High tropane alkaloid levels in cereal products: Health impairments are possible in individuals with heart problems

Opinion No 035/2014 of the BfR dated 13 November 2013

Tropane alkaloids (TA) are natural components contained in certain plants such as henbane, thorn-apple and deadly nightshade. To date, a total of more than 200 different TA have been identified in plants. Some of these alkaloids are also used in medicinal products, for example atropine (a mixture containing equal amounts of the isomers (-)-hyoscyamine and (+)-hyoscyamine), (-)-hyoscyamine and (-)-scopolamine. These alkaloids are known to affect the heart rate and the central nervous system even at low doses; drowsiness, headaches and nausea are typical symptoms.

Tropane alkaloids can also occur in cereal-based foods through the contamination of cereals with seeds of henbane or thorn-apple. For risk assessment purposes, the European Food Safety Authority (EFSA) derived an acute reference dose (ARfD) as health-based guidance value of 0.016 µg/kg body weight (b.w.) for the sum of (-)-hyoscyamine and (-)-scopolamine (group ARfD). In risk assessment, the determined levels of tropane alkaloids in food are considered in relation to the acute reference dose. On the basis of the limited data available, the EFSA concludes that for toddlers the group ARfD for the tropane alkaloids atropine, scopolamine and hyoscyamine can be exceeded up to seven times through the consumption of cereal products. The Federal Ministry of Food and Agriculture asked the BfR to conduct a health assessment on the occurrence of tropane alkaloids in food based on the exposure situation of infants, toddlers and adults in Germany. At the same time, the BfR got the mandate to check whether the ARfD for tropane alkaloids derived by the EFSA as a health-based guidance value can be used as the basis for a risk assessment for food. A further aspect to be considered was the extent to which methods are available for the analysis of tropane alkaloids in food.

In its evaluation of the EFSA opinion on tropane alkaloids in food and animal feed, the BfR concludes that the group ARfD of 0.016 µg per kilogramme of body weight for tropane alkaloids derived by the EFSA represents an adequate basis for assessing health risks posed by potential exposure to tropane alkaloid contamination in food. The institute also determines that the consumption data available for Germany only permit an estimate of the acute exposure to tropane alkaloids for toddlers. Therefore it is also only possible to estimate the health risk for this consumer group.

Referring to the incomplete data base, it must be assumed that for toddlers the group ARfD is exceeded two times (with medium consumption) or five times (with high consumption) is consumed. According to the BfR's estimations, health impairments are possible if the ARfD is exceeded to such an extent. For this reason, the BfR recommends consistent use of good agricultural practice and good manufacturing practice to ensure that tropane alkaloid levels are reduced in cereals and cereal products in order to guarantee that the ARfD is not exceeded in any consumer group, even with high consumption of these products.

To the knowledge of the BfR, no standardised and validated methods are currently available for detecting tropane alkaloids in food. The same applies to correspondingly certified reference materials. The institute sees standardising the analytical methods and making reference material available as necessary measures.

BfR risk profile: Tropane alkaloids in food (statement no. 035/2014)					
A Affected	**Persons of all age groups, including infants and toddlers Persons with cardiovascular diseases**				
B Probability of health impairments with normal or high consumption	Virtually impossible	Improbable	**Possible**	Probable	Certain
C Severity of health impairments with normal or high consumption	No impairment	Minor impairment [reversible/irreversible]	**Moderately severe impairment, reversible**	**Severe impairment, reversible**	
D Validity of available data	High: The most important data is available and consistent	**Medium: Some important data is missing or inconsistent**	Low: A large amount of important data is missing or inconsistent		
E Controllability by consumers	Control not necessary	Control possible with precautionary measures	Control possible by avoiding certain products	**Not controllable**	

Dark blue fields correspond to the properties of the risk assessed in this statement
(further details are available in the text of statement 035/2014 of the BfR dated 13 November 2013).

Explanations

The risk profile is intended to visualise the risk described in the BfR statement. It is not intended to make risk comparisons. The risk profile should only be read in connection with the statement.

