all TAs. In addition, Toxtree was used to provide further information/alerts on all 24 TAs.

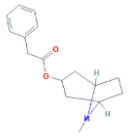
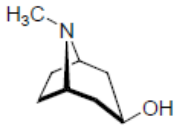
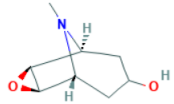
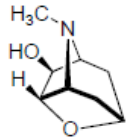
Table 1 Molecular formula and chemical structure of all 24 TAs reported as part of the FSA's survey.

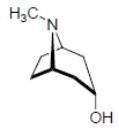
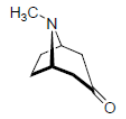
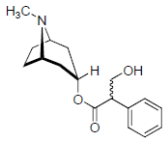
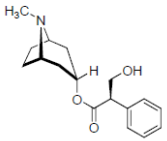
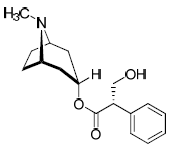
Tropane alkaloids	Molecular formula	Chemical structure	TOXTREE
6-Hydroxytropinone	C8H13NO2		
O-Acetylscopolamine	C19H23NO5		
2a-hydroxymethylatropine			
Anisodamine[a] CAS: 55869-99-3 MW: 305.37 g/mol	C17H23NO4		Cramer Class III Alert for SN1 and Michaels Acceptor Alert for SN2 and Michaels Acceptor At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Anisodine[a] CAS: MW: 319.357 g/mol	C17H21NO5		Cramer Class III Alert for SN1 and Michaels Acceptor Alert for SN2 and Michaels Acceptor Class 3 (unspecific reactivity, Verhaar scheme) Structural alert for genotoxic carcinogenicity and S typhimurium mutagenicity At least one posiive structural alert for MN assay (Class I)

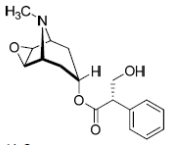
			At least one functional group found (Class I)
Apoatropine CAS: 500-55-0 MW: 271.35 g/mol	C17H21NO2		Cramer Class III Alert for SN1 and Michaels Acceptor Alert for Michaels Acceptor (Protein binding) Class 3 (unspecific reactivity, Verhaar scheme) At least one functional group found (Class I)
Aposcopolamine[a] CAS: 535-26-2 MW: 285.34 g/mol	C17H19NO3		Cramer Class III Alerst for SN1 and Michael Acceptor Alert for SN2 and Michael Acceptor Class 3 (unspecific reactivity, Verhaar scheme) Structural alert for genotoxic carcinogenicity and S typhimurium mutagenicity At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Convolamine[a] CAS: 500-56-1 MW: 305.374 g/mol	C17H23NO4		Cramer Class III Alert for SN1 and Michael Acceptor Alert for Michael Acceptor (Protein binding) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)

Convolidine[a] CAS: 63911-32-0 MW: 277.32 g/mol	C15H19NO4		Cramer Class III Alert for Michael Acceptor (both DNA and Protein binding) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Convolvine[a] CAS: 537-30-4 MW: 291.34 g/mol	C17H23NO4		Cramer Class III Alert for Michael Acceptor (both DNA and Protein binding) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Fillalbine[a] CAS: 4540-25-4 MW: 291.347 g/mol	C16H21NO4		Cramer Class III Alert for SN1 and Michael Acceptor Alert for Michael Acceptor (Protein binding) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Homatropine CAS: 80-49-9 MW: 356.26 g/mol	C16H21NO3		Cramer Class III Alert for SN1 and Michael Acceptor Alerst for SN2 and Michael Acceptor Class 3 (unspecific reactivity, Verhaar scheme) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)

Littorine[b] CAS: 21956-47-8 MW: 289.37 g/mol	C17H23NO3		Cramer Class III Alert for SN1 and Michael Acceptor Alerst for SN2 and Michael Acceptor Class 3 (unspecific reactivity, Verhaar scheme) At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Noratropine[a] CAS: 16839-98-8 MW: 275.348 g/mol	C16H21NO3		Cramer Class III Alert for SN2 and Michael Acceptor At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Norscopolamine[a] CAS:4684-28-0 MW: 289.331 g/mol	C16H19NO4		Cramer Class III Alert for SN2 and Michael Acceptor Class 3 (unspecific reactivity, Verhaar scheme) Structural alert for genotoxic carcinogenicity and S typhimurium mutagenicity At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Nortropinone CAS: MW: 125.17 g/mol	C7H11NO		