Row C – Severity of the health impairment with normal or high consumption
Severe impairments can only occur in persons with cardiovascular problems

FEDERAL INSTITUTE FOR RISK ASSESSMENT (BfR)

1 Subject of the assessment

In October 2013, the EFSA CONTAM Panel published a risk assessment on the occurrence of tropane alkaloids (TA) in food and animal feed (EFSA CONTAM Panel, 2013). The Federal Ministry of Food and Agriculture (BMEL) requested that the BfR

- submits a brief evaluation of the EFSA statement, in particular regarding the contamination of infant food and the EFSA assessment on this, and
- provides information on analysis methods for tropane alkaloids, particularly in cereals and infant food, and on the availability of reference substances.

The EFSA opinion states that TA can enter food as a result of contamination especially with seeds of the thorn-apple (*Datura stramonium*) and berries of the deadly nightshade (*Atropa belladonna*), in particular, but also with seeds of the henbane (*Hyoscyamus niger)*.

2 Findings

From the perspective of the BfR, the current EFSA statement takes all relevant data that are available at present sufficiently into consideration. The health-based guidance value of 0.016 µg/kg b.w for the sum of (-)-hyoscyamine and (-)-scopolamine that was derived as acute reference dose (group ARfD) represents an acceptable basis for a risk assessment of possible exposure to tropane alkaloids as contaminants in food. The BfR assumes that the uncertainty factor of 10 selected for the derivation also takes adequate account of possible increased sensitivity that could exist in infants, pregnant and breastfeeding women, unborn babies, sick people and the elderly, for example.

The BfR shares the EFSA's view that data on the occurrence and consumption of tropane alkaloids are incomplete. Like the EFSA, the BfR comes to the conclusion that the available consumption data is only sufficient for an exposure estimate and risk assessment for the toddler[1] age group. The data situation does not allow any corresponding estimates for infants[2] or consumers of other age groups. The estimate of acute exposure of toddlers based on the German consumption data showed that the group ARfD is exceeded approximately two times with medium consumption and approximately five times with high consumption. With respect to the occurrence of (-)-hyoscyamine and (-)-scopolamine in cereal-based infant food, it is possible only to make the statement that it is necessary to make a distinction between products that are recommended for infants and toddlers and products that are only recommended for toddlers. The latter contained higher levels of TA than the former.

Overall, the BfR is not aware of any cases so far of health impairments to infants, toddlers, or consumers of other age groups due to the consumption of products contaminated with TA. However, in consideration of the currently unclear data situation, health impairments must be considered possible in principle if the ARfD is exceeded. A potential impairment is estimated as minor to moderately severe for healthy people when the ARfD is exceeded two to five times. However, a potential impairment must be estimated as severe under certain circumstances for people who suffer from specific health problems, such as cardiovascular diseases.

The BfR therefore recommends consistent use of good agricultural practice and technological manufacturing practice (GMP) to reduce levels in cereals and cereal products as far as possible in order to ensure that the ARfD is not exceeded. This applies in particular to products intended for nutrition of infants and of toddlers.

The BfR points out that standardisation of existing methods for analysis of TA in food is necessary. Up to now, certified reference materials are also not available.

[1] Children aged from 1-3, referred to as "toddlers" in the EFSA statement

[2] Children aged < 1 year, referred to as "infants" in the EFSA statement

3 Statement of reasons

3.1 Evaluation of the EFSA statement, particularly with respect to the contamination of infant food and the EFSA assessment on this

3.1.1 Derivation of a health-based guidance value by the EFSA

More than 200 TA have been identified in various plant species. However, only very limited data are available on the occurrence of TA in food and animal feed and on the toxicity of TA, and these data mainly relate to (-)-hyoscyamine and (-)-scopolamine. In its assessment, the EFSA therefore only refers to these two alkaloids or to atropine as the racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine. (-)-Hyoscyamine and (-)-scopolamine occur naturally, for example as main components of deadly nightshade (*Atropa belladonna*) and thorn-apple species (*Datura* spp.*)*. Cases of poisoning from the consumption of food contaminated with parts of these plants have been described in literature.

As described in more detail in the EFSA report, (-)-hyoscyamine/atropine[3] and (-)-scopolamine have relevance as pharmaceuticals for oral use as muscarinic receptor antagonists (anticholinergic or vagolytic medicines) e.g. for treating spasms in the gastrointestinal tract, the biliary ducts or the urinary tract or for the prevention of kinetosis (motion sickness, travel sickness). The lowest single therapeutic dose for oral use is 1.4 µg/kg b.w. for (-)-hyoscyamine, 6 µg/kg b.w. for atropine (corresponding to approx. 3 µg/kg b.w. (-)-hyoscyamine), and 2.5 µg/kg b.w. for (-)-scopolamine. Known adverse effects of these alkaloids at the lowest oral therapeutic doses are a deceleration of the heart rate, dry mouth and reduced perspiration. At higher therapeutic oral doses an increased heart rate and mydriasis have been observed. Unlike (-)-hyoscyamine and atropine, (-)-scopolamine at levels of oral therapeutic doses causes a depression of the central nervous system (CNS). Dose-related information on the occurrence of adverse effects does not apply to patients who have diseases for which contraindications or precautionary measures relating to treatment with TA are described or who may react more sensitively than other patients. Usage restrictions with respect to oral treatment with (-)-hyoscyamine/atropine or (-)-scopolamine also apply during pregnancy or breastfeeding. When the therapeutic dose level is exceeded, all alkaloids mentioned cause CNS stimulation, psychotic reactions and hallucinations. The CNS effect only develops into a depression in the case of a massive overdose or severe poisoning. Death through respiratory arrest can then occur.

The EFSA report also describes individual therapeutic oral doses for children from two years of age for atropine and (-)-scopolamine. In relation to the body weight, these doses are above the lowest individual therapeutic oral doses for adults, but within the same order of magnitude. Oral doses for therapeutic use in younger children are not specified.

The EFSA considers the acute pharmacological and toxicological effectsto be the focus of the risk assessment of (-)-hyoscyamine and (-)-scopolamine. An acute reference dose (ARfD) for these substances is therefore derived. The derivation is based on observations in 20 healthy young male adults regarding the deceleration in the heart rate and CNS effects, such as drowsiness, headaches and nausea (Perharič et al., 2013a). A NOAEL (no observed adverse effect level) of 0.16 µg/kg b.w. and use of an uncertainty factor of 10 to consider individual differences are taken as the basis to derive a group ARfD of 0.016 µg/kg b.w. for

[3] It is assumed that (-)-hyoscyamine has approximately twice the antimuscarinic activity of atropine.

the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency.. Because TA do not accumulate and there are no indications of genotoxicity or chronic toxicity, a risk assessment based on the ARfD would also include risks posed by chronic exposure. The EFSA points out that the group ARfD lies two orders of magnitude below the lowest therapeutic doses for (-)-hyoscyamine and (-)-scopolamine.

3.1.2 Evaluation of data on occurrence by the EFSA

Data on the occurrence of TA in foodsfrom which a majority (n=112) originated in the Netherlands and a small amount (n=12) originated in Germany, was incorporated in the EFSA's statement. All samples were taken between 2010 and 2012. Concentrations below the detection limit were found in 103 samples. According to the EFSA classification, 93 samples were classed as "*Cereal-based food for infants and young children"*, 56 of which were categorised as "*Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids"*. In the majority of cases, these are products which contain a mixture of different ground cereal types, such as wheat, maize, rye, oats or rice. *"Biscuits, rusks and cookies for children"* was considered as an additional category.

The EFSA determines that adequate data on the occurrence of (-)-hyoscyamine and (-)-scopolamine is only available for the food group "*Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids"*. Based on the labelling, a distinction could be made between products that are recommended for both infants[4] and toddlers[5] and products that are only recommended for toddlers. A larger proportion of the products intended only for toddlers was contaminated with TA and there were higher TA concentrations than in those products intended also for infants.

3.1.3 Evaluation of data on exposure by the EFSA

Short-term exposure to assess the acute toxicity was established for the toddler age group based on the DONALD study (Germany) and the DIPP study (Finland). The acute exposure for people with normal consumption was 0.039 µg/kg b.w. per day based on the DONALD study and 0.107 µg/kg b.w. per day based on the DIPP study. For individuals with high consumption, the estimated result was 0.081 µg/kg b.w. per day based on the DONALD study. The data from the DIPP study for individuals with high consumption was assessed as not very reliable and was therefore not used.

The EFSA came to the conclusion that the available consumption data, which originated from Germany and Finland, were sufficient only for an exposure assessment of the consumption of the toddler age group, but not that of infants.

The EFSA opinion also cites the publication of Perharič et al. (2013b), which reports cases of poisoning after the consumption of buckwheat products contaminated with TA from *Datura stramonium*. The exposure was estimated based on the TA concentrations in contaminated buckwheat flour. According to this estimate, the intake of (-)-hyoscyamine was between 0.7 and 137.6 µg/kg b.w., while that of (-)-scopolamine was between 0.4 und 63.5 µg/kg b.w..