Phenylacetoxytropane CAS: 1690-22-8 MW: 259.349 g/mol	C16H21NO2		Cramer Class III Alert for SN1 and Michael Acceptor Alert for SN2 and Michael Acceptor At least one positive structural alert for MN assay (Class I) At least one functional group found (Class I)
Pseudotropine CAS: 135-97-7 MW: 141.21 g/mol	C8H15NO		Cramer Class III Alert for SN1 At least one functional group found (Class I)
Scopine[a] CAS: 498-45-3 MW: 155.197 g/mol	C8H13NO2		Cramer Class III Alert for SN1 Alert for SN2 Class 3 (unspecific reactivity, Verhaar scheme) Structural alert for genotoxic carcinogenicity and S typhimurium mutagenicity At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Scopoline CAS: 487-27-4 MW: 155.19 g/mol	C8H13NO2		Cramer Class III Alert for SN1 At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)

Tropine CAS: 120-29-6 MW: 141.21 g/mol	C8H15NO		Cramer Class III Alert for SN1 At least one posiive structural alert for MN assay (Class I) At least one functional group found (Class I)
Tropinone CAS: 532-24-1 MW: 139.19 g/mol	C8H13NO		Cramer Class III Alert for SN1 At least one positive structural alert for MN assay (Class I) At least one functional group found (Class I)
Atropine CAS: 51-55-8 MW: 289.37 g/mol	C17H23NO3		Cramer Class III Alert for SN1 and Michael Acceptor Slert for SN2 and Michael Acceptor At least one positive structural alert for MN assay (Class I) At least one functional group found (Class I)
(+)-Hyoscyamine CAS: 101-31-5 MW: 289.37 g/mol	C17H23NO3		See (-)-Hyoscyamine
(-)-Hyoscyamine CAS: 101-31-5 MW: 289.37 g/mol	C17H23NO3		Cramer Class III No DNA binding alerts Alert for Michael Acceptor and SN1 identified At least one positive structural alert for MN assay (Class I)

			At least one functional group found (Class I)
Scopolamine CAS: 51-34-3 MW: 303.35 g/mol	C17H21NO4		Cramer Class III No DNA binding alerts Alert for SN1 Alert for SN2 and Michael Acceptor Class 3 (unspecific reactivity, Verhaar scheme) Structural Alert for genotoxic carcinogenicity and S. typhimurium mutagenicity At least one positive structural alert for MN assay (Class I) At least one functional group found (Class I)

[a] Toxtree could not find TA by name, canonical SMILES code used instead
[b] Toxtree could not find TA by name, CAS number used instead

Additional information regarding structural parts of (-)-hyoscyamine and (-) scopolamine, which are responsible for their pharmacological effects

18. No additional information has been retrieved during a brief literature search. The following information therefore summarises the relevant information given on the mode of action and structural effects of (-)-scopolamine and (-)-hyoscyamine/(+)-hyoscyamine given by EFSA (2013).

19. TAs appear to be relatively non-selective for the five different types of muscarinic receptors (M_1 to M_5) and therapeutic concentrations seem to have little effect on nicotinic acetylcholine (ACh) receptors.

20. While (-)-scopolamine has a prominent depressing effect on the CNS, (-)-hyoscyamine rarely has been reported to have an effect on the CNS, at clinically used concentrations.

21. The anticholinergic activity of atropine has been historically attributed to the naturally occurring (-)-hyoscyamine. Various studies on the function and binding of atropine have demonstrated that the affinity of (-)-hyoscyamine for muscarinic receptor subtypes is higher than the affinity of (+)-hyoscyamine, the (-)-enantiomer being twice as potent. It has therefore been concluded that the action of hyoscyamine is stereospecific and receptor mediated.

22. In addition, dose-dependent effects (cholinomimetic and cholinolytic) have been reported for (-)-hyoscyamine and (+)-hyoscyamine and it has been suggested that the (+)-enantiomer at low concentrations binds to the presynaptic ACh receptor in a negative feedback regulation and therefore might account for the different activity of the two enantiomers.

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of potential risks from contaminants in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Additional information on other TAs reported in the FSA survey

23. The group of TAs composes of about 200 compounds, the best-known representatives are (-)-hyoscyamine and (-)-scopolamine. Both are strong antimuscarinic agents; their toxicological effect is closely related to their pharmacological effect. Toxic effects of other TAs are largely unknown and only very limited data on occurrence in food and feed is available.

24. EFSA (2013) performed a risk assessment on (-)-hyoscyamine and (-)-scopolamine, the TAs for which both, occurrence and toxicity data were available and established an acute reference dose (ARfD) of 0.016 µg/kg bw per day. EFSA considered the ARfD to be protective against long term exposure due to the lack of bioaccumulation, genotoxicity and chronic toxicity of TAs.

25. The occurrence data for the exposure assessment in the paper presented to the Committee in July, are results from a survey from the unpublished final report on monitoring of TAs in foods. Samples were taken from a wide variety of food groups and analysed for as many TAs for which reliable standards are available (FSA 102116, March 2017).

In total, 24 TAs were investigated as part of the FSA survey, for a full list see Table 1. TAs were mainly found in cereal-based samples, the proportion of cereal-based samples in which different TAs were detected varied. The percentage of samples with detectable levels > LOQ in cereal-based infant food ranged from 0% (scopine and scopoline) to 26% (tropine). Overall, the concentrations of TAs found in the survey were low, measured quantities of TAs were reported in only a limited number of samples.

26. Average concentrations (UB) of tropine in cereal-based samples range from 0.1 to 561 ng/g, average concentration (UB) of pseudotropine, which was detected at > LOQ in 23% of breakfast-based samples, range from 0.3 to 133 ng/g, norscopolamine was detected in 21% of cereal-based samples, concentrations ranging from 0.1 to 5 ng/g. The full list of average concentrations can be found in Table 1.

27. Average concentrations (UB) of (-)-hyoscyamine (atropine) was estimated to be 0.43 ng/g in cereal-based infant foods, the maximum detection being in 14.9% of cereal-based infant foods. Average concentrations (UB) of (-)-scopolamine were

estimated to be 0.19 ng/g in cereal-based infant foods, the maximum detection being in 8.5% of cereal-based infant food samples.

28. While for example tropine has been detected in a higher percentage of cereal-based samples, than (-)-hyoscyamine and (-)-scopolamine, the latter two TAs are the only TAs for which toxicological data are currently available.

29. The fact that some of the 24 TAs are reported in up to 26% of the cereal-based samples, in the absence of any toxicological data and HBGVs, add a level of uncertainty to the risks of total TAs in the diet.

Table 1 List of all 24 tropane alkaloids (TAs) investigated as part of the Food Standards Agency (FSA) survey and the corresponding average concentrations found in cereal-based infant foods and breakfast cereals.