[4] Children aged < 1 year, referred to as "infants" in the EFSA statement

[5] Children aged from 1-3, referred to as "toddlers" in the EFSA statement

3.1.4 Risk characterisation by the EFSA

Due to the restrictions explained above, the EFSA was only in a position to perform a risk assessment for the toddler age group and not for infants or consumers of other age groups.

The estimate of acute exposure of toddlers based on the limited German consumption data available showed that approximately two times the ARfD of 0.016 µg/kg b.w. is ingested with medium consumption and approximately five times the ARfD with high consumption.
Using the Finnish consumption data as a basis, an estimated acute exposure value for toddlers resulted which was six to seven times higher than the ARfD of 0.016 µg/kg b.w. with medium consumption. The Finnish data did not allow any meaningful risk analysis for toddlers with high consumption.

3.1.5 Evaluation and conclusion of the BfR

From the perspective of the BfR, the current EFSA report (EFSA CONTAM Panel, 2013) takes all relevant data that are available at present sufficiently into consideration. The derivation of a group ARfD based on a human study of healthy male adults with reference to the end points “Heart rate reduction” and “Effects on the central nervous system” is adequate.It is assumed that the selected uncertainty factor of 10 also sufficiently considers the possible increased sensitivity that could exist in infants, pregnant and breastfeeding women, unborn babies, sick people and the elderly, for example. The BfR therefore considers a group ARfD of 0.016 µg/kg b.w. for the sum of (-)-hyoscyamine and (-)-scopolamine to be an acceptable basis for a risk assessment of possible exposure to tropane alkaloids as contaminants in food. It is notable that the ARfD is approximately 80 to 150 times lower than the lowest effective therapeutic doses of (-)-hyoscyamine and (-)-scopolamine. It can be assumed here that the lowest effective therapeutic doses are not associated with serious undesired effects in the case of patients to whom no contraindications or usage restrictions (for example, due to cardiovascular diseases) apply.

The method for estimating exposure used by the EFSA corresponds to current scientific standards, and the results are outlined in a plausible manner. However, it should be noted that the estimate for toddlers was made based on only one food. Overall, the data used for estimating exposure is incomplete. The extent to which other foods could contribute to exposure is unclear. Under certain circumstances, actual exposure could be higher. A corresponding uncertainty analysis was carried out by the EFSA.

With respect to the question of how the risk associated with TA contamination of infant food should be assessed, the EFSA report makes the statement that a risk assessment of these products is not possible for infants due to insufficient consumption data for this age group. The evaluation of the data on the occurrence of TA in food merely allows the conclusion to be reached that contamination of cereal-based products for infant nutrition is at a relatively low level. For example, cereal-based products intended only for toddlers and not for infants contained higher TA concentrations and included a higher proportion of goods contaminated with TA compared to those which were also intended for infants. The BfR agrees with the statement that an assessment of the existing health risks for infants is not possible without adequate consumption data.

However, the EFSA was able to carry out a risk assessment for toddlers. Based on the German consumption data, this showed that approximately twice the ARfD of 0.016 µg/kg b.w. was ingested with medium consumption, and approximately five times the ARfD with high consumption. This means that the margine between the exposure value and the NOAEL observed in the human study is narrowed. The uncertainty factor is no longer ten, as considered necessary according to the ARfD derivation, but decreases to five or two, respectively. In healthy toddlers, the occurrence of minor to moderately severe health impairments is considered possible under these circumstances, although the occurrence of severe health problems is considered unlikely. Nevertheless, when the ARfD is exceeded to such an extent, the possibility of the occurrence of severe health impairments must be considered in persons with higher sensitivity, due to a cardiovascular disease, for example.

In this context, it should be mentioned that the EFSA opinion also describes individual therapeutic oral doses of atropine and (-)-scopolamine for children from two years of age onwards. In relation to the body weight, they are above the lowest individual therapeutic oral doses for adults, but within the same order of magnitude. No indications that children from two years of age onwards have a different sensitivity to TA than adults can be derived from this. Doses for therapeutic use in younger children are not specified. For fundamental reasons, in particular, pregnant or breastfeeding women and infants are seen as an especially sensitive consumer group for which any intake of pharmacologically active substances should be kept as low as possible.