Tropane alkaloids	**Average concentrations in Cereal-based infant foods (ng/g)**	**Average concentrations in Breakfast cereals (ng/g)**
6-Hydroxytropinone	0.38 (range from 0.15 to 2.84)	0.37 (range from 0.17 to 0.54)
O-Acetylscopolamine	0.12 (range from 0.05 to 1.72)	0.15 (range from 0.06 to 0.95)
2a-hydroxymethylatropine	0.11 (range from 0.05 to 0.77)	0.08 (range from 0.04 to 0.17)
Anisodamine	0.08 (range from 0.05 to 0.45)	0.10 (range from 0.05 to 0.76)
Anisodine	0.10 (range from 0.06 to 0.49)	0.06 (range from 0.05 to 0.09)
Apoatropine	0.16 (range from 0.06 to 0.95)	0.11 (range from 0.06 to 0.17)
Aposcopolamine	0.11 (range from 0.05 to 0.71)	0.11 (range from 0.05 to 0.33)
Atropine	0.43 (range from 0.05 to 8.07)	0.15 (range from 0.04 to 0.73)
Convolamine	0.30 (range from 0.05 to 2.8)	0.17 (range from 0.05 to 0.50)
Convolidine	1.38 (range from 0.06 to 12.8)	0.20 (range from 0.08 to 0.35)
Convolvine	0.78 (range from 0.06 to 26.0)	0.49 (range from 0.07 to 2.85)
Fillalbine	2.86 (range from 0.05 to 38.62)	0.13 (range from 0.05 to 0.24)
Homatropine	0.33 (range from 0.05 to 9.81)	0.14 (range from 0.05 to 0.70)
Littorine	0.27 (range from 0.04 to 6.94)	0.21 (range from 0.04 to 0.89)
Noratropine	0.15 (range from 0.04 to 1.00)	0.10 (range from 0.04 to 0.23)
Norscopolamine	0.59 (range from 0.07 to 7.99)	0.24 (range from 0.11 to 1.40)
Nortropinone	0.75 (range from 0.18 to 5.02)	0.55 (range from 0.23 to 1.33)
Phenylacetoxytropane	0.17 (range from 0.04 to 1.41)	0.14 (range from 0.04 to 0.38)
Pseudotropine	8.6 (range from 0.3 to 133.0)	0.93 (range from 0.35 to 3.23)
Scopine	0.36 (range from 0.15 to 2.23)	0.36 (range from 0.24 to 0.64)
Scopolamine	0.19 (range from 0.05 to 1.94)	0.11 (range from 0.04 to 0.49)
Scopoline	0.39 (range from 0.13 to 2.41)	0.52 (range from 0.21 to 1.00)
Tropine	44.5 (range from 0.1 to 560.7)	0.33 (range from 0.16 to 0.51)
Tropinone	0.56 (range from 0.06 to 2.92)	0.50 (range from 0.10 to 1.18)

Range of averages for all 24 TAs	**0.08 to 44.5**	**0.06 to 0.93**

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of potential risks from contaminants in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Tropane alkaloids

30. Unless stated otherwise, general information was derived from the European Food Safety Authority's scientific opinion (EFSA, 2013).

Background

31. Tropane alkaloids (TAs) are secondary metabolites which naturally occur in several plant families, such as Brassicaceae, Solanaceae and Erythroxylaceae. TA are found in all parts of the plant and are responsible for the toxic effects of those plants.

32. The group of TAs composes of about 200 compounds, the best-known representatives are (-)-hyoscyamine, (-)-scopolamine and atropine, a racemic mix of (-)-hyoscyamine and (+)-hyoscyamine. Plant extracts containing TAs have been and are continued to be used in veterinary and human medicine, as are (-)-hyoscyamine, (-)-scopolamine and atropine. Uses include the treatment of wounds, gout, sleeplessness and pre-anaesthesia.

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

34. TAs are readily absorbed from the gastrointestinal (GI) tract and distributed into tissues; excretion is predominantly via urine.

Toxicity

35. Both compounds inhibit the muscarinic acetylcholine receptor in the central nervous system (CNS) and autonomic nervous system (ANS). However, they differ in the ability to affect the CNS, (-)-scopolamine having a more prominent effect on the CNS.

36. In humans, toxic effects of (-)-hyoscyamine and (-)-scopolamine include inhibition of saliva, bronchial and sweat gland secretion, dilation of pupils and paralysis of accommodation, change in heart rate, inhibition of urination, reduction in GI tone and inhibition of GI secretion. In extreme cases, toxic effects can include hallucination, delirium and coma.

37. Toxic effects of other TAs are largely unknown and only very limited data on occurrence in food and feed is available.

HBGVs

38. EFSA (2013) performed a risk assessment on (-)-hyoscyamine and (-)-scopolamine, the TAs for which both, occurrence and toxicity data were available.

39. Atropine is a racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine; unlike (+)-hyoscyamine, (-)-hyoscyamine and (-)-scopolamine are naturally formed in plants. When atropine was reported in data on food and feed, EFSA used these data as (-)-hyoscyamine in their evaluation of TAs.

40. EFSA establish an acute reference dose (ARfD), as the pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time period after administration. The Panel assumed equivalent potency of (-)-hyoscyamine and (-)-scopolamine, due to their common mode of action and therefore set a group ARfD based on a human volunteer study. An uncertainty factor of 10 for interindividual differences (small study, healthy male volunteers) was applied to the no observed adverse effect level (NOAEL) of 0.16 µg/kg bw per day to derive an ARfD of 0.016 µg/kg bw per day.

41. The group ARfD is approximately two orders of magnitude lower than the lowest single therapeutic dose of (-)-hyoscyamine and (-)-scopolamine.

42. EFSA considered the ARfD to be protective against long term exposure due to the lack of bioaccumulation, genotoxicity and chronic toxicity of TAs.

43. The European Medicine Agency (EMA) and EFSA assessed the legal use of *Atropa belladonna* and atropine as authorised veterinary medicines in farm animals in 1997 and 2008. Since atropine is used infrequently and readily absorbed and eliminated, it was not considered necessary to establish a maximum residue limit (MRL) as animals are unlikely to be sent to slaughter immediately after treatment.

44. EMA and EFSA both concluded it was unlikely that residues of TAs in edible tissues (meat, milk, eggs) would be of risk to consumers.

45. Based on EFSAs conclusions that toddlers might significantly exceed the group ARfD through the diet and the fact that it is not always possible to distinguish between the enantiomers of hyoscyamine, a maximum level for atropine (reflecting the occurrence of (-)-hyoscyamine) and (-)-scopolamine of 1.0 µg/kg in cereal based food for infants and young children was derived by the European Commission (EC, 2016).

Exposure Assessment

Dietary exposure

46. The occurrence data for the exposure assessment are results from a survey from the unpublished final report on monitoring of tropane alkaloids in foods. Samples were taken from a wide variety of food groups and analysed for as many TAs for which reliable standards are available (FSA 102116, March 2017).

47. Consumption data (on a body weight basis) for the estimated dietary exposure are from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (DH, 2013) and from years 1-6 of the National Diet and Nutrition Survey (NDNS) (Bates et al., 2012 & 2014).

48. For the purposes of this scoping paper and following EFSAs approach, this assessment uses and reports atropine and (-)-scopolamine in food as (-)-hyoscyamine and (-)-scopolamine, respectively. The acute exposure assessments of infants and young children focused on (-)-hyoscyamine and (-)-scopolamine and the sum of (-)-hyoscyamine and (-)-scopolamine and the consumption of: (i) commercial infant and young children foods, (ii) breakfast cereals and (iii) teas (dry product). Consumption of these foods is assumed to be highest at the age groups of interest (children aged 4 to 18 months and 18 to 60 months) and therefore cover all other food groups.

49. Overall, the concentrations of TAs found in the survey were low, measured quantities of TAs were reported in only a limited number of samples.

50. (-)-Hyoscyamine was measured in 7 out of 47 samples (14.9%) of commercial infant and young children foods. The remainder of the samples (85.1%) were below the limit of quantification (LOQ) of 0.5 µg/kg but at or above the limit of detection (LOD) of 0.05 µg/kg. (-)-Scopolamine was measured in only 4 out of 47 samples (8.5%); the concentrations found in the remainder of the samples (91.5%) were below the LOQ of 0.5 µg/kg but at or above the LOD of 0.1 µg/kg.

51. (-)-Hyoscyamine was measured in 2 out of 29 samples (6.9%) of breakfast cereal. The concentrations in the remainder of the samples were below the LOQ of 0.5 µg/kg (93.1%), below the LOD of 0.05 µg/kg (17.2%), at the LOD (3.4%) or between the LOD and LOQ (72.4%). (-)-Scopolamine was measured in 1 out of 29 samples (3.4%); the remainder of the samples were below the LOQ (96.6%), below the LOD (58.6%), at the LOD (10.3%) or between the LOD and LOQ (27.6%).

52. (-)-Hyoscyamine was measured in 9 of the 29 samples (31%) of teas (dry product). The remainder of the samples were below the LOQ (69%) of which 65.5% were at the LOD. (-)-Scopolamine was measured in 5 of 29 samples (17.2%); the remainder of the samples was below the LOQ (82.8%), of which 69% were at the LOD.

53. Tea infusions were prepared from a selection of 20 tea samples and analysed for TAs. On average, it was found that 47% of the alkaloids transferred from the dry tea to the infusion (Stratton et al., 2017).