On the basis of the present data situation, there are currently no specific indications of health impairments to infants, toddlers and consumers of other age groups through the consumption of TA-contaminated products. However, taking into account the current, albeit unsatisfactory, data situation, such health impairments must be considered possible in principle if the ARfD is exceeded. The severity of a possible impairment where twice to five times the ARfD is exceeded is assessed as minor to moderately severe in healthy individuals, but must be assessed as severe under certain circumstances for individuals suffering from specific diseases, such as cardiovascular diseases.

The BfR therefore recommends adherence to the specified ARfD and consistent use of agricultural and technological good manufacturing practice (GMP) in order to reduce TA levels. This applies in particular to products for the nutrition of infants and of toddlers.

Further, efforts to improve the data base on the occurrence of TA in different food categories and on their consumption by consumers of all age groups are seen as necessary.

3.2 Information on methods for the analysis of tropane alkaloids, particularly in cereals and infant food

For the analysis of the group of tropane alkaloids, which consists of more than 200 compounds with a tropane ring as their common basic structure, detection methods have mainly been developed for the individual compounds (-)-scopolamine, (-)-hyoscyamine and atropine. Atropine is a racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine.

A number of studies on the occurrence and the composition of tropane alkaloids in the various plants show that the tropane alkaloid pattern varies in the different plant parts and is also dependent on the geographical origin (Doncheva et al., 2006; Berkovet al., 2006). (-)-Scopolamine and (-)-hyoscyamine were identified as main agents in the plants studied to

date. The studies to identify and separate the individual compounds were performed primarily with GC-MS (gas chromatography with mass spectrometry). The relative proportion of individual compounds in relation to the total tropane alkaloids detected was considered to estimate the quantitative composition.

Detection methods were developed for plant extracts, cereals, buckwheat, fruits, flour, cereal-based foods such as crackers, pasta, biological materials such as plasma or urine, and for animal feed.

GC-MS and LC-MS/MS (liquid chromatography with tandem mass spectrometry) were used for the quantitative determination of (-)-scopolamine, (-)-hyoscyamine and atropine (Breton et al., 2005; Caligiani et al., 2011; Jandric et al., 2011; John et al., 2010). With some LC methods, the racemic mixture atropine was separated on a chiral column (Breton et al., 2005). Some of these methods have been validated in the BfR. A standardised method or one which has been validated in a round robin test is not available.

To extract the analytes from the sample matrix, acidified extraction agents are often used, as well as mixtures of organic solvents, e.g. dichloromethane, or acetonitrile/water mixtures. The extraction can be carried out using a Soxhlet extractor, ultrasound or incubation of the samples over a longer period of time. Liquid-liquid extractions, for example using Extrelut, solid phase extraction or dispersive solid phase extraction, are described for further sample purification. Other purification steps reported in literature are filtration or degreasing of the sample. The analytes can be derivatised for analysis with GC. Due to the volatility of tropane alkaloids, it is also possible to analyse the underivatised compounds. For analysis with LC MS/MS, separation of the analytes takes place primarily with RP-18 columns and detection takes place by means of electrospray ionisation and tandem mass spectrometry.

Standard addition or internal standardisation with nicotine as an internal standard are used for quantification (Caligiani et al., 2011).

(-)-Scopolamine, (-)-hyoscyamine and atropine, in particular, can be obtained commercially as analytical reference substances. A small number of other tropane alkaloids (e.g. homatropine or anisodamine) can be procured from suppliers of phytochemicals or manufacturers of special chemicals. Atropine is available as an isotope-labelled compound.

The following conclusions can be drawn from the available literature on analysis methods for the detection of tropane alkaloids:

- Further studies on the occurrence and pattern of tropane alkaloids in the different plants and plant parts must be carried out. The results from these studies also determine the further procedure for developing suitable analysis methods, particularly with respect to the selection of the required analytes. Conclusions can be drawn from these studies as to the food and animal feed products for which control of the tropane alkaloid level is necessary. This applies both to food and animal feed products containing plants in which tropane alkaloids naturally occur and to those products which are contaminated with these plants.
- Subsequently, standardisation of methods is desirable, particularly with respect to control of the relevant food and feed products.
- Certified reference materials are advantageous for checking the quality of an analysis. These materials are not available to date.

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