54. Average concentrations of (-)-hyoscyamine were estimated to be 0.18 ng/g lower bound (LB) and 0.43 ng/g upper bound (UB) (cereal-based infant foods), 0.02 ng/g LB and 0.15 ng/g UB (breakfast cereals) and 6.58 ng/g LB and 6.66 ng/g UB (teas, dry product) and used in the exposure assessment. Average levels of (-)-scopolamine were estimated to be 0.04 ng/g LB and 0.19 ng/g UB (cereal-based infant foods), 0.03 ng/g LB and 0.11 ng/g (breakfast cereals) and 2.45 ng/g LB and 2.55 ng/g UB (teas, dry product).

55. The following tables provide the mean and 97.5th percentile estimated acute exposures (UB) to (-)-hyoscyamine, (-)-scopolamine and the sum of (-)-hyoscyamine and (-)-scopolamine from consumption of cereal-based infant foods (Table 1), breakfast cereals (Table 2), teas (dry product; Table 3) and the combination of all 3 food categories (Table 4) for children aged 4 to 18 months and 18 to 60 months.

Table 1 Estimated TAs acute exposure for children aged 4 to 60 months from consumption of cereal-based infant foods, using data from the unpublished FSA report (retail survey; FSA 102116, 2017).

	Exposure LB-UB (ng/kg bw/day)					
	4 to 18 m-olds (n=2683)			**18 to 60 m-olds (n=1015)**		
	Number of consumers	**Mean**	**97.5th Percentile**	**Number of consumers**	**Mean**	**97.5th Percentile**
Hyoscyamine	1997	1.2-3.0	4.9-12	308	0.41-0.99	1.8-4.3
Scopolamine	1997	0.28-1.3	1.1-5.2	308	0.092-0.44	0.40-1.9
Total Exposure	**1997**	**1.5-4.3**	**6.0-17**	**308**	**0.50-1.4**	**2.2-6.1**

Table 2 Estimated TAs acute exposure for children aged 4 to 60 months from consumption of breakfast cereals, using data from the unpublished FSA report (retail survey; FSA 102116, 2017).

	Exposure LB-UB (ng/kg bw/day)					
	4 to 18 m-olds (n=2683)			**18 to 60 m-olds (n=1015)**		
	Number of consumers	**Mean**	**97.5th Percentile**	**Number of consumers**	**Mean**	**97.5th Percentile**

Hyoscyamine	1134	0.074-0.55	0.39-2.9	686	0.054-0.40	0.23-1.7
Scopolamine	1134	0.11-0.41	0.59-2.2	686	0.080-0.29	0.34-1.3
Total Exposure	**1134**	**0.18-0.96**	**0.98-5.1**	**686**	**0.13-0.70**	**0.57-3.0**

Table 3 Estimated TAs acute exposure for children aged 4 to 60 months from consumption of teas (dry product), using data from the unpublished FSA report (retail survey; FSA 102116, 2017).

	Exposure LB-UB (ng/kg bw/day)					
	4 to 18 m-olds (n=2683)			**18 to 60 m-olds (n=1015)**		
	Number of consumers	**Mean**	**97.5th Percentile**	**Number of consumers**	**Mean**	**97.5th Percentile**
Hyoscyamine	153	0.83-0.84	2.7	177	0.77-0.78	2.1-2.2
Scopolamine	153	0.31-0.32	1.0	177	0.29-0.30	0.79-0.82
Total Exposure	**153**	**1.1-1.2**	**3.7-3.8**	**177**	**1.1**	**2.9-3.0**

Table 4 Estimated TAs acute exposure for children aged 4 to 60 months from consumption of breakfast cereals, infant foods and teas (dry product), using data from the unpublished FSA report (retail survey; FSA 102116, 2017).

	Exposure LB-UB (ng/kg bw/day)					
	4 to 18 m-olds (n=2683)			**18 to 60 m-olds (n=1015)**		
	Number of consumers	**Mean**	**97.5th Percentile**	**Number of consumers**	**Mean**	**97.5th Percentile**
Hyoscyamine	2442	1.1-2.6	4.6-11*	836	0.33-0.76	1.8 -3.0*
Scopolamine	2442	0.28-1.2	1.1-4.9*	836	0.15-0.41	0.63-1.8*
Total Exposure	2442	**1.4-3.8**	**5.7-16**	**836**	**0.48-1.2**	**2.3-4.8**

* Determined from a distribution of consumption of any combination of categories rather than by summation of the respective individual 97.5th percentile consumption value for each of the three food categories

Human breast milk

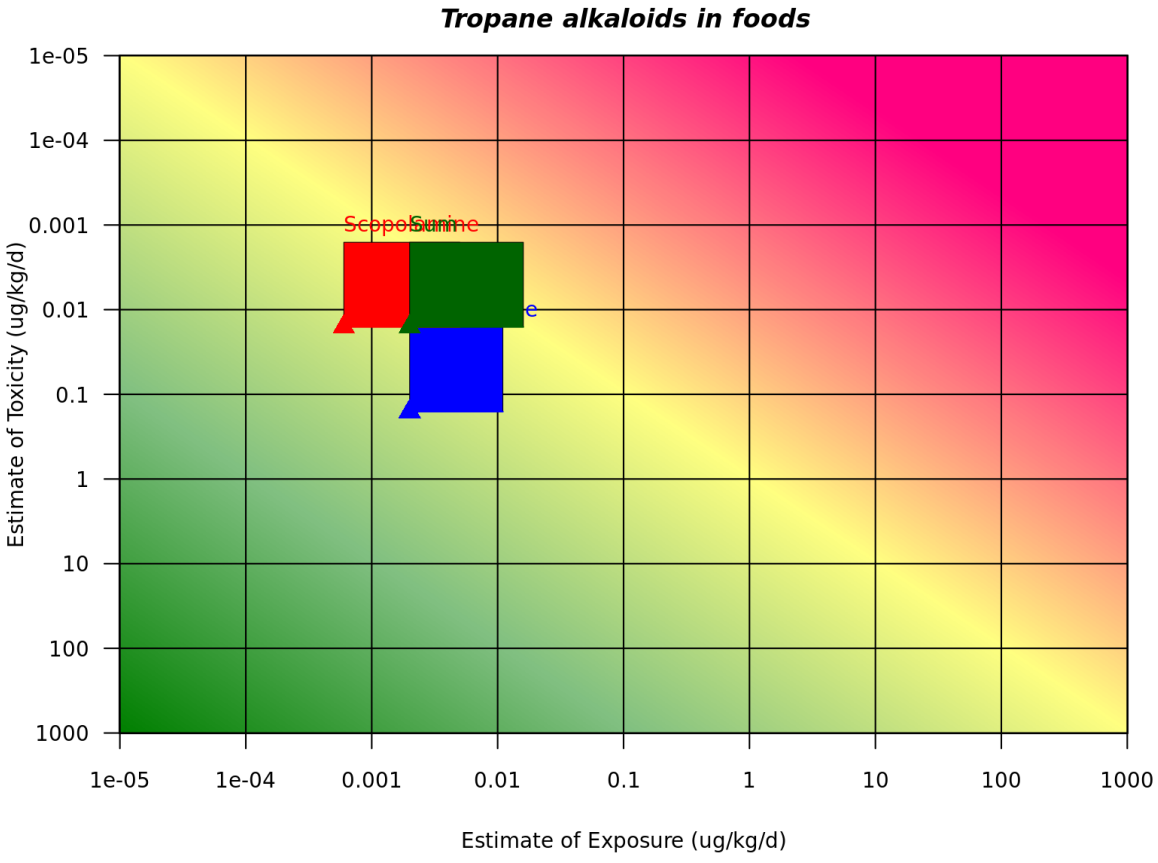
56. Little to no information is available of the transfer of TAs to breast milk; the limited information available reports that only limited amounts of tropane alkaloids, namely atropine, (-)-hyoscyamine and (-)-scopolamine are excreted into breast milk (EFSA, 2013). A literature search including the years since the last EFSA opinion on TAs has not resulted in any additional information.

Infant formula

57. No data is available on concentrations of TAs in infant formula.

Risk21

58. Figure 1 shows the 97.5th percentile estimated acute exposure for (-)-hyoscyamine, (-)-scopolamine and the sum of (-)-hyoscyamine and (-)-scopolamine for the consumption of breakfast cereals, infant foods and tea across all age groups.



Risk characterisation

59. EFSA established an ARfD of 0.016 µg/kg (16 ng/kg) bw per day based on the rapid onset of pharmacological effects; no HBGV was set for long term exposure as EFSA considered the ARfD to be effective in the absence of bioaccumulation, genotoxicity and chronic toxicity.

60. In infants and young children, the UB mean and 97.5th percentile estimated acute exposures to (-)-hyoscyamine and (-)-scopolamine and the sum of (-)-hyoscyamine and (-)-scopolamine for each individual food category and the sum of all three categories were below the ARfD. The only exceptions are the 97.5th percentile (UB) estimated exposures to the sum of (-)-hyoscyamine and (-)-scopolamine in cereal-based infant foods and all three food categories combined where exposures are at or close to the ARfD; however these are UB exposures, reflecting limited detection of (-)-hyoscyamine and (-)-scopolamine rather than being based on actual measured consentrations. The ARfD is based on a human (male) volunteer study and derived from a NOAEL with the application of an UF of 10 for interindividual differences. The exposures are unlikely to be of toxicological concern.

61. The limited information available on the transfer to and concentrations of TAs in breast milk does not indicate a toxicological concern.

62. No data on the concentration of TAs in infant formula is available; given the source of TAs and the assessment by the EMA and EFSA that it is unlikely for residues of TAs in milk to be of risk to the consumer, it is highly unlikely that TAs would be detected in infant formula or that levels reported would be of risk to infants.

Uncertainties in the risk characterisation

63. Although numerous TAs have been tested for and reported in the FSA unpublished report (2017), due to the lack of toxicity data, this risk assessment, only focused on (-)-hyoscyamine and (-)-scopolamine. Thus, the total dietary exposure of infants and young children to a combination of all TAs may be substantially underestimated. The estimated exposures are based on LB and UB concentrations, which reflect the uncertainties associated with concentrations being below the LOQ in the majority of the samples.

64. Insufficient data on the racemisation and degradation of TAs under conditions used for food preparation as well as the effects of *in vivo* racemisation or potential toxicity of degradation products further add to the overall uncertainty regarding the total dietary exposure.

Conclusions

65. EFSA established an ARfD of 16 ng/kg bw per day based on the rapid onset of pharmacological effects.

66. Overall, the levels of TAs detected in foods in the 2014 (unpublished) survey were low, with very few incidences of (-)-hyoscyamine and (-)-scopolamine at or above the LOQ. The average levels reported for (-)-hyoscyamine and (-)-scopolamine in cereal-based infant foods, breakfast cereals and teas (dry) were below the permitted maximum level of 1.0 µg/kg in cereal based food for infants and young children derived by the European Commission (EC, 2016). However, 4 out of 66 samples (3/46 from the EFSA survey, 1/20 from the FSA survey) were found to exceed the maximum level; the highest level found was 3.73 µg/kg (-)-hyoscyamine.

67. All estimated acute exposures of infant and young children to (-)-hyoscyamine and (-)-scopolamine or the sum of (-)-hyoscyamine and (-)-scopolamine are close to or below the ARfD of 16 ng/kg bw per day. The exposures are unlikely to be of toxicological concern.

68. Limited information is available on the transfer of TAs into breast milk; the limited information available prior to the EFSA opinion in 2013 does not indicate significant concentrations of TAs in breast milk. A recent literature search could not detect any new data or newer information on either the transfer to or concentration of TAs in breast milk since the 2013 EFSA opinion. The limited information available currently indicates no toxicological concern regarding TAs in breast milk.

Questions to be asked to the Committee

v) Do the Committee agree with the ARfD established by EFSA in 2013?

vi) Do the Committee agree with EFSAs conclusions that an ARfD would also protect against long term exposure?

vii) Do the Committee consider it sufficient to include a brief summary of the important points (HBGVs, exposure, conclusions) in the overarching statement?

viii) Do the members have any other comments?

Secretariat

July 2018